



Association between glucose fluctuation during 2-hour oral glucose tolerance test, inflammation and oxidative stress markers, and β -cell function in a Chinese population with normal glucose tolerance

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Backgrounds: Glucose fluctuation (GF) may have detrimental effects in individuals with diabetes; however, clinical data on the association between short-term GF, inflammation/oxidative stress markers, and islet β -cell function based on a population with normal glucose tolerance (NGT) are insufficient. Therefore, we aimed to explore these associations in a Chinese population of 209 individuals with NGT in a cross-sectional analysis.

Methods: Individuals were categorized based on GF tertiles, calculated as the maximum-minimum glucose levels among four time points (0, 30, 60, 120 min) during 2-hour oral glucose tolerance test (OGTT). Plasma inflammation markers tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), and oxidative stress markers superoxide dismutase (SOD), and 8-oxo-2'-deoxyguanosine (8-oxo-dG) were measured. Islet β -cell function was estimated according to the disposition index (DI) at the early (30 min) and total (120 min) phase of the OGTT, adjusted for insulin sensitivity.

Results: Individuals in the middle and highest tertile of GF had reduced β -cell function, and increased plasma SOD and TNF- α levels compared with those in the lowest tertile of GF ($P < 0.05$). Multiple linear regression analysis indicated that GF was positively associated with TNF- α , 8-oxo-dG and SOD levels, but negatively associated with β -cell function, whereas IL-6, TNF- α , 8-oxo-dG and SOD levels were negatively associated with β -cell function ($P < 0.05$).

Conclusions: GF may increase inflammation and oxidative stress markers in individuals with NGT, which could contribute to reduced β -cell function. Thus, maintaining glucose stability after a meal may have beneficial effects on delaying β -cell dysfunction, suggesting that diet and exercise strategies to decrease diet related GF are warranted.

Keywords: β -cell function; glucose fluctuation; inflammation; oxidative stress

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Introduction

Type 2 diabetes mellitus (T2DM), characterized by progressive deterioration of islet β -cell function, imposes a heavy social economic and public health burden globally. Individuals with impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are considered to be at greater risk for developing future T2DM than those with normal glucose tolerance (NGT); however, a large proportion of individuals with NGT could also develop diabetes after 5 years or longer (1). Moreover, mounting evidence indicates a degree of β -cell dysfunction in individuals with prediabetes (including IFG and IGT), as well as in some individuals with NGT (2-4). Therefore, it is an urgent task to identify the diabetes risk factors of individuals with NGT to implement effective and timely interventions that can prevent or delay the development of diabetes.

Diabetes and prediabetes may be diagnosed based on the value of fasting plasma glucose (FPG) and/or 2-hour postprandial plasma glucose (PPG) after an oral glucose tolerance test (OGTT). A high plasma glucose level can cause β -cell dysfunction by changing the pulsatility pattern of insulin secretion (5). Moreover, high glucose levels lead to upregulated expression of ATP-conducting mitochondrial outer membrane voltage-dependent anion channel-1 (VDAC1), resulting in cellular ATP depletion and impaired glucose-stimulated insulin secretion (6). Although overexpressed VDAC1 does not cause islet apoptosis itself, high VDAC1 levels combined with a high glucose concentration (20Mm), markedly increase the apoptosis rate (6). In recent years, there has been increasing awareness of the clinical value in assessment of glucose fluctuation (GF), which is regarded as a type of glycemic disorder, separate from high FPG and PPG, present in individuals with diabetes and prediabetes (7). GF is closely associated with oxidative stress and an inflammatory response, which may in turn aggravate β -cell dysfunction and diabetic complications (8-12). A German study based on a population with T2DM suggested that glycemic variability was strongly related to postprandial β -cell function for individuals taking oral hypoglycemia drugs (13). Another study using an *in vitro* model further demonstrated that intermittent high glucose could promote β -cell dysfunction

by increasing oxidative stress, leading to apoptosis (14). GF was also suggested to have harmful effects to endothelial function in both healthy controls and in individuals with T2DM (15). In most clinical studies, GF is estimated based on the mean amplitude of glycemic excursions (MAGE) computed from 3-day continuous glucose monitoring, however, the most common short-term GF may occur after a meal. Indeed, Polonsky and colleagues calculated the short-term GF by subtracting the minimum from the maximum glucose level during the 2-hour OGTT, which was associated with the plasma levels of oxidative stress markers in both individuals with diabetes and NGT (16). Another study used the difference between the maximum and minimum glucose levels during the 4-hour OGTT as a GF indicator in patients with coronary artery disease, which was closely associated with β -cell function and inflammation markers (8). Thus, the difference between maximum and minimum glucose levels during the 2-hour OGTT may be an accurate short-term indicator of GF and could have important downstream effects for the body.

Although most previous studies on GF have focused on its role in diabetes, GF is also closely associated with future incident cardiovascular disease and malignancies in the nondiabetic population (17,18), demonstrating the need for more attention to be paid to the role of GF in individuals with NGT. Moreover, identifying the risk factors of diabetes in individuals with NGT will help in devising strategies for delaying the development of diabetes. Toward this end, the aim of the present study was to explore the association between short-term GF, estimated as the maximum-minimum glucose levels during the 2-hour OGTT, plasma inflammation and oxidative stress markers, and β -cell function in a Chinese population with NGT. These findings can provide more evidence on the role of short-term GF in the development and progression of diabetes.

We present the following article in accordance with the MDAR checklist and the STROBE reporting checklist (available at <http://dx.doi.org/10.21037/atm-20-6119>).

Methods

Study population and clinical measurements

This study was based on a T2DM project conducted in the

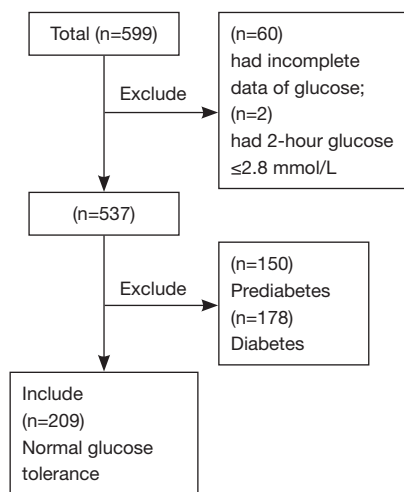


Figure 1 The flow chart of the population-based study.

Changping district of Beijing from 2014 to 2015, which is described in detail elsewhere (19). A total of 599 participants were recruited and completed questionnaires related to baseline characteristics, including age, gender and medical history. Detailed physical examination including body mass index (BMI) and blood pressure, were also performed. After a 2-hour 75-g OGTT, diabetes and prediabetes were diagnosed in accordance with the 1999 World Health Organization criteria. Participants diagnosed with diabetes or prediabetes, a 2-hour PPG ≤ 2.8 mmol/L, or missing data of glucose levels were excluded from the present analysis, whereas some individuals with missing data of markers of inflammation [tumor necrosis factor- α (TNF- α , n=35) and interleukin-6 (IL-6, n=36)] and oxidative stress [8-oxo-2'-deoxyguanosine (8-oxo-dG, n=36) and superoxide dismutase (SOD, n=35)] were nevertheless included in the analysis. Finally, a total of 209 adults of Chinese Han ethnicity aged from 19 to 73 years with NGT (FPG < 6.1 mmol/L and 2-hour PPG < 7.78 mmol/L) were enrolled in the study (Figure 1), including 64 men and 145 women.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Peking Union Medical College Hospital (Approval Number: ZS-1274) and informed consent was taken from all individual participants.

Biochemical measurements

Blood samples for plasma glucose measurement were obtained at 0, 30, 60, and 120 minutes after a 75-g oral

glucose load. Plasma glucose concentrations were assayed using a glucose oxidase assay. Insulin concentrations were quantified by a Chemiluminescent enzyme immunoassay (ADVIA Centaur XP, Siemens) according to the manufacturer instructions. Hemoglobin A1c (HbA1c) was analyzed in whole blood using high-performance liquid chromatography (D10 Hemoglobin Testing System, BioRad). An automated analyzer was used to determine serum uric acid (UA) and lipids concentrations. Plasma inflammation markers (TNF- α and IL-6) and oxidative stress markers (8-oxo-dG and SOD) were determined by respective kits (Cloud-Clone Corp, Houston, TX, USA).

Variables and calculation

Basal insulin resistance was estimated by homeostatic model assessment of insulin resistance (HOMA-IR):

$$\text{HOMA-IR} = \frac{\text{Fasting INS (uIU/mL)} \times \text{FPG (mmol/L)}}{22.5} \quad (20).$$

Basal and glucose stimulated insulin sensitivity was estimated by Matsuda insulin sensitivity index: (ISI_M):

$$\text{ISI}_M = \frac{10000}{\sqrt{\frac{(\text{FPG [mg/dL]} \times \text{Fasting INS [uIU/mL]}) \times (\text{Mean OGTT glucose concentration [mg/dL]} \times \text{Mean OGTT insulin concentration (uIU/mL)})}{(21)}}}$$

The disposition index (DI) was used as the measure of β -cell function adjusted by insulin sensitivity, calculated in the early phase at 30 min (DI₃₀):

$$\text{DI}_{30} = \left(\frac{\text{InsulinAUC}_{30}}{\text{GlucoseAUC}_{30}} \right) \times \text{ISI}_M, \text{ and in the total phase at}$$

$$120 \text{ min (DI}_{120}\text{): } \text{DI}_{120} = \left(\frac{\text{InsulinAUC}_{120}}{\text{GlucoseAUC}_{120}} \right) \times \text{ISI}_M \quad (22).$$

Statistical analysis

Normally distributed quantitative variables are presented as the mean \pm standard deviation (SD), and categorical variables are presented as number (percentage). Non-normally distributed variables are presented as the median [interquartile range (IQR)] or transformed using the natural logarithm as appropriate. Quantitative parameters were compared among three groups using the Kruskal-Wallis test or one-way analysis of variance with a post-hoc Bonferroni test. Differences among three groups were evaluated by the χ^2 test for categorical variables. Correlations between GF, inflammation and oxidative stress markers, and β -cell

secretion and insulin resistance index were analyzed using Spearman's correlation coefficient. Multiple linear regression analysis was used to explore the associations between GF, inflammation and oxidative stress markers, and β -cell function. Model 1 was adjusted for age, gender, BMI and systolic blood pressure (SBP). Model 2 was adjusted for the covariates of model 1 plus triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and UA. Model 3 was adjusted for the covariates of model 2 plus the 2-hour PPG. β coefficients and 95% confidence intervals (CIs) were calculated per 1-SD increment of GF, and inflammation and oxidative stress markers. Individuals with missing data of markers of inflammation and oxidative stress were not included in analyses involving the related markers.

Statistical analyses were conducted using SPSS 22.0 software. A two-sided P value <0.05 was considered statistically significant.

Results

Association of GF with clinical and demographic characteristics

Clinical and demographic characteristics of the participants based on tertiles of GF are shown in *Table 1*. The median GF of individuals was 2.37 mmol/L (IQR, 1.54–2.80), 4.07 mmol/L (IQR, 3.76–4.49) and 5.96 mmol/L (IQR, 5.34–7.38) in the lowest, middle and highest tertile, respectively. Compared with individuals in the lowest GF tertile, those in the highest tertile were more likely to be older, male, and also had higher levels of UA and HbA1c (all $P<0.05$). In terms of β -cell function, individuals in higher GF tertile had lower DI_{30} and DI_{120} values ($P<0.001$) than those in the lowest tertile of GF, whereas there were no significant differences in insulin resistance index among the three GF groups.

Individuals with higher GF also exhibited higher values of plasma TNF- α and SOD than those in the lowest GF tertile ($P<0.05$), whereas the differences in IL-6 and 8-oxo-dG among the three groups did not reach statistical significance.

Correlations of GF, inflammation and oxidative stress markers, and β -cell function and insulin resistance index

The correlations between GF, inflammation and oxidative stress markers, and β -cell function and insulin resistance index are provided in *Table 2*. GF was negatively correlated

with DI_{30} ($r=-0.493$, $P<0.001$) and DI_{120} ($r=-0.431$, $P<0.001$). The level of plasma IL-6 was also negatively correlated with both DI_{30} ($r=-0.152$, $P=0.046$) and DI_{120} ($r=-0.210$, $P=0.005$), whereas the plasma TNF- α level was only negatively correlated with DI_{120} ($r=-0.183$, $P=0.016$). In terms of oxidative stress markers, 8-oxo-dG was negatively correlated with DI_{30} ($r=-0.152$, $P=0.046$) and SOD was negatively correlated with DI_{120} ($r=-0.187$, $P=0.014$). Neither the inflammation markers nor the oxidative stress markers were correlated with the insulin resistance index.

Multiple linear regression analysis of the association between glucose fluctuation and β -cell function

The coefficients of multiple linear regression analysis for GF and β -cell function estimated by DI_{30} and DI_{120} are presented in *Table 3*. GF was negatively associated with DI_{30} ($\beta = -29.818$, 95% CI: -39.519 to -20.118, $P<0.001$) and DI_{120} ($\beta = -20.676$, 95% CI: -28.870 to -12.482, $P<0.001$) in Model 1 after adjustment for age, gender, BMI and SBP. With further adjustment for TG, HDL-C and UA in Model 2, the negative trend remained significant ($P<0.001$). After additional adjustment for 2-hour PPG in Model 3, the negative association between GF and β -cell function remained significant ($P<0.001$).

Multiple linear regression analysis of the association between GF and markers of inflammation and oxidative stress

The coefficients of multiple linear regression analysis for GF and inflammation markers are provided in *Table 4*. After adjustment for age, gender, BMI and SBP, GF was positively associated with TNF- α in Model 1 ($\beta = 2.204$, 95% CI: 0.719 to 3.689, $P=0.004$), and the association remained significant ($\beta = 2.314$, 95% CI: 0.786 to 3.842, $P=0.003$) independent of age, gender, BMI, SBP, lipids, UA and 2-hour PPG in Model 3. IL-6 was also positively associated with GF, but the P value did not reach statistical significance in any model (all $P>0.05$, Model 1 to 3).

The coefficients of multiple linear regression analysis for GF and oxidative stress markers are provided in *Table 5*. After adjustment for age, gender, BMI and SBP, GF was positively associated with SOD ($\beta = 4.178$, 95% CI: 1.341 to 7.105, $P=0.004$) and 8-oxo-dG ($\beta = 6.240$, 95% CI: 0.835 to 11.646, $P=0.024$) in Model 1. These associations remained significant independent of age, gender, BMI, SBP, lipids, UA and 2-hour PPG in Model 3 ($P<0.05$).

Table 1 Association of glucose fluctuation with Clinical and demographic characteristics

	Lowest GF (n=69)	Middle GF (n=71)	Highest GF (n=69)	P
GF (mmol/L)	2.37 (1.54–2.80)	4.07 (3.76–4.49)*	5.96 (5.34–7.38) [#]	<0.001
Gender, n (%)				<0.001
Female	56 (81.2)	56 (78.9)	33 (47.8)	
Male	13 (18.8)	15 (21.1)	36 (52.2)	
Age (years)	46.42±10.87	50.08±11.1	53.55±10.72*	0.001
BMI (kg/m ²)	24.87±3.46	25.72±3.65	25.44±3.6	0.356
SBP (mmHg)	121.06±16.98	126.96±17.96	126.96±17.24	0.073
DBP (mmHg)	76.49±9.64	75.82±10.32	74.87±9.31	0.619
TC (mmol/L)	5.13±0.95	5.40±1.19	5.39±0.91	0.215
LnTG (mmol/L)	0.14±0.50	0.25±0.55	0.26±0.55	0.304
HDL-C (mmol/L)	1.35±0.54	1.35±0.28	1.29±0.27	0.542
LDL-C (mmol/L)	2.58±0.72	2.78±0.78	2.79±0.66	0.179
UA (umol/L)	262.64±74.09	282.33±79.62	298.82±75.75*	0.023
HbA1c (%)	5.2 (5.0–5.5)	5.4 (5.2–5.6)	5.5 (5.15–5.7)*	0.009
FPG (mmol/L)	5.42 (5.13–5.65)	5.57 (5.37–5.76)	5.43 (5.26–5.80)	0.081
2h-PPG (mmol/L)	6.11 (5.11–6.83)	6.28 (5.69–7.15)	5.90 (4.62–6.83)	0.054
LnHOMA-IR	0.77±0.43	0.84±0.47	0.71±0.67	0.353
Ln (ISI _M)	1.59±0.42	1.43±0.46	1.42±0.64	0.105
DI ₃₀	229.89±82.97	174.12±45.55*	151.77±70.92*	<0.001
DI ₁₂₀	226.24±67.03	212.11±42.47*	209.07±62.12*	<0.001
TNF-α (fmol/mL) (n=174)	19.70 (14.45–25.06)	27.83 (18.45–35.50)*	25.75 (18.98–34.53)*	<0.001
IL-6 (pg/mL) (n=173)	3.05 (1.98–4.90)	3.73 (2.48–4.48)	3.53 (2.11–5.55)	0.651
8-oxo-dG (pg/mL) (n=173)	36.63 (23.09–53.40)	36.63 (20.73–56.03)	43.81 (26.46–63.56)	0.324
SOD (U/mL) (n=174)	56.41 (45.63–66.08)	60.16 (53.47–70.13)	62.52 (55.34–75.12)*	0.030

Data were presented as mean ± SD, n (%) or median (interquartile range). *Compared with the lowest tertile of GF, a two-side P<0.05 was considered statistically significant; [#]Compared with the middle tertile of GF, a two-side P<0.05 was considered statistically significant. GF, glucose fluctuation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose; PPG, postprandial plasma glucose; HOMA-IR, homeostatic model assessment of insulin resistance; ISI_M, Matsuda insulin sensitivity index; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; 8-oxo-dG, 8-oxo-2'-deoxyguanosine; SOD, superoxide dismutase.

Multiple linear regression analysis of the association between inflammation/oxidative stress markers and β-cell function

The coefficients of multiple linear regression analysis for inflammation/oxidative stress markers and β-cell function are provided in *Table 6*. TNF-α was negatively associated with DI₁₂₀ (β = -9.455, 95% CI: -17.895 to -1.015, P=0.028),

whereas IL-6 was negatively associated with both DI₃₀ (β = -10.933, 95% CI: -21.812 to -0.053, P=0.049) and DI₁₂₀ (β = -11.672, 95% CI: -20.025 to -3.319, P=0.006) after adjustment for age, gender, BMI and SBP in Model 1. These associations remained valid after adjustment for TG, HDL-C and UA in Model 2 and with further adjustment for 2-hour PPG in Model 3.

Table 2 Correlations of glucose fluctuation, inflammation and oxidative stress markers, and β -cell function and insulin sensitivity index

	DI ₃₀		DI ₁₂₀		LnHOMA-IR		LnISI _M	
	r	P	r	P	r	P	r	P
GF (n=209)	-0.493	<0.001	-0.431	<0.001	-0.032	0.643	-0.134	0.052
TNF- α (n=174)	-0.136	0.073	-0.183	0.016	-0.086	0.260	0.008	0.912
IL-6 (n=173)	-0.152	0.046	-0.210	0.005	0.025	0.746	0.027	0.725
8-oxo-dG (n=173)	-0.152	0.046	-0.072	0.346	-0.027	0.720	0.029	0.709
SOD (n=174)	-0.109	0.153	-0.187	0.014	-0.097	0.205	0.024	0.756

A two-side $P < 0.05$ was considered statistically significant. HOMA-IR, homeostatic model assessment of insulin resistance; ISI_M, Matsuda insulin sensitivity index; GF, glucose fluctuation; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; 8-oxo-dG, 8-oxo-2'-deoxyguanosine; SOD, superoxide dismutase.

Table 3 β coefficients and 95% confidence intervals of multiple linear regression models of glucose fluctuation with β -cell function

	DI ₃₀		DI ₁₂₀	
	β (95% CI)	P	β (95% CI)	P
Model 1	-29.818 (-39.519, -20.118)	<0.001	-20.676 (-28.870, -12.482)	<0.001
Model 2	-30.006 (-39.653, -20.359)	<0.001	-20.347 (-28.550, -12.144)	<0.001
Model 3	-35.964 (-44.814, -27.115)	<0.001	-26.205 (-33.418, -18.991)	<0.001

Model 1 was adjusted for age, gender, body mass index and systolic blood pressure. Model 2 was adjusted for the covariates of model 1 plus triglycerides, high-density lipoprotein cholesterol and uric acid. Model 3 was adjusted for the covariates of model 2 plus 2-hour postprandial plasma glucose. A two-side $P < 0.05$ was considered statistically significant. β coefficients and 95% confidence intervals (CI) were calculated as per 1-SD increment of glucose fluctuation.

Table 4 β coefficients and 95% confidence intervals of multiple linear regression models of glucose fluctuation with inflammation markers

	TNF- α (n=174)		IL-6 (n=173)	
	β (95% CI)	P	β (95% CI)	P
Model 1	2.204 (0.719, 3.689)	0.004	0.292 (-0.215, 0.799)	0.257
Model 2	2.143 (0.650, 3.636)	0.005	0.254 (-0.254, 0.762)	0.325
Model 3	2.314 (0.786, 3.842)	0.003	0.253 (-0.268, 0.774)	0.339

TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6. Model 1 was adjusted for age, gender, body mass index and systolic blood pressure. Model 2 was adjusted for the covariates of model 1 plus triglycerides, high-density lipoprotein cholesterol and uric acid. Model 3 was adjusted for the covariates of model 2 plus 2-hour postprandial plasma glucose. A two-side $P < 0.05$ was considered statistically significant. β coefficients and 95% confidence intervals (CI) were calculated as per 1-SD increment of glucose fluctuation.

SOD was negatively associated with DI₁₂₀ in all three models. Moreover, 8-oxo-dG was marginally (but not significantly) associated with DI₃₀ after adjustment for age, gender, BMI and SBP in Model 1 (Table 6). With further adjustment for TG, HDL-C and UA in Model 2, the associations between 8-oxo-dG and DI₃₀ still did not reach statistical significance ($P = 0.109$). However, the negative

association between DI₃₀ and 8-oxo-dG became significant after additional adjustment for 2-hour PPG ($\beta = -11.269$, 95% CI: -21.917 to -0.620, $P = 0.038$) in Model 3) (Table 6).

Discussion

Our present study based on individuals with NGT

Table 5 β coefficients and 95% confidence intervals of multiple linear regression models of glucose fluctuation with oxidative stress markers

	8-oxo-dG (n=173)		SOD (n=174)	
	β (95% CI)	P	β (95% CI)	P
Model 1	6.240 (0.835, 11.646)	0.024	4.178 (1.341, 7.105)	0.004
Model 2	6.057 (0.652, 11.461)	0.028	4.082 (1.237, 6.927)	0.005
Model 3	5.654 (0.140, 11.167)	0.045	4.564 (1.662, 7.467)	0.002

8-oxo-dG, 8-oxo-2'-deoxyguanosine; SOD, superoxide dismutase Model 1 was adjusted for age, gender, body mass index and systolic blood pressure. Model 2 was adjusted for the covariates of model 1 plus triglycerides, high-density lipoprotein cholesterol and uric acid. Model 3 was adjusted for the covariates of model 2 plus 2-hour postprandial plasma glucose. A two-side $P < 0.05$ was considered statistically significant. β coefficients and 95% confidence intervals (CI) were calculated as per 1-SD increment of glucose fluctuation.

Table 6 β coefficients and 95% confidence intervals of multiple linear regression models of inflammation and oxidative stress markers with β -cell function

	DI ₃₀		DI ₁₂₀	
	β (95% CI)	P	β (95% CI)	P
TNF- α (n=174)				
Model 1	-5.574 (-16.573, 5.424)	0.318	-9.455 (-17.895, -1.015)	0.028
Model 2	-6.520 (-17.597, 4.558)	0.247	-9.245 (-17.683, -0.807)	0.032
Model 3	-5.831 (-16.395, 4.733)	0.277	-8.612 (-16.459, -0.766)	0.032
IL-6 (n=173)				
Model 1	-10.933 (-21.812, -0.053)	0.049	-11.672 (-20.025, -3.319)	0.006
Model 2	-11.333 (-22.339, -0.327)	0.044	-10.669 (-19.080, -2.258)	0.013
Model 3	-11.732 (-22.172, -1.292)	0.028	-11.038 (-18.796, -3.279)	0.006
8-oxo-dG (n=173)				
Model 1	-10.407 (-21.393, 0.580)	0.063	-6.439 (-15.352, 2.474)	0.156
Model 2	-9.137 (-20.322, 2.048)	0.109	-5.127 (-14.079, 3.826)	0.260
Model 3	-11.269 (-21.917, -0.620)	0.038	-7.355 (-15.512, 0.803)	0.077
SOD (n=174)				
Model 1	-9.276 (-20.175, 1.623)	0.095	-9.891 (-18.164, -1.618)	0.019
Model 2	-8.529 (-19.569, 2.510)	0.129	-9.240 (-17.543, -0.936)	0.029
Model 3	-7.079 (-17.651, 3.492)	0.188	-7.897 (-15.636, -0.158)	0.046

TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; 8-oxo-2'-deoxyguanosine; SOD, superoxide dismutase Model 1 was adjusted for age, gender, body mass index and systolic blood pressure. Model 2 was adjusted for the covariates of model 1 plus triglycerides, high-density lipoprotein cholesterol and uric acid. Model 3 was adjusted for the covariates of model 2 plus 2-hour postprandial plasma glucose. A two-side $P < 0.05$ was considered statistically significant. β coefficients and 95% confidence intervals (CI) were calculated as per 1-SD increment of inflammation and oxidative stress markers.

demonstrated that individuals in the higher tertiles of short-term GF (using the difference between maximum and minimum glucose at various time points during the 2-hour OGTT) had reduced β -cell function but higher

plasma levels of TNF- α and SOD compared with those of individuals in the lowest GF tertile. Moreover, multiple linear regression analysis indicated that GF was negatively associated with β -cell function but positively associated with

TNF- α , SOD and 8-oxo-dG levels, and these inflammation and oxidative stress markers were also negatively associated with β -cell function. Although the positive association between IL-6 and GF did not reach statistical significance, IL-6 was also negatively associated with β -cell function. In addition, individuals in the lowest GF tertile were more likely to be female and younger (mean age 46.42 years), which may be related to high estrogen levels that have been shown to contribute to a decrease in oxidative stress and inflammation (23-25). However, multiple linear regression analysis indicated that gender and age were not significantly associated with inflammation or oxidative stress markers in the whole population (data not shown).

Both experimental and clinical studies have established that GF was closely associated with β -cell function (13,14,26-28); however, most of these clinical studies included only individuals with diabetes and GF was estimated according to MAGE, which is typically calculated from 3-day continuous glucose monitoring. Nevertheless, Murata *et al.* (8) found that GF during the 4-hour OGTT was closely associated with β -cell function in patients with different degrees of glucose tolerance and coronary artery disease, suggesting that short-term GF may be another potential marker to estimate β -cell function. Our present study including only individuals with NGT provides further evidence that estimation of shorter-term GF during the 2-hour OGTT was significantly associated with β -cell function, which may be the main mechanism related to oxidative stress and inflammation. Increased oxidative stress was observed in the Goto-Kakizaki rat diabetes model, displaying high basal glucose levels and diminished glucose-stimulated insulin release both *in vivo* and *in vitro* (29,30). Individuals with abnormal glucose tolerance also show altered levels of oxidative stress markers such as SOD and 8-oxo-dG. SOD is a type of antioxidant, and its levels were shown to be increased in individuals with T2DM compared with those of healthy controls, which is considered to indicate a compensatory mechanism in response to elevated oxidative stress (31). 8-oxo-dG is a product of DNA oxidative damage, which was also shown to be elevated in subjects with abnormal glucose tolerance (19). Moreover, a clinical study indicated that GF caused a significant increase in oxidative stress and endothelial dysfunction compared with a constant high glucose state in both healthy controls and T2DM patients (15). Our present data support these previous studies by showing a positive association of GF during the 2-hour OGTT with SOD and 8-oxo-dG in

individuals with NGT, and these oxidative stress markers were negatively associated with β -cell function. Therefore, short-term GF may be also one cause of β -cell dysfunction in individuals with NGT via oxidative stress. This also supports the previous *in vitro* results that intermittent high glucose had more of an impact on β -cell dysfunction than sustained hyperglycemia due to increased oxidative stress (14). We also found that the levels of TNF- α , as an inflammatory cytokine, were positively associated with GF but negatively associated with β -cell function in individuals with NGT, which is consistent with a study in diabetic rats showing that GF enhanced the expression of inflammation factors such as TNF- α (32) as another critical signal linked to β -cell dysfunction (33). Since the present cross-sectional analysis can only demonstrate that the negative association between GF and β -cell function was closely linked to inflammation and oxidative stress, larger longitudinal mechanistic studies are needed to confirm the causal relationships. Nevertheless, these findings suggested that diet and exercise strategies to maintain glucose stability after a meal would be warranted to prevent β -cell dysfunction.

In addition to the aggravation of β -cell dysfunction, GF has been shown to play critical roles in the occurrence and development of diabetic complications (12,34). Glucose variability was an independent risk for future incident cardiovascular disease even in individuals without diabetes (18). Interestingly, we found that GF was independently associated with oxidative stress and inflammation markers in patients with NGT, which may have detrimental effects promoting the development of metabolic disorders. From this point of view, diet and exercise intervention to regulate GF, and ultimately decrease inflammation and oxidative stress to delay β -cell dysfunction, should be a general recommendation, irrespective of an individual's glucose tolerance status.

These findings therefore offer a new perspective for diabetes prevention and treatment, while drawing attention to the role of GF after a meal. Our study also had some limitations. First, the cross-sectional design makes it difficult to yield a causal relationship, and longitudinal mechanistic studies are warranted. Nevertheless, the major results are supported by previous clinical and preclinical studies (14,15,29-33). Second, we only included Chinese Han individuals, and thus further study is needed to explore whether the results apply to other ethnicities. However, restricting the sample to a single race also reduced the

possibility of introducing other confounding factors common in multiethnic studies.

Conclusions

In conclusion, as a simple indicator of GF, the difference between maximum and minimum glucose levels during the 2-hour OGTT was positively associated with inflammation and oxidative stress markers but negatively associated with β -cell function in a Chinese population with NGT. GF may increase plasma inflammation and oxidative stress markers in individuals with NGT, which could contribute to reduced β -cell function. Thus, maintaining glucose stability after a meal may have beneficial effects even in individuals with NGT, and diet and exercise strategies to decrease diet related GF are warranted to delay or prevent the development of diabetes.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/atm-20-6119>). The authors declare that they have no conflicts of interest.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Peking Union Medical College Hospital

(Approval Number: ZS-1274) and informed consent was taken from all individual participants.

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