

Impaired secretion of glucagon-like peptide 1 during oral glucose tolerance test in patients with newly diagnosed type 2 diabetes mellitus

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ABSTRACT

الأهداف: لتقييم إفراز الببتيد شبيه الجلوكاجون (GLP-1) بعد اختبارات تحمل الغلوكوز عن طريق الفم (OGTTs) في الموضوعات شُخصت حديثاً من النوع 2 السكري (T2DM)، ضعف تحمل الجلوكوز (IGT)، وتحمل الغلوكوز العادي (NGT) إلى توضيح التغييرات في إفراز GLP-1 أثناء T2DM.

الطريقة: في هذه الدراسة المقطعية، قُسم 80 موضوعاً في المجموعات NGT، IGT، وT2DM بعد خضوعه 75 OGTT من مارس إلى ديسمبر 2014م في كلية الطب ومستشفى الأولى التابعة، جامعة شيجهتسي وشينجيانغ، الصين. وقد تم قياس البلازما الكلي GLP-1 في 0، 30، 60، 120، و 180 دقيقة. وتم قياس HOMA-IR، islet، تقييم نموذج الاستتباب مقاومة الأنسولين (HOMA-IR)، β -cell function (HOMA- β)، Gutt index، Matsuda index، incremental GLP-1 (Δ GLP-1)، والمناطق الحاضمة لمنحنيات (AUC_{glp-1})، الجلوكوز (AUC_g)، والأنسولين (AUC_{ins}).

النتائج: كانت البلازما الكلي GLP-1 في 30-120 دقيقة و Δ GLP-1 في 30-120 دقيقة أقل في مجموعة من T2DM في المجموعات IGT and NGT ($p < 0.05$) وكانت ذروة مستويات GLP-1 انخفاض 35% في المجموعة T2DM من مجموعة NGT. البلازما الكلي Δ GLP-1، GLP-1، و AUC_{glp-1} تتناسب عكسياً مع HOMA-IR و AUC_g ، وإيجابية مع HOMA- β ، β ، Gutt index، Matsuda index، ($p < 0.05$) AUC_{ins} .

الخلاصة: شخصت حديثاً GLP-1 بعد أن إفراز ضعف 75 OGTT في المرضى الذين يعانون T2DM، يتناسب عكسياً مع الأشعة تحت الحمراء وارتفاع السكر في الدم، وارتبطت إيجابياً مع وظيفة خلايا β وحساسية للأنسولين.

Objectives: To assess glucagon-like peptide 1 (GLP-1) secretion after oral glucose tolerance tests (OGTTs) in subjects with newly diagnosed type 2 diabetes mellitus (T2DM), impaired glucose tolerance (IGT), and normal glucose tolerance (NGT) to clarify changes in GLP-1 secretion during the course of T2DM.

Methods: In this cross sectional study, 80 subjects were divided into the NGT, IGT, and T2DM groups after undergoing a 75 g OGTT from March to December 2014 at the School of Medicine, First Affiliated Hospital, Shihezi University, Xinjiang, China. Plasma total GLP-1 was measured at 0, 30, 60, 120, and 180 minutes. Homeostasis model assessment of insulin resistance (HOMA-IR), islet β -cell function (HOMA- β), Gutt index, Matsuda index, incremental GLP-1 (Δ GLP-1), and areas under the curves of GLP-1 (AUC_{glp-1}), glucose (AUC_g), and insulin (AUC_{ins}) were calculated.

Results: Plasma total GLP-1 at 30-120 minutes and Δ GLP-1 at 30-120 minutes were lower in the T2DM group than in the IGT and NGT groups ($p < 0.05$). Peak GLP-1 levels were 35% lower in the T2DM group than in the NGT group. Plasma total GLP-1, Δ GLP-1, and AUC_{glp-1} correlated negatively with HOMA-IR and AUC_g , and positively with HOMA- β , Gutt index, Matsuda index, and AUC_{ins} ($p < 0.05$).

Conclusion: The GLP-1 secretion after 75 g OGTT was impaired in newly diagnosed T2DM patients, inversely proportional to IR and hyperglycemia, and positively correlated with β -cell function and insulin sensitivity.

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Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder with multiple pathophysiological abnormalities. Currently, T2DM is one of the most common chronic diseases in almost every country. China has the largest population of diabetes patients. The prevalence of diabetes among adults in China is 11.6%, which is equivalent to 114 million patients.¹ Glucagon-like peptide-1 (GLP-1) is secreted by L-cells in the distal parts of the intestines in response to nutrient ingestion.² The GLP-1 regulates blood glucose levels deriving from several mechanisms. It stimulates insulin secretion from the pancreatic β -cells in a glucose-dependent manner and suppresses glucagon secretion.^{3,4} The GLP-1 also affects gastrointestinal motility, enhances satiety, promotes weight loss, and increases β -cell mass.⁵⁻⁹ Therefore, impaired GLP-1 secretion may contribute to the initiation and development of DM. As a consequence of these properties, GLP-1 based therapies (GLP-1 agonists and dipeptidyl peptidase-4 inhibitors) are currently playing a cornerstone role in the treatment of T2DM. Since GLP-1 plays an important role in the pathophysiology of DM, the assessment of GLP-1 secretory responses in individuals with different glycometabolism states is of great interest. The outcomes of GLP-1 secretion after the administration of an oral glucose load in individuals with and without diabetes are controversial. Toft-Nielsen et al¹⁰ reported a 53% reduction in integrated incremental GLP-1 concentrations in T2DM patients relative to healthy controls, while participants with impaired glucose tolerance (IGT) had an intermediate GLP-1 response. However, other studies^{11,12} have reported that GLP-1 secretion was not reduced in response to an oral glucose tolerance test (OGTT), or meal test in patients with T2DM. The mode of GLP-1 secretion in Asians may be different. Thus far, limited data are available on comparisons of GLP-1 levels among Chinese individuals with newly diagnosed T2DM, IGT, and normal glucose tolerance (NGT). Therefore, this study was conducted to measure GLP-1 levels during a standard OGTT in Chinese subjects with newly diagnosed T2DM, IGT, and NGT, in order to characterize the changes in GLP-1 secretion during the course of T2DM development. We also investigated

the relationship between GLP-1 secretion, insulin resistance (IR), and insulin β -cell function.

Methods. This cross sectional study was carried out between March and December 2014. Data were collected from the Endocrinology and Metabolism Department at the School of Medicine, First Affiliated Hospital, Shihezi University, Shihezi, Xinjiang, China. A total of 80 participants, aged 35-71 years, and suspected to have T2DM were considered eligible for this study. All participants were of Han Chinese ethnicity and had not been treated with any hypoglycemic or hypolipidemic agents. Individuals with malignancy, serious hepatic diseases, gastrointestinal diseases, or other major diseases were excluded. On the basis of the results of a 75 g OGTT, participants were divided into T2DM group (n=35), IGT group (n=22), and NGT group (n=23). The diagnoses of IGT and T2DM were based on the World Health Organization diagnostic criteria.¹³ Before the study, informed consent was obtained from every participant. This study was conducted in accordance with the principles of the Helsinki Declaration. It was approved by the Human Research Ethics Committee of the First Affiliated Hospital, School of Medicine, Shihezi University.

Anthropometric and biochemical measurements. In all participants, anthropometric and body composition measurements were performed before breakfast. Body mass index (BMI) was calculated as weight divided by height squared. Waist and hip circumferences were measured for the calculation of the waist-to-hip ratio (WHR). A standard 75 g OGTT was conducted for all subjects after overnight fasting (longer than 10 hours). In brief, 75 g glucose was ingested within 5 minutes. Blood samples were collected in the fasting state, and 30, 60, 120, and 180 minutes after taking 75 g glucose. Blood samples were withdrawn from the cubital vein and collected in evacuated sample tubes containing preservatives (such as, EDTA-containing tubes for biochemical measurements, EDTA and dipeptidyl peptidase-4 inhibitor-containing tubes for GLP-1 determination). The tubes were kept on ice until centrifugation. Separated plasma samples were frozen and stored at -70°C until further analysis. In addition, one ml fresh whole blood samples were collected for the measurement of hemoglobin A1c (HbA1c) levels using an automatic HbA1c analyzer (D10, Bio-Rad, California, USA). Plasma glucose (at each OGTT time point), and fasting plasma total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were

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analyzed enzymatically using an autoanalyzer (Hitachi, Tokyo, Japan). Fasting plasma C-peptide (FCP) and insulin (at each OGTT time point) were detected using electrochemiluminescence (E170, Roche, Switzerland). Plasma total GLP-1 (at each OGTT time point) was determined using an enzyme-linked immunosorbent assay kit, as per the manufacturer's instructions (Westang Biological Technology, Shanghai, China). The intra- and inter-assay coefficients of variation were less than 10.3%, and the sensitivity was typically less than 0.1 pmol/L.

Statistical analysis and calculations. Data analyses were carried out using Statistical Package for Social Sciences version 17 (SPSS Inc., Chicago, IL, USA). The results were expressed as the mean \pm standard deviation (SD), or as median and interquartile range, if the data were not normally distributed. Normal distribution of the data was tested. One-way analysis of variance followed by the Student-Neuman-Keuls method was used to test between-group differences in normally distributed data. Comparisons of the constituent ratios were assessed using the chi-square test. The rank sum test was used for nonparametric data, and the Kruskal-Wallis test was used for multiple comparisons. Spearman rank correlation analysis was used to explore the correlations between total GLP-1 levels (at each OGTT time point), incremental GLP-1 (Δ GLP-1) levels, and area under the curve (AUC_{glp-1}) with homeostatic model assessment (HOMA)-IR, $HOMA-\beta$, Matsuda index, Gutt index, AUC_g , and AUC_{ins} . Multivariate linear regression was also carried out to adjust for confounding factors for GLP-1 levels (at each OGTT time point). *P*-values less than 0.05 were considered to indicate a statistically significant difference. The AUCs were calculated using the trapezoidal method. The Δ GLP-1 levels were calculated by subtracting the fasting GLP-1 level from the GLP-1 levels at 30, 60, 120, and 180 minutes. The HOMA-IR was used to evaluate IR, and $HOMA-\beta$ was used to estimate basal insulin secretion.¹⁴ Meanwhile, Matsuda index¹⁵ and Gutt indices¹⁶ were chosen for assessment of insulin sensitivity. Formulas are as follows:

$$HOMA-IR = \frac{FINS \times FPG}{22.5}$$

$$HOMA-\beta = \frac{FINS \times 20}{FPG - 3.5}$$

$$Gutt \text{ index} = \frac{75000 + (FPG - 2hPG) \times 0.19 \times BW}{120 \times \log \left(\frac{[FINS + 2hINS] / 2}{(FPG + 2hPG) / 2} \right)}$$

$$Matsuda \text{ index} = \frac{10000}{\sqrt{FINS \times FPG \times I \times G}}$$

where: FPG = fasting plasma glucose level, 2hPG = 2-hour postprandial blood glucose level of OGTT, G = mean plasma glucose level of OGTT, FINS (μ U/mL) = fasting insulin level, 2hINS = 2-hour insulin level of OGTT, I (μ U/mL) = mean insulin level of OGTT, BW (Kg) = body weight. Plasma glucose levels were mg/dl in Matsuda index formula, and mmol/l in other formulas.

Results. General clinical characteristics. The general characteristics of the subjects in the 3 groups is shown in Table 1. Age and gender distribution did not significantly differ among the groups ($p > 0.05$ for all). Systolic blood pressure (SBP), diastolic blood pressure (DBP), and LDL-C levels were significantly higher in the T2DM group than in the NGT group ($p < 0.05$ for all). The TG, TC, FPG, and HbA1c levels were higher in the T2DM than in the IGT and NGT groups ($p < 0.05$ for all). Compared with the NGT group, the T2DM exhibited low HDL-C level ($p < 0.05$). The HOMA-IR was higher and $HOMA-\beta$, Gutt and Matsuda indices were lower in the T2DM group than in the IGT and NGT groups ($p < 0.05$ for all). Moreover, $HOMA-\beta$, Gutt and Matsuda indices were lower in the IGT than in the NGT group ($p < 0.05$ for all). Other variables, such as height, weight, waist circumference, hip circumference, BMI, WHR, FCP, and FINS were similar in all groups ($p > 0.05$ for all).

Total GLP-1, glucose, and insulin levels during OGTT. The fasting and 180 minutes plasma total GLP-1 level in the T2DM group was lower than in the NGT group; GLP-1 levels at 30-120 min were lower in the T2DM group than in the IGT and NGT groups (Figure 1A). However, these levels did not differ between the IGT and NGT groups ($p > 0.05$). In each group, peak GLP-1 secretion occurred at approximately 30 minutes during OGTT. The peak level of total GLP-1 in the T2DM group was 35% lower than that in the NGT group. Glucose and insulin levels markedly differed among the 3 groups ($p < 0.05$ for all, Figures 1B and 1C). In addition, Δ GLP-1 levels were compared in this study (Figure 1D). The Δ GLP-1_{30 min}, Δ GLP-1_{60 min}, and Δ GLP-1_{120 min} levels were lower in the T2DM group than in the IGT and NGT groups. The Δ GLP-1_{180 min} level in the T2DM group was lower than that in the NGT group ($p < 0.05$). No significant differences in Δ GLP-1 levels were observed between the IGT and NGT groups ($p > 0.05$). We also compared AUC_{glp-1} among the 3 groups (Figure 1E). The AUC_{glp-1} was obviously lower in the T2DM group than in the IGT and NGT groups ($p < 0.05$). Furthermore, there was a 32.8% reduction in AUC_{glp-1} in the T2DM group relative to the NGT group.

Table 1 - General clinical characteristics of the subjects included in a study in China ($\bar{x} \pm$ standard deviation).

Demographics	T2DM	IGT	NGT	P-value
N, (male/female)	35 (17/18)	22 (14/8)	23 (12/11)	0.531
Age, years	52.1 \pm 10.2	50.1 \pm 11	49.2 \pm 10.6	0.641
Height, cm	167.0 \pm 7.6	167.6 \pm 8.3	166.1 \pm 7.6	0.811
Weight, kg	72.5 \pm 15.0	73.4 \pm 14.1	70.7 \pm 13.6	0.720
BMI, kg/m ²	26.5 \pm 4.4	26.0 \pm 3.8	25.6 \pm 4.7	0.752
Waist circumference, cm	92.6 \pm 10.3	92.4 \pm 10.6	91.7 \pm 10.9	0.944
Hip circumference, cm	98.1 \pm 8.6	98.0 \pm 9.0	98.3 \pm 9.4	0.994
WHR	0.94 \pm 0.04	0.94 \pm 0.06	0.93 \pm 0.07	0.773
SBP, mmHg	132 \pm 15*	131 \pm 14	123 \pm 15	0.076
DBP, mmHg	82 \pm 10*	81 \pm 5	76 \pm 9	0.064
<i>Laboratory assays</i>				
TG, mmol/L	2.70 \pm 1.24* [†]	2.10 \pm 0.87	1.94 \pm 1.06	0.024
TC, mmol/L	5.36 \pm 2.09* [†]	4.44 \pm 0.95	3.94 \pm 0.88	0.003
LDL-C, mmol/L	2.98 \pm 0.75*	2.70 \pm 0.75	2.37 \pm 0.60	0.008
HDL-C, mmol/L	1.10 \pm 0.28*	1.40 \pm 0.63	1.60 \pm 0.59	0.001
FPG, mmol/L	9.64 \pm 2.54* [†]	5.48 \pm 0.62	4.99 \pm 0.50	0.000
HbA1c, %	8.9 \pm 2.3* [†]	5.9 \pm 0.5	5.2 \pm 0.3	0.000
FINS, pmol/L	77.75 \pm 21.08	87.06 \pm 24.80	81.29 \pm 24.47	0.340
FCP, nmol/L	0.39 \pm 0.17	0.43 \pm 0.15	0.41 \pm 0.16	0.473
<i>Medical history, n (%)</i>				
Cigarette smoking	12 (34.3)	11 (50.0)	7 (30.4)	0.348
Hypertension	10 (28.6)	5 (22.7)	2 (8.7)	0.191
<i>Insulin sensitivity and islet cell function</i>				
HOMA-IR	4.68 \pm 1.55* [†]	2.94 \pm 0.89	2.49 \pm 0.74	0.000
HOMA- β [‡]	33.52 (22.31, 60.35)* [†]	115.98 (90.76, 152.30)*	146.98 (90.61, 229.42)	0.000
Gutt	34.03 \pm 7.60* [†]	57.15 \pm 10.00*	77.11 \pm 11.18	0.000
Matsuda	2.82 \pm 0.71* [†]	3.67 \pm 0.92*	4.35 \pm 0.83	0.000

Data are expressed as mean \pm standard deviation. T2DM - type 2 diabetes mellitus, IGT - impaired glucose tolerance, NGT - normal glucose tolerance, BMI - body mass index, WHR - waist-to-hip ratio, SBP - systolic blood pressure, DBP - diastolic blood pressure, TG - triglycerides, TC - total cholesterol, LDL-C - low-density lipoprotein cholesterol, HDL-C - high-density lipoprotein cholesterol, FPG - fasting plasma glucose, HbA1c - hemoglobin A1c, FINS - fasting plasma insulin, FCP - fasting plasma C-peptide. *significant difference ($p < 0.05$) versus NGT, [†]significant difference ($p < 0.05$) versus IGT. [‡]HOMA- β value was not normally distributed even after logarithmic transformation, so they were expressed as mean and interquartile range

Table 2 - Analysis of the correlation of GLP-1, Δ GLP-1, AUC_{glp-1} with HOMA-IR, HOMA- β , Gutt index, Matsuda index, AUC_g , and AUC_{ins} .

Variables	HOMA-IR		HOMA- β		Gutt		Matsuda		AUC_g		AUC_{ins}	
	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value
FGLP-1	-0.326	0.003	0.242	0.031	0.340	0.002	0.297	0.007	-0.272	0.015	0.231	0.039
GLP-1 _{30min}	-0.283	0.011	0.225	0.045	0.404	0.000	0.324	0.003	-0.336	0.002	0.307	0.006
GLP-1 _{60min}	-0.322	0.004	0.124	0.274	0.345	0.001	0.322	0.004	-0.294	0.008	0.209	0.063
GLP-1 _{120min}	-0.368	0.001	0.345	0.002	0.473	0.000	0.461	0.000	-0.440	0.000	0.300	0.007
GLP-1 _{180min}	-0.181	0.107	0.347	0.002	0.322	0.004	0.179	0.113	-0.359	0.001	0.283	0.011
Δ GLP-1 _{30min}	-0.226	0.003	0.160	0.156	0.350	0.001	0.275	0.014	-0.294	0.008	0.272	0.015
Δ GLP-1 _{60min}	-0.268	0.016	0.056	0.621	0.299	0.007	0.274	0.014	-0.250	0.025	0.169	0.133
Δ GLP-1 _{120min}	-0.249	0.026	0.200	0.076	0.349	0.002	0.353	0.001	-0.341	0.002	0.217	0.054
Δ GLP-1 _{180min}	-0.048	0.674	0.193	0.086	0.184	0.102	0.057	0.614	-0.250	0.026	0.190	0.091
AUC_{glp-1}	-0.267	0.017	0.443	0.000	0.396	0.000	0.251	0.025	-0.412	0.000	0.447	0.000

r - Spearman correlation coefficient. GLP-1 - glucagon-like peptide-1, FGLP-1 - fasting GLP-1; Δ GLP-1 - incremental GLP-1, AUC_g - area under the curve of glucose, AUC_{ins} - area under the curve of insulin, AUC_{glp-1} - area under the curve of GLP-1

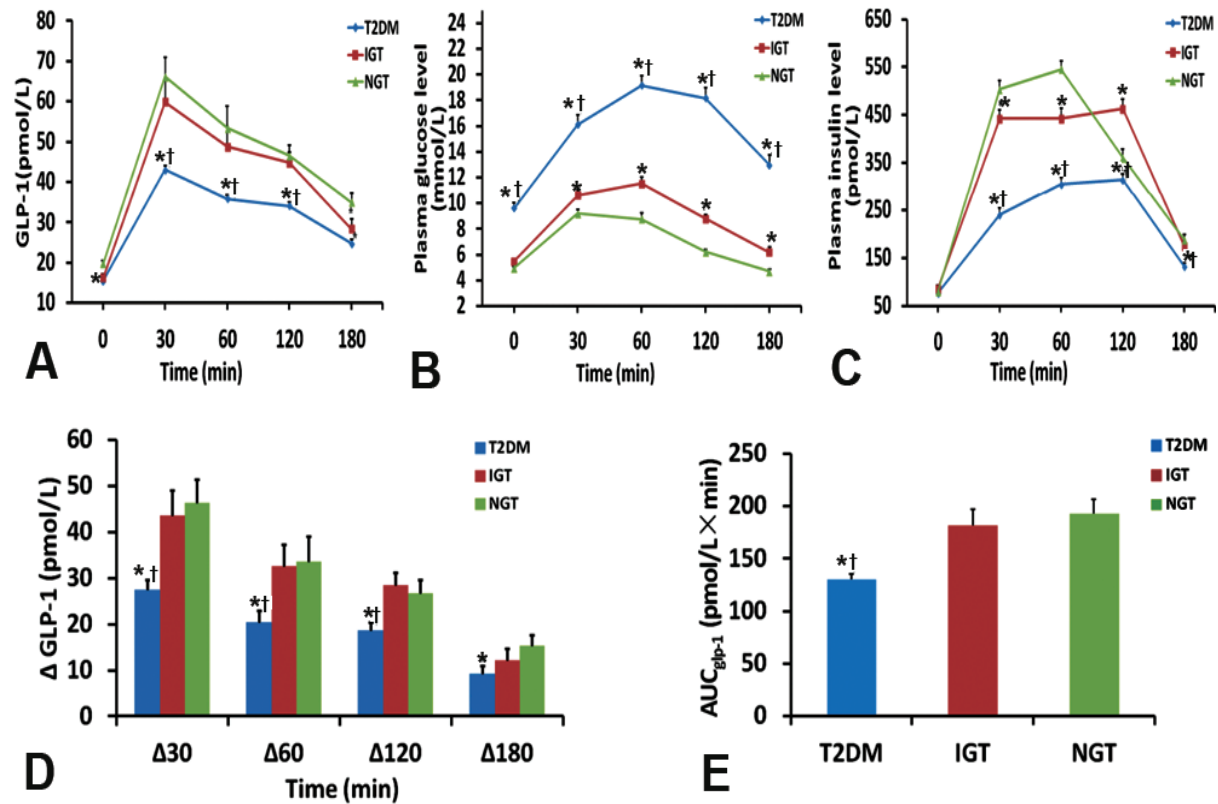


Figure 1 - Comparisons of glucagon-like peptide-1 (GLP-1), glucose and insulin levels, incremental (Δ) GLP-1 levels, and AUC_{glp-1} during OGTT among the 3 groups: A) GLP-1 levels, B) glucose levels, C) insulin levels, D) incremental GLP-1 levels from 30 to 180 minutes, and E) AUC_{glp-1} . *significant difference ($p < 0.05$) versus normal glucose tolerance (NGT), †significant difference ($p < 0.05$) versus impaired glucose tolerance (IGT). T2DM - type 2 diabetes mellitus

Correlation of total GLP-1, Δ GLP-1, and AUC_{glp-1} with HOMA-IR, HOMA- β , Gutt index, Matsuda index, AUC_g , and AUC_{ins} . The total GLP-1 levels at each OGTT time point, Δ GLP-1 levels, and AUC_{glp-1} correlated with HOMA-IR, HOMA- β , Gutt index, Matsuda index, AUC_g , and AUC_{ins} in all subjects are shown in Table 2. The FGLP-1, $GLP-1_{30min}$, $GLP-1_{60min}$, $GLP-1_{120min}$, Δ GLP-1_{30min}, Δ GLP-1_{60min}, Δ GLP-1_{120min}, and AUC_{glp-1} were inversely proportional with HOMA-IR ($p < 0.05$). The FGLP-1, $GLP-1_{30min}$, $GLP-1_{120min}$, $GLP-1_{180min}$, and AUC_{glp-1} positively correlated with HOMA- β ($p < 0.05$). All variables, but Δ GLP-1_{180min}, positively correlated with Gutt index ($p < 0.05$). The FGLP-1, $GLP-1_{30min}$, $GLP-1_{60min}$, $GLP-1_{120min}$, Δ GLP-1_{30min}, Δ GLP-1_{60min}, Δ GLP-1_{120min} and AUC_{glp-1} positively correlated with Matsuda index ($p < 0.05$). Every variable negatively correlated with AUC_g ($p < 0.05$). The FGLP-1, $GLP-1_{30min}$, $GLP-1_{120min}$, $GLP-1_{180min}$, Δ GLP-1_{30min}, and AUC_{glp-1} were positively proportional with AUC_{ins} ($p < 0.05$). These results indicated that increased IR, decreased insulin sensitivity, and pancreatic β -cell}}}

function may contribute to defective GLP-1 secretion, and vice versa.

Multivariate linear regression analysis. To determine whether total GLP-1 levels at each OGTT time point were associated with various clinical factors, multivariate linear regression models were used. The total GLP-1 levels (at each OGTT time point) were selected as the dependent variables, and age, BMI, WHR, SBP, DBP, TG, TC, LDL-C, HDL-C, FPG, FINS, FCP, and HbA1c were set as the independent variables. The FGLP-1 was found to be negatively correlated with FPG ($t = -2.866$, $p = 0.005$), and DBP ($t = -2.436$, $p = 0.017$). The $GLP-1_{30min}$ and $GLP-1_{120min}$ negatively correlated with FPG ($t = -2.810$, $p = 0.006$ and $t = -3.849$, $p = 0.000$). The $GLP-1_{60min}$ and $GLP-1_{180min}$ negatively correlated with HbA1c ($t = -2.585$, $p = 0.012$ and $t = -3.191$, $p = 0.002$).

Discussion. We assessed GLP-1 responses to 75 g OGTT among subjects with different glucose tolerances to determine whether GLP-1 secretion was

related to IR and pancreatic islet β -cell function. We found that the GLP-1 levels at each OGTT time point, Δ GLP-1 levels, and AUC_{glp-1} were lower in the T2DM group than in the IGT and NGT groups, but that these levels did not differ between the IGT and NGT groups. Compensatory GLP-1 secretion may occur in the prediabetic state, and decompensation may result in overt diabetes. The GLP-1 secretion was correlated with hyperglycemia, IR, and islet β -cell dysfunction. Glucotoxicity and IR may impair GLP-1 secretion, and low GLP-1 levels may aggravate β -cell dysfunction, IR, and hyperglycemia. This study provided evidence that Chinese patients with newly diagnosed T2DM had decreased GLP-1 secretion.

It was reported that the GLP-1 levels after a glucose load are lower in patients with impaired fasting glucose (IFG) + IGT and DM than in subjects with NGT.¹⁷ However, the authors of that study¹⁷ tested GLP-1 levels at only 2 time points (in the fasting state and 2 hours after glucose load). Their results were in partial agreement with our present study. In the present study, we performed a standard 75 g OGTT and measured GLP-1 levels at 5 successive time points. We found a reduction of GLP-1 secretion in T2DM patients. However, GLP-1 secretion did not differ between subjects with IGT and NGT. This indicates that impaired GLP-1 secretion may be a phenomenon typically observed in T2DM. However, recent studies have reported either no abnormalities at all, or increased GLP-1 secretion in T2DM patients.^{11,18-20} The possible reasons for this are as follows: first, hypoglycemic agents, especially metformin, have been shown to increase GLP-1 secretion.²¹ Bile acid sequestrants, such as colestevlam, have been reported to enhance GLP-1 secretion.²² Researchers should consider these types of confounding factors when investing GLP-1 secretion.

In this study, we selected patients with newly diagnosed, untreated T2DM; thus, their GLP-1 levels represented the natural secretion state in these patients. Second, some subject-specific factors have been found to be significantly related to GLP-1 secretion, such as, age, body weight, and BMI.^{20,23} Therefore, the T2DM group should be matched to healthy controls, as well, as possible. In this study, the general characteristics (age, gender, body weight, BMI) were matched very well. Third, genetic background may influence GLP-1 secretion. There are several genes that were reported to affect the physiology of GLP-1.^{24,25} Recently, Sleddering *et al*²⁶ determined that the GLP-1 levels after a glucose load were higher in young healthy South Asians living in the UK than in their Caucasian counterparts. Our

study provided evidence that Chinese patients with newly diagnosed T2DM had impaired GLP-1 secretion as compared with that in matched subjects with IGT and NGT. Whether the GLP-1 secretion pattern in the Han Chinese population differs from that in other ethnicities is unknown. Further studies are required to assess differences in GLP-1 secretion among various races. We observed that in each group, peak GLP-1 secretion occurred at approximately 30 minutes during OGTT, which is consistent with previous findings.²⁷ Peak GLP-1 level was almost 35% lower in the T2DM group than in the NGT group. Furthermore, AUC_{glp-1} was 32.8% lower in the T2DM group than in the NGT group, which was more than that reported by Toft-Nielsen *et al*.¹⁰ However, there was no difference in peak GLP-1 and AUC_{glp-1} between the IGT and NGT groups. We hypothesized that compensatory GLP-1 secretion may occur in the prediabetic state. With disease progression, the function of the L cells in the intestines may decline, leading to a decrease in GLP-1 level. The exact mechanism underlying it remains to be investigated in future studies.

The present results also suggested that the GLP-1 levels and incremental GLP-1 levels during OGTT, as well as AUC_{glp-1} were correlated to plasma glucose level, pancreatic islet β -cell function and IR. This indicates that hyperglycemia, IR, and decreased pancreatic β -cell function may contribute to defective GLP-1 secretion, and vice versa. Some authors have suggested that reduced incretin effect was not a primary event in the development of T2DM, but rather a consequence of the diabetic state.¹⁷ Glucotoxicity and IR may impair GLP-1 secretion, and low GLP-1 levels may aggravate β -cell dysfunction, IR, and hyperglycemia. In addition, GLP-1 levels negatively correlated with DBP. It is well known that hypertension is an important component of metabolic disorder and correlated with hyperglycemia and IR. Thus, glycometabolic disorder, IR, β -cell dysfunction, and defective GLP-1 secretion form a vicious cycle in T2DM.

A limitation of this study is its small sample size, due to which the correlation of GLP-1 levels with age and BMI may not have been as apparent as in previous reports.^{11,20} Another limitation is that glucagon and non-esterified fatty acid levels were not measured; both of these have been reported to be confounding factors for GLP-1 secretion.²⁰

In conclusion, GLP-1 secretion in response to 75 g OGTT was impaired in patients with newly diagnosed, untreated T2DM, as compared with that in matched subjects with IGT and NGT. The GLP-1 secretion

was inversely proportional to IR and hyperglycemia, and positively correlated with pancreatic islet β -cell function and insulin sensitivity. This study provided the GLP-1 secretion mode of newly diagnosed T2DM. These findings are meaningful for the prevention and treatment of T2DM.

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