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Article

The Relationship Between Glucagon Levels and Visceral Adiposity in Type 2 Diabetes: A Retrospective Cross-Sectional Study From China

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Abstract

Aims/Background: Visceral fat accumulation contributes to type 2 diabetes mellitus (T2DM) by impairing β-cell function and disrupting insulin resistance. Glucagon (GCG) dysregulation is a hallmark of pancreatic α-cell failure. This study evaluated the association between glucagon levels and visceral fat area (VFA) in T2DM. **Methods**: This retrospective cross-sectional study enrolled 211 patients with T2DM who were treated at Affiliated Hospital of Nantong University between August and October 2022. All the participants underwent a 3-hour oral glucose tolerance test after discontinuing all hypoglycemic treatments. Blood samples were collected at different time points, and plasma glucagon levels were assessed using a chemiluminescence approach, while VFA was evaluated using bioelectrical impedance analysis. Visceral fat obesity was set at VFA $\geq$111 cm^2 in men and $\geq$96 cm^2 in women. Finally, regression analyses were applied to explore the relationship between glucagon and VFA. **Results**: A paradoxical increase in glucagon was observed, with 54.98% of patients reaching their peak at 30 min post-glucose load. VFA showed a positive correlation with GCG (0 min, peak, the area under the curve [AUC]$_{0–180\ min}$), insulin (0 min, 120 min, AUC$_{0–180\ min}$), c-peptide (CP) (0 min, 120 min, AUC$_{0–180\ min}$), insulin resistance (IR), body mass index, ultrasound attenuation parameter, alanine aminotransferase, aspartate aminotransferase, total cholesterol, triglycerides, and low-density lipoprotein, whereas a negative association was determined with diabetes duration (all $p < 0.05$). Logistic regression revealed that GCG$_{0\ min}$ was independently associated with an increased risk of visceral obesity in T2DM patients. Notably, the association persisted after additional adjustment for homeostatic model assessment of insulin resistance (HOMA-IR) (Model 2: odds ratio = 1.060, $p = 0.028$) and adjustment for CP$_{0\ min}$ (Model 3: OR = 1.075, $p = 0.014$). **Conclusion**: Glucagon dysregulation is strongly linked to visceral adiposity in T2DM, suggesting α-cell dysfunction as a potential therapeutic target.

Keywords: type 2 diabetes mellitus; glucagon; visceral fat area; α-cell dysfunction; insulin resistance; hyperglucagonaemia

1. Introduction

Obesity, particularly the accumulation of visceral fat, is a major contributor to the progression of type 2 diabetes mellitus (T2DM) [1]. Body mass index (BMI) is commonly used to categorize obesity, but it does not differentiate fat mass from lean mass or reflect how fat is distributed across the whole body [2]. These limitations are clinically significant because the metabolic outcomes of obesity depend heavily on the fat distribution pattern. Compared with BMI, visceral fat exhibits a strong association with insulin resistance and T2DM risk [3]. Excess fat indicates ectopic lipid deposition and promotes metabolic dysfunction through dysregulated systemic endocrine signaling and increased release of pro-inflammatory factors, thereby exacerbating insulin resistance and elevating the risk of T2DM. Moreover, elevated visceral fat accumulation is associated with several obesity-related conditions, such as metabolic syndrome, atherosclerosis, non-alcoholic steatohepatitis, and cardiovascular disease [1].

Hyperglucagonaemia has been observed across multiple ethnicities in metabolic conditions such as obesity, insulin resistance, and diabetes [4]. T2DM is increasingly recognized as a "bihormonal" disorder characterized by insulin deficiency and glucagon (GCG) excess [4]. Visceral fat accumulation has been shown to worsen metabolic dysfunction by impairing both basal and postload insulin secretion and sensitivity [5]. A few studies have explored glucagon secretion among the obese cohort [6,7]. For example, in a Danish cohort of young individuals, central obesity, evaluated by waist-to-hip ratio, was linked to higher fasting glucagon and inadequate postprandial suppression, especially among participants who had insulin resistance [6]. Similarly, a study involving Hispanic American adults with BMI-defined obesity reported impaired insulin-induced suppression of glucagon secretion [7]. However, these studies investigated general obesity measures and did not assess fat distribution, making it challenging to distinguish the metabolic effects of overall obesity from those of visceral obesity. Consequently, the true relationship between visceral fat and glucagon secretion remains unclear, particularly in Asian populations, where relevant studies are still limited.

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Therefore, this study aims to investigate dynamic patterns of GCG secretion and their association with visceral fat area (VFA) in Chinese patients with T2DM. Specific objectives of this study were as follows: (1) to compare dynamic GCG secretion patterns between patients with and without visceral obesity, (2) to evaluate the association between circulating GCG levels and visceral adipose tissue (VAT) accumulation, and (3) to discuss the potential of α-cell dysfunction as a therapeutic target.

2. Methods

2.1 Study Participants

This retrospective study enrolled 211 patients with T2DM who were treated at Affiliated Hospital of Nantong University between August and October 2022.

The inclusion criteria were as follows: (1) diagnosis of diabetes as per American Diabetes Association criteria (2025) [8]; (2) age 18 years or above; (3) poor blood glucose control with glycosylated hemoglobin (HbA1c) $\geq$7%; (4) completion of a 3-hour 75 g oral glucose tolerance test (OGTT) and bioelectrical impedance analysis (BIA) during the same hospitalization; and (5) provision of written informed consent.

The exclusion criteria included: (1) type 1 diabetes mellitus (T1DM); (2) secondary diabetes; (3) acute diabetic complications; (4) use of medications within the last 3 months that significantly affect glucagon or glucose metabolism, such as glucagon-like peptide-1 (GLP-1) analogues or receptor agonists, dipeptidyl peptidase-4 inhibitors (DPP4i), glucagon receptor antagonists (GRAs) or agonists, sodium–glucose cotransporter-2 inhibitors (SGLT2i), exogenous insulin, insulin secretagogues (sulfonylureas or meglitinides), somatostatin analogues, pramlintide, or systemic glucocorticoids; (5) a history of surgery or significant trauma within the past three months; (6) severe cardiac, pulmonary, hepatic, or renal impairment; (7) active inflammatory disease, infection, or malignancy; (8) a history of other endocrine disorders affecting metabolism (e.g., uncontrolled hyperthyroidism or Cushing's syndrome); (9) a history of heavy alcohol consumption (>40 g/day for men or >20 g/day for women); and (10) pregnancy or lactation.

2.2 Biochemical and Anthropometric Measures

Participants' baseline clinical and anthropometric characteristics were documented, including sex, age, height, weight, systolic blood pressure (SBP) and diastolic blood pressure (DBP), and disease duration. Blood pressure was measured twice, and the average of the two readings was used for further analysis. BMI was calculated as weight (kg) divided by height squared (m^2).

The next morning after admission, fasting blood samples were obtained. Serum creatinine (Scr), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total cholesterol (TC) were measured using an automated biochemical analyzer (Hitachi 7600-020; Hitachi, Tokyo, Japan). Serum cystatin C (Scys C) was detected by a modular analyzer (Cobas 8000, Roche Diagnostics GmbH, Mannheim, Germany). High-performance liquid chromatography was employed to measure HbA1c (Bio-Rad Laboratories, Hercules, CA, USA). Urinary albumin and creatinine levels were assessed by immunonephelometry using a BN II analyzer (BN II System, Siemens Diagnostics, Erlangen, Germany), and the urine albumin-to-creatinine ratio (UACR) was then calculated.

Estimated glomerular filtration rate (eGFR) was determined according to the 2012 Chronic Kidney Disease Epidemiology Collaboration creatinine-cystatin C equation, as shown below: $eGFR = 135 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-0.601} \times \min(Scys\ C/0.8, 1)^{-0.375} \times \max(Scys\ C/0.8, 1)^{-0.711} \times 0.995^{Age}$ [$\times$0.969 for female] [$\times$1.08 for black]. The parameters α and κ were set as –0.207 and 0.9 for males, and –0.248 and 0.7 for females, respectively. The terms min and max denote the lowest or highest value of the expression (Scr/κ or 1) [9].

2.3 The Conduction of OGTT and Measurement of Plasma Glucagon Concentration

All participants underwent a 3-hour 75 g OGTT after discontinuing all hypoglycemic treatments for at least three days before this assessment. Venous blood samples were collected at 0, 30, 60, 120, and 180 min to measure plasma glucagon, insulin (INS), c-peptide (CP), and glucose (GLU) levels.

For plasma glucagon levels detection, blood samples were collected in specialized tubes containing ethylenediaminetetraacetic acid-K2 (EDTA-K2) anticoagulant and a protease inhibitor. Immediately after collection, samples were gently inverted and mixed more than 10 times to ensure adequate anticoagulant and inhibitor dispersion. The samples were then kept at room temperature for 1 hour and centrifuged for 15 min at 3000 rpm within 4 hours after collection. The supernatant was obtained and analyzed for glucagon using an automated chemiluminescence analyzer (HomoG 100, Jinde Biotech, Guangzhou, China). The assay had a variation coefficient of <10% and an analytical sensitivity of <2.0 pmol/L. For each individual, peak GCG (GCG_{peak}) was defined as the highest observed GCG concentration across all OGTT time points.

CP and INS levels were determined using a Roche Cobas e 411 analyzer (d-68305, Mannheim, Germany). Plasma glucose was assessed using standard laboratory procedures (ADVIA® 2400, Siemens, Tarrytown, NY, USA). Insulin resistance was determined using the homeostatic model assessment of insulin resistance (HOMA-IR), calculated as: fasting INS (μU/mL) × fasting plasma glucose (mmol/L) / 22.5. The area under the curve (AUC)

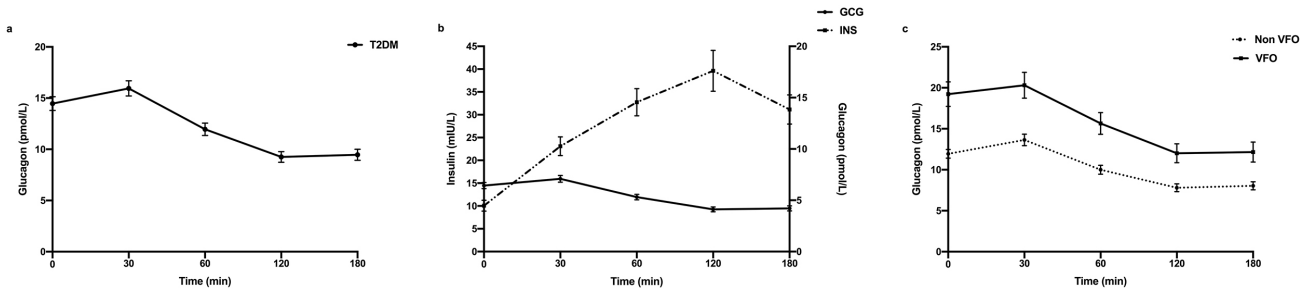

Fig. 1. The pattern of glucagon and insulin secretion in patients with T2DM. (a) GCG secretion trend in T2DM group. (b) GCG and INS secretion trend in T2DM group. (c) GCG secretion trend in two subgroups. T2DM, type 2 diabetes mellitus; GCG, glucagon; INS, insulin; VFO, visceral fat obesity. Error bars represent the standard error of the mean (SEM).

for OGTT-derived measures was calculated using the trapezoidal rule.

2.4 Vibration-Controlled Transient Elastography (VCTE)

VCTE, a non-invasive assessment tool, was employed to evaluate hepatic steatosis and fibrogenic remodeling of the liver tissue (FibroScan 402; Echosens, Paris, France). Liver stiffness measurement (LSM) served as an indicator of fibrogenic remodeling, and the ultrasound attenuation parameter (UAP) was used to determine the degree of hepatic steatosis.

2.5 Bioelectrical Impedance Analysis (BIA)

Dual-BIA (InBody 770; InBody Co., Ltd., Seoul, Republic of Korea), a simple and reliable technique for evaluating visceral fat accumulation, was used to quantify VFA. Unlike traditional BIA, which relies on a single current pathway, dual-BIA utilizes two current paths to comprehensively determine the electrical conductivity of the abdominal region and its surface. This method shows a strong correlation with computed tomography (CT), which is recognized as the benchmark for assessing visceral fat accumulation. Visceral fat obesity (VFO) was defined as VFA $\geq$111 cm^2 in men and $\geq$96 cm^2 in women [10].

2.6 Statistical Analysis

Statistical analysis was conducted using SPSS version 26.0 (IBM, Chicago, IL, USA). The Kolmogorov–Smirnov test was used to detect the normality of continuous variables. Continuous variables with a normal distribution were reported as mean $\pm$ standard deviation (SD), and non-normally distributed variables were presented as median with the first and third quartiles (Q1, Q3). For continuous variables that conform to a normal distribution, the independent samples *t*-test is used for comparisons between groups; for continuous variables that do not conform to a normal distribution, the Mann–Whitney U test is used for comparisons between groups. Categorical and qualitative variables were reported as counts (n) and were analyzed using the chi-square test.

Spearman correlation analysis was performed to identify factors potentially associated with VFA. Before inclusion in the regression model, all VFA-related variables were assessed for multicollinearity. Significant multicollinearity was defined as a variance inflation factor (VIF) $>$5 or a tolerance $<$0.2. To resolve multicollinearity while preserving biological relevance, we employed a two-step strategy: (1) within the homologous variable clusters (INS, CP, and GCG series), only the single most biologically representative variable was retained; and (2) among remaining non-homologous variables, those with the highest VIF were excluded iteratively until all VIF values were $\leq$5. Binary logistic regression analysis was used to identify the independent risk factors for VFO in patients with T2DM. A p-value $<$ 0.05 was considered statistically significant.

3. Results

3.1 Glucagon Kinetics in T2DM Stratified by Visceral Fat Level

First, we plotted the glucagon secretion curve of patients with T2DM during the 75 g OGTT. After glucose loading, a paradoxical increase in glucagon was found. Overall, 54.98% of the T2DM patients reached their glucagon peak at 30 min, followed by a gradual decrease that coincided with the delayed peak in insulin secretion (Fig. 1). The remaining individuals showed peak glucagon levels at other time points: 33.65% at 0 min, 6.16% at 60 min, 1.90% at 120 min, and 3.32% at 180 min. Then, the patients were categorized based on VFA using sex-specific cut-offs (111 cm^2 for men and 96 cm^2 for women) into non-VFO and VFO groups. Although the overall pattern of GCG secretion was roughly similar between groups, plasma glucagon levels were consistently higher in the VFO group at every measured time point than in the non-VFO group (Fig. 1).

3.2 Clinical and Metabolic Characteristics of VFO Status

Based on the observed glucagon secretion pattern, we selected fasting glucagon, peak glucagon, and total glucagon secretion as the primary glucagon-related variables for further analysis. Compared with the non-VFO

Table 1. Comparisons of anthropometry and metabolic indicators between the VFO and non-VFO groups.

Variable	Non-VFO group (n = 138)	VFO group (n = 73)	$t/Z/\chi^2$	p-value
Sex (male/female)	102/36	35/38	14.139	<0.001
Age (years)	56.98 ± 14.58	53.92 ± 16.27	1.393	0.165
Diabetes duration (years)	7.00 (2.00, 12.00)	4.00 (0.50, 10.00)	–2.048	0.041
SBP (mmHg)	135.00 (124.00, 150.00)	139.00 (128.50, 149.50)	–0.997	0.319
DBP (mmHg)	82.51 ± 11.26	80.38 ± 12.34	1.265	0.207
BMI (kg/m^2)	23.37 ± 3.11	27.92 ± 3.89	–8.633	<0.001
UAP (dB/m)	254.69 ± 40.53	282.62 ± 43.11	–4.656	<0.001
LSM (kPa)	5.42 (3.45, 6.53)	5.10 (2.00, 7.38)	–0.072	0.942
ALT (U/L)	19.50 (13.00, 31.00)	26.00 (15.00, 44.00)	–2.191	0.028
AST (U/L)	21.00 (16.00, 28.00)	23.00 (18.50, 31.50)	–2.059	0.040
UACR (mg/g)	2.08 (1.03, 6.89)	2.63 (1.08, 11.39)	–1.391	0.164
Scr (mg/dL)	0.72 (0.61, 0.90)	0.70 (0.55, 0.87)	–1.190	0.234
Scys C (mg/L)	0.71 (0.61, 0.88)	0.70 (0.60, 0.875)	–0.262	0.793
eGFR (mL/min/1.73 m^2)	108.94 (90.18, 124.50)	113.86 (87.29, 128.26)	–0.697	0.486
TC (mmol/L)	4.60 (3.80, 5.40)	4.90 (4.40, 5.85)	–2.375	0.018
TG (mmol/L)	1.33 (0.98, 2.01)	1.79 (1.17, 2.67)	–2.988	0.003
HDL (mmol/L)	1.10 (0.95, 1.24)	1.04 (0.92, 1.23)	–0.715	0.475
LDL (mmol/L)	3.11 ± 0.93	3.33 ± 0.94	–1.649	0.101
$GLU_{0\ min}$ (mmol/L)	8.91 ± 2.37	9.30 ± 2.49	–1.077	0.283
$GLU_{120\ min}$ (mmol/L)	20.25 (17.08, 22.60)	21.00 (17.65, 23.35)	–0.896	0.370
$AUC_{GLU\ 0–180\ min}$	3107.25 (2679.38, 3544.13)	3255.00 (2676.00, 3612.00)	–0.709	0.478
$INS_{0\ min}$ (mIU/L)	4.84 (2.21, 8.95)	6.92 (4.62, 15.18)	–3.525	<0.001
$INS_{120\ min}$ (mIU/L)	18.45 (9.96, 35.12)	26.96 (12.26, 46.19)	–2.406	0.016
$AUC_{INS\ 0–180\ min}$	2806.2 (1566.11, 5368.95)	3801.00 (2125.95, 7156.17)	–2.698	0.007
$CP_{0\ min}$ (μg/L)	1.65 (0.89, 2.46)	2.01 (1.51, 3.29)	–3.189	0.001
$CP_{120\ min}$ (μg/L)	4.50 (2.93, 6.78)	5.08 (3.56, 7.42)	–1.858	0.063
$AUC_{CP\ 0–180\ min}$	686.40 (437.81, 1015.43)	790.95 (552.60, 1130.7)	–2.212	0.027
HbA1c (%)	9.10 (7.50, 11.20)	9.10 (7.75, 10.90)	–0.525	0.600
HOMA-IR	1.98 (0.80, 3.74)	3.06 (1.65, 6.30)	–3.635	<0.001
$GCG_{0\ min}$ (pmol/L)	10.94 (7.12, 15.39)	16.32 (11.55, 22.05)	–5.012	<0.001
GCG_{peak} (pmol/L)	13.15 (9.95, 19.02)	17.88 (13.61, 27.31)	–4.288	<0.001
$AUC_{GCG\ 0–180\ min}$	1494.30 (1087.99, 2302.39)	2321.55 (1339.13, 3060.68)	–4.167	<0.001

Values are presented as mean ± standard deviation or median (Q1, Q3). χ^2 test for sex comparison; The t-test was used for normally distributed variables; the Mann–Whitney U test for non-normally distributed variables. A p-value < 0.05 was considered significant. VFO, visceral fat obesity; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; UAP, ultrasound attenuation parameter; LSM, liver stiffness measurement; ALT, alanine aminotransferase; AST, aspartate aminotransferase; UACR, urine albumin-to-creatinine ratio; Scr, serum creatinine; Scys C, serum cystatin C; eGFR, estimated glomerular filtration rate; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GLU, glucose; AUC, area under the curve; INS, insulin; CP, c-peptide; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; GCG, glucagon; Q1, the first quartile; Q3, the third quartile.

group, the VFO group comprised a higher proportion of women ($p < 0.001$) and had higher BMI, UAP, ALT, AST, TC, TG, and HOMA-IR (all $p < 0.05$), along with a shorter duration of diabetes ($p = 0.041$, Table 1). Furthermore, patients with VFO had greater insulin (basal, two-hour postprandial, and total insulin secretion), higher c-peptide levels (fasting and total c-peptide secretion), and increased glucagon secretion (basal, peak, and total glucagon secretion) than those without VFO (all $p < 0.05$). However, no significant between-group differences were observed for age, SBP, DBP, LSM, UACR, Scr, Scys C, eGFR, HDL, LDL, $GLU_{0\ min}$, $GLU_{120\ min}$, $AUC_{GLU\ 0–180\ min}$, $CP_{120\ min}$ or HbA1c.

3.3 Spearman Correlation Analysis of VFA in T2DM

Spearman correlation analysis (Table 2) revealed that VFA had a positive connection with BMI, UAP, ALT, AST, TC, TG, LDL, $INS_{0\ min}$, $INS_{120\ min}$, $AUC_{INS\ 0–180\ min}$, $CP_{0\ min}$, $CP_{120\ min}$, $AUC_{CP\ 0–180\ min}$, HOMA-IR, $GCG_{0\ min}$, GCG_{peak}, and $AUC_{GCG\ 0–180\ min}$ (all $p < 0.05$). In contrast, VFA was negatively correlated with diabetes duration ($p = 0.006$).

Table 2. Correlation analysis of VFA in patients with T2DM.

Variable	VFA[a]	
	r	*p*-value
Female sex	0.105	0.128
Age (years)	–0.128	0.063
Diabetes duration (years)	–0.187	0.006
SBP (mmHg)	0.110	0.111
DBP (mmHg)	0.068	0.325
BMI (kg/m^2)	0.769	<0.001
UAP	0.467	<0.001
LSM	0.033	0.638
ALT (U/L)	0.246	<0.001
AST (U/L)	0.211	0.002
UACR (mg/g)	0.086	0.211
Scr (mg/dL)	–0.037	0.589
Scys C (mg/L)	–0.023	0.737
eGFR (mL/min/1.73 m^2)	0.084	0.223
TC (mmol/L)	0.167	0.015
TG (mmol/L)	0.307	<0.001
HDL (mmol/L)	–0.132	0.056
LDL (mmol/L)	0.162	0.018
$GLU_{0\ min}$ (mmol/L)	0.102	0.141
$GLU_{120\ min}$ (mmol/L)	0.029	0.678
$AUC_{GLU\ 0–180\ min}$	0.034	0.627
$INS_{0\ min}$ (mIU/L)	0.372	<0.001
$INS_{120\ min}$ (mIU/L)	0.235	0.001
$AUC_{INS\ 0–180\ min}$	0.266	<0.001
$CP_{0\ min}$ (µg/L)	0.327	<0.001
$CP_{120\ min}$ (µg/L)	0.190	0.006
$AUC_{CP\ 0–180\ min}$	0.228	0.001
HbA1c (%)	0.009	0.892
HOMA-IR	0.386	<0.001
$GCG_{0\ min}$ (pmol/L)	0.373	<0.001
GCG_{peak} (pmol/L)	0.330	<0.001
$AUC_{GCG\ 0–180\ min}$	0.331	<0.001

[a]Spearman correlation analysis for non-normally distributed variables. A p-value < 0.05 was considered significant. VFA, visceral fat area; T2DM, type 2 diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; UAP, ultrasound attenuation parameter; LSM, liver stiffness measurement; ALT, alanine aminotransferase; AST, aspartate aminotransferase; UACR, urine albumin-to-creatinine ratio; Scr, serum creatinine; Scys C, serum cystatin C; eGFR, estimated glomerular filtration rate; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GLU, glucose; AUC, area under the curve; INS, insulin; CP, c-peptide; HOMA-IR, homeostatic model assessment of insulin resistance; GCG, glucagon.

3.4 Multicollinearity Management and Identification of Independent Predictors of VFA

As shown in **Supplementary Table 1**, we conducted a collinearity assessment for factors associated with VFA. For clusters of variables derived from the same physiological measure (INS, CP, and GCG series), we first retained only the single most biologically representative variable from each cluster ($GCG_{0\ min}$ for basal α-cell function as our primary exposure, $CP_{0\ min}$ for basal β-cell function, HOMA-IR for insulin resistance).

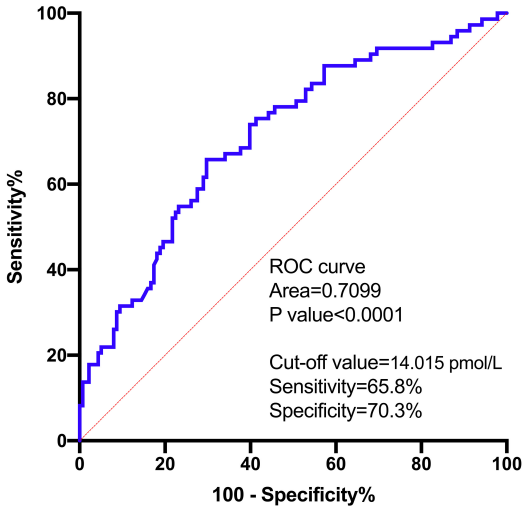

Fig. 2. Diagnostic performance of $GCG_{0\ min}$ to identify VFO in T2DM patients. GCG, glucagon; VFO, visceral fat obesity; T2DM, type 2 diabetes mellitus; ROC, receiver operating characteristic.

For the remaining non-homologous variables (ALT, AST, TC, LDL), we applied an iterative VIF approach, removing the variable with the highest VIF at each step and recalculating until all variables achieved a VIF $\leq$5. In practice, the process led to the sequential exclusion of ALT and TC. Collinearity diagnostics for the final set of retained variables were presented in Table 3. The remaining variables were then entered into a multiple linear regression model to assess the relationship between VFA and plasma glucagon levels. In the adjusted models 2 and 3, VFA remained positively associated with $GCG_{0\ min}$, BMI, and female sex, after controlling for diabetes duration, UAP, AST, TG, LDL, $CP_{0\ min}$, and HOMA-IR (Table 4).

A binary logistic regression model was established to further assess the relationship between $GCG_{0\ min}$ and the risk of VFO. In this regression model, VFA was included as a binary dependent variable based on sex-specific cut-off values. Because the VFO definition accounted for sex differences, sex was not included as a covariate. As summarized in Table 5, higher $GCG_{0\ min}$ and greater BMI were independently associated with a higher risk of VFO among patients with T2DM.

3.5 Diagnostic Value of $GCG_{0\ min}$ for Identifying Visceral Fat Obesity

Given the established independent association between $GCG_{0\ min}$ and visceral adiposity, we examined

Table 3. Collinearity diagnostics of variables included in the final regression models.

Variable	Collinearity statistics	
	Tolerance	VIF
Sex	0.908	1.101
Diabetes duration	0.926	1.079
BMI	0.562	1.779
UAP	0.656	1.525
AST	0.956	1.047
TG	0.870	1.149
LDL	0.897	1.114
$CP_{0\ min}$	0.676	1.478
HOMA-IR	0.850	1.177
$GCG_{0\ min}$	0.688	1.453

The variance inflation factor for all variables included in the model was less than 5, indicating no significant multicollinearity. VIF, variance inflation factor; BMI, body mass index; UAP, ultrasound attenuation parameter; AST, aspartate aminotransferase; TG, triglycerides; LDL, low-density lipoprotein; CP, c-peptide; HOMA-IR, homeostatic model assessment of insulin resistance; GCG, glucagon.

whether it could serve as a diagnostic biomarker for visceral fat obesity. Receiver operating characteristic (ROC) curve analysis (Fig. 2) revealed that the optimal cut-off value for diagnosing VFO was 14.015 pmol/L, indicating a sensitivity of 65.8% and a specificity of 70.3%. The AUC was 0.7099 (95% confidence interval [CI], 0.636–0.783; $p < 0.0001$).

4. Discussion

This study indicates a significant association between dynamic glucagon secretion and visceral fat area in Chinese patients with T2DM. Glucagon showed an abnormal early peak after a 75 g glucose load. More importantly, visceral fat obesity was significantly associated with consistently higher glucagon levels, including fasting levels, postprandial peak responses, and total glucagon exposure. Fasting glucagon was observed to be an independent risk factor for visceral fat obesity, with an optimal diagnostic value of 14.015 pmol/L. This association persisted even after adjusting for traditional adiposity indices and metabolic factors, highlighting the critical involvement of α-cell dysfunction in the pathophysiology of visceral obesity in T2DM.

In healthy individuals, glucagon secretion is inversely regulated by glycemia: it rises during hypoglycemia and is inhibited during hyperglycemia. Typical fasting glucagon levels are around 10 pmol/L, and they can be robustly reduced to about 1 pmol/L during hyperglycemia [4]. In contrast, our study identified a dysregulated glucagon pattern in patients with T2DM, with persistently higher overall secretion and an abnormal early postprandial peak. This pattern is consistent with evidence of intrinsic α-cell dysfunction observed in isolated islets from patients with T2DM, where glucagon secretion paradoxically increases in response to increased glucose [11]. The lack of postprandial glucagon suppression in our patients also aligns with previous clinical observations. For instance, Chen et al. [7] reported that glucagon suppression after glucose loading was significantly lower in patients with T2DM (3.3%) compared with prediabetes (9.1%) or normal glucose tolerance (10.4%) in an obese population. These findings suggested that postprandial glucagon suppression progressively worsens as glucose intolerance deteriorates, and that this impairment is driven primarily by the severity of insulin resistance rather than by hyperglycemia alone [7].

This disrupted kinetic pattern suggests a failure of the normal feedback loop in which postprandial hyperglycemia and insulin secretion should suppress α-cell activity. Impaired insulin signal transduction pathway may be the main explanation behind α-cell insulin resistance. Elevated free fatty acids (FFAs), a hallmark of visceral adiposity, can directly promote α-cell insulin resistance by strongly downregulating the expression of key signaling molecules, including insulin receptor substrate (IRS) 2 and phosphatidylinositol 3 kinase (PI3K) (by 28.5% and 21.3%, respectively) [12]. Furthermore, elevated FFAs can induce endoplasmic reticulum stress in α-cells, thus activating glycogen synthase kinase 3 (GSK3). Onyango reported that activated GSK3 drives phosphorylation, ubiquitination and degradation of IRS1, thereby disrupting a critical signaling node [13]. These lipotoxic effects significantly weaken insulin's capacity to suppress glucagon output. FFA may also amplify inflammation. Elevated FFA has been linked to activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-mediated inflammatory pathway, supported by the reported association between FFA levels and NF-κB messenger RNA (mRNA) overexpression in islet cells from diet-induced obese (DIO) mice [14]. Notably, pioglitazone was reported to ameliorate α-cell insulin resistance in this setting by inhibiting the overexpression of NF-κB mRNA [14]. These metabolic disturbances and inflammatory responses likely reinforce each other, creating a vicious cycle that sustains α-cell insulin resistance and inappropriate glucagon production.

Beyond intracellular metabolic disturbances, the dysregulation of intercellular communication aggravates α-cell dysfunction in T2DM. One crucial regulatory mechanism involves ephrin type-A receptor/ephrin-A ligand (EphA/Ephrin-A) juxtacrine signaling: ephrin-A5 ligands on β-cells activate EphA receptors (primarily EphA4) on neighboring α-cells. This activation stimulates ras homologous family member A (RhoA), a key suppressor of glucagon exocytosis. Activation of this axis increases cortical F-actin density and suppresses Ca^{2+}-triggered glucagon secretion [15]. In diabetes, β-cell deficiency and dysfunction reduce the availability of EphA5 ligand, leading to the impairment of this inhibitory control. Additionally, somatostatin-mediated inhibition of α-cells is disrupted in

Table 4. Multiple linear regression analysis of the relationship between VFA and GCG in T2DM patients.

		Standard β	t	p-value
Model 1	$GCG_{0\ min}$	0.159	3.337	0.001
	Female	0.232	5.524	<0.001
	Diabetes duration	0.041	0.958	0.339
	BMI	0.663	12.549	<0.001
	UAP	0.088	1.745	0.083
	AST	0.045	1.091	0.276
	TG	–0.012	–0.275	0.783
	LDL	0.061	1.442	0.151
Model statistics: F = 49.505, $p < 0.001$; R^2 = 0.662, adjusted R^2 = 0.649; n = 211				
Model 2	$GCG_{0\ min}$	0.161	3.394	0.001
	Female	0.243	5.685	<0.001
	Diabetes duration	0.041	0.970	0.333
	BMI	0.678	12.581	<0.001
	UAP	0.088	1.744	0.083
	AST	0.047	1.142	0.255
	TG	–0.011	–0.266	0.791
	LDL	0.051	1.184	0.238
	HOMA-IR	–0.058	–1.327	0.186
Model statistics: F = 44.366, $p < 0.001$; R^2 = 0.665, adjusted R^2 = 0.650; n = 211				
Model 3	$GCG_{0\ min}$	0.167	3.387	0.001
	Female	0.231	5.492	<0.001
	Diabetes duration	0.039	0.910	0.364
	BMI	0.670	12.437	<0.001
	UAP	0.091	1.794	0.074
	AST	0.049	1.159	0.248
	TG	–0.008	–0.172	0.863
	LDL	0.057	1.343	0.181
	$CP_{0\ min}$	–0.032	–0.651	0.516
Model statistics: F = 43.926, $p < 0.001$; R^2 = 0.663, adjusted R^2 = 0.648; n = 211				

Model 1: adjusted for sex, diabetes duration, BMI, UAP, AST, TG, and LDL. Model 2: adjusted for Model 1 plus HOMA-IR. Model 3: adjusted for Model 1 plus $CP_{0\ min}$. VFA, visceral fat area; GCG, glucagon; T2DM, type 2 diabetes mellitus; BMI, body mass index; UAP, ultrasound attenuation parameter; AST, aspartate aminotransferase; TG, triglycerides; LDL, low-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; CP, c-peptide.

T2DM. Expression of somatostatin receptor 2 (SSTR2) on the surface of pancreatic α-cells is significantly reduced, diminishing paracrine suppression by somatostatin and reducing the coordinated inhibitory effects of somatostatin and insulin on glucagon release. Together, these defects impair normal physiological suppression of glucagon and likely contribute to both fasting and postprandial hyperglucagonemia in T2DM [16].

α-Cell dysfunction and the resulting hyperglucagonemia are well-known contributors to diabetes. In recent years, the glucagon receptor (GCGR) has emerged as a potential therapeutic target [17]. GRAs have been reported to reduce glucose levels by inhibiting glucagon-induced hepatic glucose production and, in parallel, elevating GLP-1 levels [18]. This beneficial effect appears consistently across various species. For example, in a dog study, GRA treatment reduced hepatic glucose production by about 60% [19]. Similar effects have also been observed in metabolically healthy, overweight cats [20]. Additionally, evidence indicates that GCGR suppression may promote intestinal L cell proliferation and induce GLP-1 production through a GLP-1 receptor-protein kinase A signaling pathway [21].

Recently, an unexpected effect was reported that goes much beyond the initial hypoglycemic expectations: GRAs may also promote functional β-cell regeneration. Wei et al. [22] demonstrated that GCGR inhibition increases circulating glucagon concentrations, which can activate β-cell GLP-1 receptors, thereby improving insulin secretion and β-cell regeneration. Additionally, studies in diabetic mice reveal that GRAs increase hepatic secretion of fibroblast growth factor 21 (FGF21), which may further stimulate β-cell regeneration via activating the PI3K/(protein kinase B)

Table 5. Binary logistic regression analysis of risk factors for VFO in T2DM patients.

		With or without VFO			
		B	SE	OR (95% CI)	*p*-value
Model 1	$GCG_{0\ min}$	0.058	0.027	1.060 (1.006, 1.117)	0.028
	BMI	0.368	0.071	1.445 (1.257, 1.662)	<0.001
	Diabetes duration	0.032	0.023	1.033 (0.987, 1.080)	0.161
	UAP	–0.001	0.005	0.999 (0.989, 1.009)	0.848
	AST	0.009	0.007	1.010 (0.996, 1.024)	0.181
	TG	0.048	0.097	1.049 (0.868, 1.269)	0.621
	LDL	0.212	0.204	1.237 (0.829, 1.844)	0.298
Model 2	$GCG_{0\ min}$	0.059	0.027	1.060 (1.006, 1.117)	0.028
	BMI	0.369	0.072	1.446 (1.257, 1.665)	<0.001
	Diabetes duration	0.032	0.023	1.033 (0.987, 1.080)	0.161
	UAP	–0.001	0.005	0.999 (0.989, 1.009)	0.848
	AST	0.010	0.007	1.010 (0.996, 1.024)	0.179
	TG	0.049	0.098	1.050 (0.867, 1.271)	0.618
	LDL	0.210	0.206	1.233 (0.824, 1.846)	0.309
	HOMA-IR	–0.003	0.026	0.997 (0.948, 1.050)	0.921
Model 3	$GCG_{0\ min}$	0.072	0.029	1.075 (1.015, 1.139)	0.014
	BMI	0.386	0.073	1.471 (1.274, 1.697)	<0.001
	Diabetes duration	0.028	0.023	1.029 (0.983, 1.077)	0.222
	UAP	–0.001	0.005	0.999 (0.989, 1.009)	0.902
	AST	0.011	0.008	1.011 (0.996, 1.026)	0.162
	TG	0.074	0.109	1.076 (0.869, 1.333)	0.499
	LDL	0.175	0.209	1.192 (0.792, 1.794)	0.401
	$CP_{0\ min}$	–0.241	0.184	0.786 (0.548, 1.126)	0.189

Binary logistic regression analysis was conducted with VFO as a categorized binary dependent variable defined using sex-specific VFA cut-off values ($\geq$111 cm^2 for men and $\geq$96 cm^2 for women). Model 1: adjusted for diabetes duration, BMI, UAP, AST, TG, and LDL. Model 2: adjusted for Model 1 plus HOMA-IR. Model 3: adjusted for Model 1 plus $CP_{0\ min}$. VFO, visceral fat obesity; T2DM, type 2 diabetes mellitus; SE, standard error; OR, odds ratio; CI, confidence interval; GCG, glucagon; BMI, body mass index; UAP, ultrasound attenuation parameter; AST, aspartate aminotransferase; TG, triglycerides; LDL, low-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; CP, c-peptide; VFA, visceral fat area.

Akt pathway [23]. Thus, these results suggest dual benefit of GRAs, with short-term improvement in glycemia through reduced hepatic glucose output and long-term recovery of β-cell function via β-cell regenerative pathways involving FGF21.

Numerous clinical trials support the metabolic efficacy of GRAs. Several studies, including those assessing the small molecule LGD-6972 (RVT-1502), have shown a reduction in HbA1c and fasting plasma glucose levels in those with T2DM, with good tolerability and a low risk of hypoglycemia [24]. Potential efficacy may extend to T1DM, for example, volagidemab, a fully human monoclonal antibody targeting the glucagon receptor, has been explored as a first-in-class therapeutic approach in this clinical context [25].

Limitation

Despite these promising findings, this study has several limitations. The causal relationship between elevated GCG levels and VFO could not be established because this was a single-center and retrospective investigation. Furthermore, the sample size of participants with VFO was relatively limited, which may have reduced statistical power and contributed to significant differences in some estimates. Larger-scale, multicenter studies with prospective follow-up are therefore required to validate our findings and improve generalizability. Finally, additional mechanistic investigations are needed to elucidate the underlying pathways linking increased glucagon secretion with visceral adiposity in patients with T2DM.

5. Conclusion

Our findings offer strong clinical evidence that elevated fasting glucagon levels are independently associated with visceral adiposity in Chinese patients with T2DM. This observation supports the idea that α-cell dysfunction is a key pathophysiological component linking visceral obesity to diabetes. In practice, especially for patients with visceral fat obesity, dynamic assessment of glucagon secretion should not be overlooked. Ultimately, targeting glucagon dysregulation may offer a promising therapeutic approach for treating T2DM in the presence of visceral adiposity.

Key Points

• In this cohort of Chinese patients with type 2 diabetes, fasting glucagon levels, postprandial peak, and overall glucagon secretion were each significantly and positively correlated with visceral fat area measured by bioelectrical impedance analysis.

• Fasting glucagon was identified as an independent risk factor for visceral fat obesity, with an optimal diagnostic threshold of 14.015 pmol/L.

• A distinctive paradoxical glucagon peak at 30 min was observed after a 75 g oral glucose load in patients, while patients with visceral fat obesity exhibited consistently higher glucagon levels at all time points.

Availability of Data and Materials

The datasets analyzed during the current study are not publicly available due to patient privacy concerns, but are available from the corresponding authors on reasonable request.

Author Contributions

Conceptualization, PH, YS, MYL, XQZ and YJG; methodology, PH, YS, XQZ and YJG; software, PH; validation, YS and XQZ; formal analysis, PH, YS and TTX; investigation, PH and TTX; resources, ZXQ, WRL, MYL, XQZ and YJG; data curation, PH, YS, TTX, ZXQ and WRL; writing—original draft, PH; visualization, PH and YS; supervision, XQZ; project administration, MYL and XQZ; funding acquisition, XQZ and YJG. All authors contributed to revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This present study was conducted adhering to the Declaration of Helsinki's tenets and authorized by Affiliated Hospital of Nantong University's ethics committee (2018-k016). All participants provided written informed consent after receiving details about the study objectives.

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Conflicts of Interest

Zongxian Qi, Weirong Lin and Minyou Li are employees of Guangzhou Jinde Biotech Company Limited. The glucagon detection equipment and reagents used in this study were provided by Guangzhou Jinde Biotech Company Limited. This commercial affiliation did not influence the design, execution, analysis, or reporting of this study. The other authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/BJHM62016.

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