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Differences in the insulin counterregulatory hormones between obese and nonobese male patients with type 2 diabetes mellitus

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Obesity is associated with both high and low levels of hypoglycemia, and impairment of counterregulatory hormones may predispose individuals to hypoglycemia. This study aimed to explore differences in the responsiveness of insulin counterregulatory hormones to hypoglycemia between men with or without obesity who have been newly diagnosed with type 2 diabetes mellitus (T2DM). This study enrolled 25 men newly diagnosed with T2DM who were hospitalized in the Department of Endocrinology and Metabolism between January 2022 and December 2022. All participants were treated with intensive insulin pump therapy to achieve glycemic control within one week, then a hyperinsulinemic-hypoglycemic clamp was used to evaluate insulin counter-regulatory hormones for hypoglycemia. Based on the body mass index, 10 and 15 patients were included in the obese and nonobese groups, respectively. During the hyperinsulinemic-hypoglycemic clamp test, the obese group showed a significant lower multiple of adrenocorticotropic hormone elevation than the nonobese group ($P = 0.040$). Regarding the proportion of hormone response multiples reaching the target, those who reached the reaction multiple were lower in the obese group than those in the non-obese group, although the differences were not statistically significant (all $P > 0.05$). The responses of insulin counterregulatory hormones to hypoglycemia in men with obesity and newly diagnosed T2DM were significantly lower than those in men with T2DM but without obesity.

Keywords Insulin counterregulatory hormones, Hypoglycemia, Hyperinsulinemic-hypoglycemic clamp, Obesity, Newly diagnosed T2DM

The prevalence of type 2 diabetes mellitus (T2DM) is increasing yearly, and recent researches have mainly focused on protecting pancreatic islet β-cell function, improving insulin resistance, weight management, and intensive glycemic control. The ADVANCE clinical trial, Veterans Affairs Diabetes Trial (VADT), and Action to Control Cardiovascular Risk in Diabetes (ACCORD) studies have not confirmed whether intensive glucose control can reduce cardiovascular events or mortality[1–3]. However, the results of the above-mentioned studies showed a correlation between severe hypoglycemia and mortality. The Diabetes Control and Complications (DCCT) study also demonstrated that 60% of patients with diabetes who received intensive insulin therapy had a three times higher risk of severe hypoglycemia than those receiving conventional insulin therapy[4]. In addition to the use of insulin and sulfonylurea drugs, the impairment of counterregulatory hormones may predispose patients to hypoglycemia[5]. In a healthy population, the concentration of counter regulatory hormones such as glucagon and adrenaline increase to counteract hypoglycemia; otherwise, the body may experience irreversible damage owing to prolonged hypoglycemia[6]. Previous studies have shown that patients with T2DM have an abnormal hypothalamic-pituitary-adrenal (HPA) axis, resulting in high basal adrenocorticotropin levels[7].

Obesity is an important factor in T2DM pathogenesis. In T2DM with cardiac autonomic neuropathy, obesity is associated with a lower risk of severe hypoglycemia when compared with non-obesity[8]. Among adults with T2DM and nonalcoholic fatty liver disease, those with severe hypoglycemia had a lower mean (standard

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deviation) body mass index (BMI) than those without severe hypoglycemia[9]. Conversely, individuals with obesity have a higher rate of reactive hypoglycemia than normal weight group in a 75 g prolonged oral glucose tolerance test, in which insulin resistance may play an important role[10]. Additionally, the counterregulatory hormones levels during hypoglycemia in patients with obesity remain controversial. In a study by Lassman et al., glucagon (GCG) and of C-peptide (CP) secretion in response to insulin hypoglycemia did not differ between the obese and control groups. However, the integrated response of adrenocorticotropic hormone (ACTH) was higher, while the secretory response of growth hormone (GH) was lower in the obese group[11]. Insulin-induced hypoglycemia elicited a significant increase in plasma GCG levels in patients with obesity but with no diabetes and in lean normal men. Moreover, the response to GCG after insulin injection was significantly lower in participants with obesity than in normal controls[12]. Inconsistent findings were observed in a study by Klement et al. in which the ACTH, norepinephrine, and GCG responses to hypoglycemia were stronger in men with obesity than in lean men[13]. Weaver et al. reported an augmented peak increase in the ACTH response in women with obesity in response to hypoglycemia despite normal cortisol (Cor) responses[14]. Slightly different from the above-mentioned findings, significantly elevated Cor and ACTH responses were observed in patients with overweight/obese vs. lean participants during the hypoglycemic clamps[15]. In patients with T2DM, GCG and ACTH levels were higher, whereas GH levels were lower during the hyperglycemic clamp during hypoglycemia than in those with normoglycemia[16]. Therefore, this study was aimed to explore the differences in insulin counterregulatory hormones during hypoglycemia between men with and without obesity who have T2DM after achieving glycemic control.

Methods

Study design and population

This study was approved by the Institutional Ethical Committee of Naning First Hospital, Nanjing Medical University, in compliance with the Declaration of Helsinki guidelines (KY20220124-07). All participants provided informed consent before inclusion in the study. Male patients aged between 18 and 65 years with newly diagnosed T2DM who were hospitalized in the Department of Endocrinology and Metabolism at Nanjing First Hospital between January 2022 and December 2022 were recruited for this study. All patients were diagnosed with diabetes according to the World Health Organization 1999 criteria. The exclusion criteria were as follows: (1) patients taking oral hypoglycemic drugs or insulin before admission; (2) patients with concurrent infections; (3) patients with other diseases, such as hypertension, hypothyroidism, pituitary dysfunction, adrenal cortical dysfunction, or pancreatic disease; and (4) patients with liver or kidney function damage. Finally, 25 participants (aged 24–48 years) were enrolled.

Experimental procedures

Patient data were collected after admission, including age, height, weight, body mass, and blood pressure. BMI was calculated as weight divided by height squared (kg/m^2). According to the Chinese standard, obesity was defined as BMI ≥ 28 kg/m^2Superscript>[17]. Blood samples were collected from all patients after overnight fasting, and fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), fasting insulin (FINS), fasting Cp (FCp), liver and kidney function, and lipid levels were measured. Postprandial blood glucose (PBG), 2-hour insulin (INS120) and C-peptide (Cp120) were examined after a standard meal with steamed bread. After blood sample collection, all patients were given insulin pump intensive treatment to achieve glycemic control (fasting blood glucose < 7.0 mmol/L, postprandial blood glucose < 11.1 mmol/L, with a compliance rate of over 80%, and no hypoglycemia related to the use of hypoglycemic drugs). On the second day after glycemic control, a hyperinsulinemic-hypoglycemic clamp test was performed. The insulin pump was stopped 12 h before the clamp test, and the patient arrived at the clamp room with a 12-h overnight fast. A venous catheter was placed in the forearm vein for insulin and glucose infusion, and a second intravenous catheter was placed on the other side for blood sampling. Throughout the study, insulin was continuously administered at a dose of 3 mIU/kg at a rate of 1.5 mU/kg/min, and the infusion rate of 20% glucose solution was adjusted to achieve the target blood glucose level[18]. During this process, blood glucose levels were measured every 5 min. When the blood glucose level remained stable between 5 and 5.5 mmol/L, blood samples were drawn to examine the insulin, Cp, GCG, GH, insulin-like growth factor 1 (IGF-1), Cor and ACTH levels. Then, the peripheral blood glucose was adjusted to less than 2.8 mmol/L and remained for 30 min. Blood samples were obtained again to check insulin, Cp, GCG, GH, IGF-1, Cor and ACTH levels. Finally, insulin infusion was stopped, and glucose infusion was continued until the patient's blood glucose returned to normal. In Chiodera et al. study, the plasma concentration of ACTH was strikingly increased by hypoglycemia with the mean peak level 3.5 times higher than baseline[19]. The insulin-induced hypoglycemia test (insulin tolerance test, ITT) is considered the gold standard for the diagnosis of Cor and GH deficiencies. In which, a peak Cor level > 18 μg/dL and GH level > 5 μg/L were normal responses[20,21]. In the hyperinsulinemic-hypoglycemic clamp test, the peak GCG level 1.5 times lower than baseline was diagnosed inappropriate GCG secretion[22].

Blood parameter analyses

FBG, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cr), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were analyzed by standard enzymatic assays (Olympus AU5400 autoanalyzer; Beckman Coulter, Japan). HbA1c was measured using a high-performance liquid chromatography assay (Bio-Rad Laboratories, Inc. Hercules, CA, USA). Insulin, Cp, GH, IGF-1, Cor and ACTH were detected using a chemiluminescent immunometric assay (Modular Analytics E170; Roche Diagnostics GmbH, Mannheim, Germany), and GCG was detected using a radioimmunoassay (IBL International, Hamburg, Germany).

Statistical analysis

Statistical analyses were conducted using SPSS 22.0 software. Normally distributed quantitative data are expressed as mean ± standard deviation, asymmetrically distributed quantitative data are expressed as media (interquartile range), and categorical data are expressed as n (%). The independent sample T-test, Mann-Whitney U test, and Fisher's exact test were used to compare normally distributed quantitative variables, asymmetrically distributed quantitative variables, and categorical variables between the two groups, respectively. A two-sided P-value of < 0.05 indicated statistically significant differences.

Results

Clinical characteristics

A total of 25 subjects were enrolled in this study, with 15 men in the nonobese group (N group) and 10 cases in the obese group (O group) according to a BMI of over or less than 28.0 kg/m^2. The clinical characteristics of the two groups are presented in Table 1. The BMI, TG and FCp of the O group were significantly higher than those of N group (both $P < 0.05$). No differences were observed in age, ALT, AST, Cr, TC, TG, HDL-C, LDL-C, HbA1c, FINS, INS120 and Cp120 (all $P > 0.05$).

Comparison of insulin counterregulatory hormones levels between the two groups

1. The levels of insulin counterregulatory hormones during euglycemia and hypoglycemia in the hyperinsulinemic-hypoglycemic clamp test are shown in Fig. 1. During hypoglycemia, the O group showed a lower multiple of ACTH elevation than the N group with a statistically significant difference ($P = 0.040$, Table 2). No statistically significant differences were observed for the other indicators (all $P > 0.05$, Table 2).

2. Regarding the proportion of hormone response multiples reaching the target after the hyperinsulinemic-hypoglycemic clamp test, those who reached the reaction multiple were lower in the O group than those in the N group, although the differences were not statistically significant (all $P > 0.05$, Table 3).

Discussion

In the present study, insulin counterregulatory hormones were first examined in response to hypoglycemia in men with and without obesity who were newly diagnosed T2DM. The results revealed that the O group had a significantly lower multiple of ACTH elevation than the N group in the hypoglycemia state during the hyperinsulinemic-hypoglycemic clamp test. Moreover, the O group had a lower proportion of participants who reached the desired response multiple compared with the N group, although difference was not statistically significant.

As a common acute complication in patients with diabetes mellitus, hypoglycemia is associated with irreversible brain damage or substantial morbidity and mortality[23]. When the blood glucose is < 3.8 mmol/L, key glucose counterregulatory systems are activated, in which GCG plays a major role. Bolli et al. proposed inappropriate GCG secretion as an increase of less than 1.5 times in a hypoglycemia state compared with the baseline during the hyperinsulinemic-hypoglycemic clamp test[22]. A previous study showed that patients with non-insulin-dependent and insulin-dependent diabetes have an impaired GCG response to hypoglycemia,

Index	N group ($n = 15$)	O group ($n = 10$)	*P* value
Age (years)	38.87 ± 6.38	34.5 ± 5.04	0.082
BMI (kg/m2)	25.96 (23.40–26.92)	32.04 (30.32–33.95)	< 0.001
ALT (U/L)	28.0 (16.00–40.00)	41.00 (29.50–67.00)	0.078
AST (U/L)	18.00 (16.00–23.00)	16.00 (13.50–45.50)	0.976
Cr (μmol/L)	63.04 ± 8.82	59.94 ± 6.82	0.376
TC (mmol/L)	5.62 (4.56–6.66)	5.45 (4.55–7.35)	0.835
TG (mmol/L)	1.79 (1.26–3.08)	4.57 (2.45–5.38)	0.013
HDL-C (mmol/L)	1.07 ± 0.25	0.92 ± 0.25	0.168
LDL-C (mmol/L)	3.15 ± 0.91	3.54 ± 1.07	0.349
HbA1c (%)	10.09 ± 1.53	9.98 ± 2.19	0.887
FINS (uU/mL)	14.65(7.78–21.10)	17.79(9.80–30.74)	0.425
FCp (ng/mL)	1.45 ± 0.82	2.41 ± 1.38	0.044
INS120 (uU/mL)	39.28 (31.10–75.19)	70.74 (36.93–100.65)	0.257
Cp120 (ng/mL)	3.70 (1.77–4.54)	4.27 (2.48–7.93)	0.120

Table 1. Clinical characteristics. Data are presented as mean ± SD and median (interquartile range) as appropriate. Student's t test for comparison of normally distributed quantitative variables, and Mann-Whitney U test for comparison of asymmetrically distributed quantitative variables between O group and N group. Abbreviations: BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatinine; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HbA1c, glycated hemoglobin; FINS, fasting insulin; FCp, fasting C-peptide; INS120, postprandial 2-hour insulin; Cp120, postprandial 2-hour C-peptide.

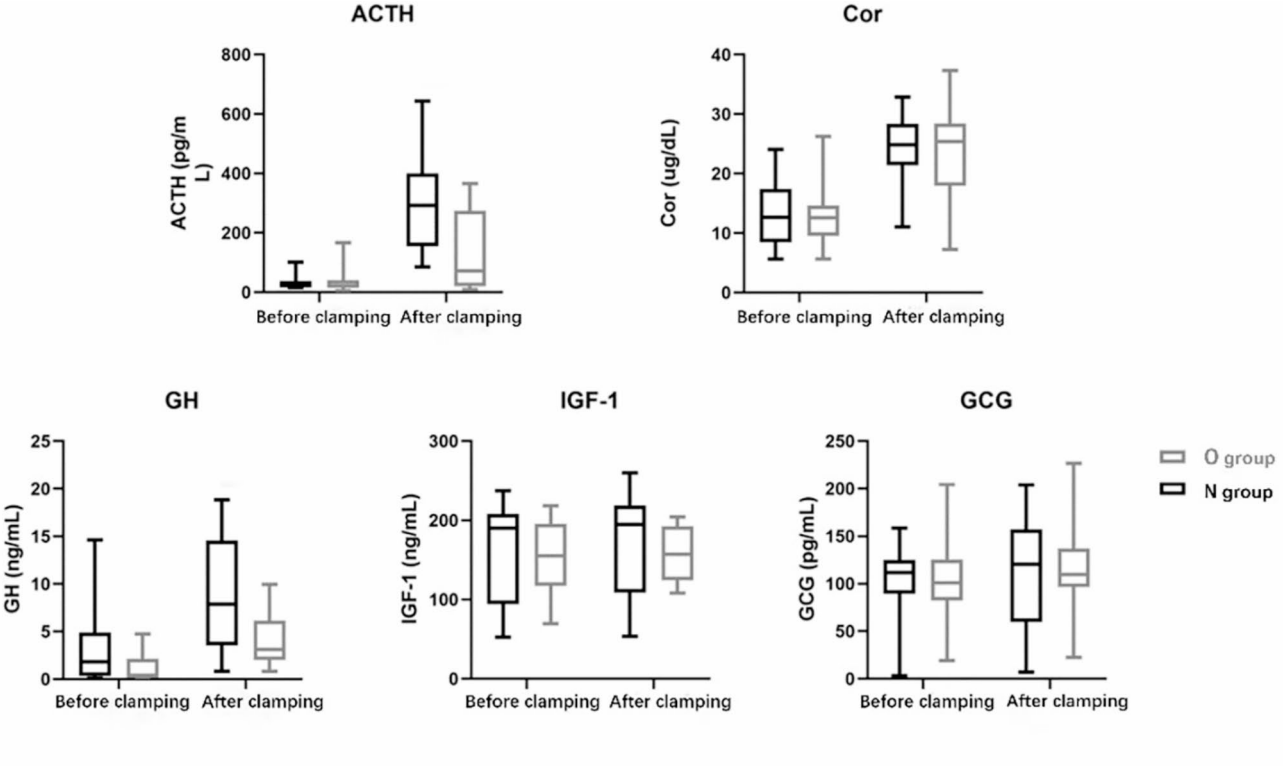

Fig. 1. Differences in the insulin counterregulatory hormones levels between two groups before and after hyperinsulinemic-hypoglycemic clamp test. Abbreviations: ACTH, adrenocorticotropic hormone; Cor, cortisol; GH, growth hormone; IGF-1, insulin-like growth factor 1; GCG, glucagon.

Index	*N* group	O group	*P* value
ACTH (multiples)	12.16 (4.04–17.26)	2.98 (0.83–11.04)	0.040
Cor (multiples)	1.62 (1.38–2.66)	2.13 (1.08–2.36)	1.000
GH (multiples)	5.38 (1.19–27.52)	7.16 (1.35–17.92)	0.907
IGF-1 (multiples)	1.07 (0.99–1.13)	1.03 (0.90–1.10)	0.356
GCG (multiples)	1.18 (1.05–1.56)	1.10 (1.05–1.24)	0.245

Table 2. Differences in hormone response multiple levels after hyperinsulinemic-hypoglycemic clamp test between two groups. Data are presented as median (interquartile range). Mann-Whitney U test for comparison of asymmetrically distributed quantitative variables between O group and N group. Abbreviations: ACTH, adrenocorticotropic hormone; Cor, cortisol; GH, growth hormone; IGF-1, insulin-like growth factor 1; GCG, glucagon.

which is related to the duration of diabetes and insulin resistance[22,24]. In patients with obesity and impaired glucose tolerance, circulating GCG was correlated with FBG, TG and fat mass, duration of diabetes, glycemic control, and renal function, suggesting that obesity is related to GCG secretion levels[25,26]. Animal experiments have also shown that the GCG level in obese Zucker rats decreases and that GLP-1/GCG/CCK2 receptors triagonist can significantly inhibit obesity and treat diabetes[27,28].

Other hormones, such as GH and Cor, also protect against the development of hypoglycemia, and the magnitude of the response is significantly related to the degree and duration of hypoglycemia[29]. GH, a peptide hormone secreted by the anterior pituitary gland, is involved in human growth, development, and metabolism. GH is inhibited by glucose and fatty acids, while increased during sleep, stress, exercise, and hypoglycemia[30]. Children usually have a threshold of 10 μg/L owing to their greater ability to secrete GH, and GH deficiency is usually diagnosed based on levels < 5 μg/L[21,31]. When severe hypoglycemia occurs, Cor levels below 18 μg/dL are considered abnormal responses[32]. In a study by Rhyou et al., many patients with T2DM had impaired Cor and/or GH responses during severe hypoglycemia. The impaired GH response was associated with advanced age, short duration of diabetes, and higher BMI, which suggested that a higher BMI is a risk factor for impaired Cor and GH response to severe hypoglycemia in patients with T2DM[33]. In patients with obesity, the GH secretion response was gradually impaired during the insulin-induced hypoglycemia test, and serum somatomedin activity was significantly decreased in patients with obesity and an average body weight exceeding 100%[34]. Moreover, the

	ACTH > 3.5 multiples	ACTH < 3.5 multiples	*P* value
N group	14	1	0.121
O group	6	4	
	Cor > 18 μg/dL	Cor < 18 μg/dL	
N group	13	2	1.000
O group	8	2	
	GH > 5 ng/mL	GH < 5 ng/mL	
N group	10	5	0.111
O group	3	7	
	GCG > 1.5 multiples	GCG < 1.5 multiples	
N group	5	10	0.345
O group	1	9	

Table 3. Differences in the proportion of hormone response multiples reaching the target after hyperinsulinemic-hypoglycemic clamp test between two groups. Data are presented as n (%). χ 2 test for comparison of qualitative variables between O group and N group. Abbreviations: ACTH, adrenocorticotropic hormone; Cor, cortisol; GH, growth hormone; GCG, glucagon.

GH response induced by GH-releasing factor in patients with obesity was significantly impaired compared with that in normal-weight individuals[35]. Research have also shown an average peak ACTH level 3.5 times higher than baseline in insulin induces hypoglycemia[19]. Patients with obesity and normal glucose metabolism have lower plasma Cor and ACTH levels in the morning than patients who were not obese. Moreover, ACTH levels in patients with obesity are insensitive to glucocorticoid negative feedback regulation especially at night[36,37]. In premenopausal women with obesity, the ACTH-Cor relationship is characterized by decreased sensitivity and efficacy, thus explaining non-elevated serum Cor concentration despite increased plasma ACTH levels[38]. The various insulin counterregulatory hormones mentioned above, such as Cor, GH, and ACTH, are mainly regulated by the central nervous system. In contrast, GCG is primarily regulated by glucose levels in the islet environment, and also regulated by the central nervous system through other antiregulatory hormones and the autonomic nervous system[39–41]. Consistent with the results of previous studies, the secretion levels of GH, ACTH, Cor and GCG are impaired during the insulin-induced hypoglycemia test in men with T2DM who were obese, which may be related to the impaired control of the hypothalamus[42–44].

The innovation of our study was to exclude the blood glucose effect on insulin counter-regulatory hormones using intensive insulin pump therapy to achieve glycemic control. Additionally, the hyperinsulinemic-hypoglycemic clamp test was used to induce hypoglycemia to unify the hypoglycemia levels of patients and detect the corresponding insulin counter-regulatory hormones concentrations promptly. However, the studies had several limitations. The small sample size could have led to type II errors, which may not entirely represent men with T2DM with or without obesity. GCG, a counter-regulatory hormone rapidly released during hypoglycemia, is secreted when blood glucose levels are below 3.8 mmol/L. We collected blood samples 30 min into the hypoglycemic state because of the financial resources of our study, possibly missing peak GCG secretion. Plasma ACTH levels rose sharply in response to hypoglycemia, with a mean peak response 3.5 times higher than baseline at 45 min. Cor and GH should also be observed if the blood glucose level is 2.2 mmol/L for more than 2 h, but, in our study, this was risky and challenging for patients with diabetes from an ethical perspective. Moreover, a nine-point self-monitoring of blood glucose other than the continuous glucose monitoring (CGM) system was used in our study because of the limited cost.

In conclusion, men who are obese with newly diagnosed T2DM had an impaired insulin regurgitator response during hypoglycemia, suggesting that more attention should be paid to hypoglycemia risk in these patients. Given the limitations of this study and the harmful effects of severe hypoglycemia on health, further studies with a larger sample size and multipoint detection of insulin counterregulatory hormones after hypoglycemia are necessary to verify our findings.

Data availability

All data generated or analyzed during this study are included in this published article.

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References

1. Group, A. C. et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl. J. Med.* **358**, 2560–2572 (2008).
2. Duckworth, W. et al. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl. J. Med.* **360**, 129–139 (2009).
3. Action to Control Cardiovascular Risk in Diabetes Study. Effects of intensive glucose lowering in type 2 diabetes. *N Engl. J. Med.* **358**, 2545–2559 (2008).
4. Nathan, D. M. & Group, D. E. R. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. *Diabetes Care.* **37**, 9–16 (2014).

5. Silbert, R., Salcido-Montenegro, A., Rodriguez-Gutierrez, R., Katabi, A. & McCoy, R. G. Hypoglycemia among patients with type 2 diabetes: epidemiology, risk factors, and Prevention Strategies. *Curr. Diab Rep.* **18**, 53 (2018).
6. Cryer, P. E. Glucose counterregulation: prevention and correction of hypoglycemia in humans. *Am. J. Physiol.* **264**, E149–155 (1993).
7. Chiodini, I. et al. Hypothalamic-pituitary-adrenal activity in type 2 diabetes mellitus: role of autonomic imbalance. *Metabolism* **55**, 1135–1140 (2006).
8. Zhang, W. et al. Cardiac autonomic neuropathy modified the association between obesity and hypoglycemia in type 2 diabetes. *Endocrine* (2024).
9. Lee, J. Y. et al. Analysis of severe hypoglycemia among adults with type 2 diabetes and nonalcoholic fatty liver disease. *JAMA Netw. Open.* **5**, e220262 (2022).
10. Lv, X. et al. Identification of reactive hypoglycemia with different basic BMI and its causes by prolonged oral glucose tolerance test. *Diabetes Metab. Syndr. Obes.* **13**, 4717–4726 (2020).
11. Lassmann, V., Garcia, C., Vialettes, L., Vague, P. & B. & Impaired pancreatic polypeptide response to insulin hypoglycemia in obese subjects. *Horm. Metab. Res.* **17**, 663–666 (1985).
12. Coiro, V. et al. Glucagon response to insulin-induced hypoglycemia in obese men. *Horm. Metab. Res.* **20**, 656–657 (1988).
13. Klement, J. et al. Effects of blood glucose on delay discounting, food intake and counterregulation in lean and obese men. *Psychoneuroendocrinology* **89**, 177–184 (2018).
14. Weaver, J. U., Kopelman, P. G., McLoughlin, L., Forsling, M. L. & Grossman, A. Hyperactivity of the hypothalamo-pituitary-adrenal axis in obesity: a study of ACTH, AVP, beta-lipotrophin and cortisol responses to insulin-induced hypoglycaemia. *Clin. Endocrinol. (Oxf).* **39**, 345–350 (1993).
15. Lundqvist, M. H. et al. Altered hormonal and autonomic nerve responses to hypo- and hyperglycaemia are found in overweight and insulin-resistant individuals and may contribute to the development of type 2 diabetes. *Diabetologia* **64**, 641–655 (2021).
16. Lundqvist, M. H., Pereira, M. J., Almby, K., Hetty, S. & Eriksson, J. W. Regulation of the Cortisol Axis, Glucagon, and growth hormone by glucose is altered in Prediabetes and Type 2 diabetes. *J. Clin. Endocrinol. Metab.* **109**, e675–e688 (2024).
17. Geriatric, E., Metabolism Branch of the Chinese Geriatrics & National Clinical Research Center of Geriatric. Compilation Group of Expert Consensus of the, D. & Treatment measures for the Chinese Elderly patients with type, D. [Expert consensus of the diagnosis and treatment measures for the Chinese elderly patients with type 2 diabetes (2018 Edition)]. *Zhonghua Nei Ke Za Zhi.* **57**, 626–641 (2018).
18. Jiang, L. L. et al. The effects of add-on exenatide to insulin on glycemic variability and hypoglycemia in patients with type 1 diabetes mellitus. *J. Endocrinol. Invest.* **41**, 539–547 (2018).
19. Chiodera, P. & Coiro, V. Inhibition by dexamethasone of arginine vasopressin and ACTH responses to insulin-induced hypoglycemia and cigarette smoking in normal men. *Acta Endocrinol. (Copenh).* **123**, 487–492 (1990).
20. Lee, H. C., Yoon, H. K., Kim, J. H., Kim, Y. H. & Park, H. P. Comparison of intraoperative cortisol levels after preoperative hydrocortisone administration versus placebo in patients without adrenal insufficiency undergoing endoscopic transsphenoidal removal of nonfunctioning pituitary adenomas: a double-blind randomized trial. *J. Neurosurg.* **134**, 526–534 (2021).
21. Molitch, M. E. et al. Evaluation and treatment of adult growth hormone deficiency: an endocrine Society clinical practice guideline. *J. Clin. Endocrinol. Metab.* **96**, 1587–1609 (2011).
22. Bolli, G. B., Tsalikian, E., Haymond, M. W., Cryer, P. E. & Gerich, J. E. Defective glucose counterregulation after subcutaneous insulin in noninsulin-dependent diabetes mellitus. Paradoxical suppression of glucose utilization and lack of compensatory increase in glucose production, roles of insulin resistance, abnormal neuroendocrine responses, and islet paracrine interactions. *J. Clin. Invest.* **73**, 1532–1541 (1984).
23. Davis, S. N. et al. Effects of severe hypoglycemia on Cardiovascular outcomes and Death in the Veterans affairs Diabetes Trial. *Diabetes Care*. **42**, 157–163 (2019).
24. Bolli, G. et al. Abnormal glucose counterregulation in insulin-dependent diabetes mellitus. Interaction of anti-insulin antibodies and impaired glucagon and epinephrine secretion. *Diabetes* **32**, 134–141 (1983).
25. Ortega, F. J. et al. Circulating glucagon is associated with inflammatory mediators in metabolically compromised subjects. *Eur. J. Endocrinol.* **165**, 639–645 (2011).
26. Hamasaki, H. & Morimitsu, S. Association of Glucagon with Obesity, Glycemic Control and renal function in adults with type 2 diabetes Mellitus. *Can. J. Diabetes*. **45**, 249–254 (2021).
27. Bryce, G. F., Johnson, P. R., Sullivan, A. C. & Stern, J. S. Insulin and glucagon: plasma levels and pancreatic release in the genetically obese Zucker rat. *Horm. Metab. Res.* **9**, 366–370 (1977).
28. Zhao, S. et al. A GLP-1/glucagon (GCG)/CCK(2) receptors tri-agonist provides new therapy for obesity and diabetes. *Br. J. Pharmacol.* **179**, 4360–4377 (2022).
29. Nye, E. J. et al. The insulin hypoglycemia test: hypoglycemic criteria and reproducibility. *J. Neuroendocrinol.* **13**, 524–530 (2001).
30. Kargi, A. Y. & Merriam, G. R. Diagnosis and treatment of growth hormone deficiency in adults. *Nat. Rev. Endocrinol.* **9**, 335–345 (2013).
31. Growth, H. & Research, S. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. *J. Clin. Endocrinol. Metab.* **85**, 3990–3993 (2000).
32. Schneider, H. J., Aimaretti, G., Kreitschmann-Andermahr, I., Stalla, G. K. & Ghigo, E. *Hypopituitarism Lancet* **369**, 1461–1470 (2007).
33. Rhyu, Y. A. et al. Impaired cortisol and growth hormone counterregulatory responses among severe hypoglycemic patients with type 2 diabetes Mellitus. *Endocrinol. Metab. (Seoul).* **34**, 187–194 (2019).
34. Grzywa, M. Serum somatomedin activity and growth hormone level in obese men: dependence on degree of obesity and hyperlipidemia. *Exp. Clin. Endocrinol.* **88**, 325–333 (1986).
35. Kopelman, P. G., Noonan, K., Goulton, R. & Forrest, A. J. Impaired growth hormone response to growth hormone releasing factor and insulin-hypoglycaemia in obesity. *Clin. Endocrinol. (Oxf).* **23**, 87–94 (1985).
36. Praveen, E. P. et al. Morning cortisol is lower in obese individuals with normal glucose tolerance. *Diabetes Metab. Syndr. Obes.* **4**, 347–352 (2011).
37. Jessop, D. S., Dallman, M. F., Fleming, D. & Lightman, S. L. Resistance to glucocorticoid feedback in obesity. *J. Clin. Endocrinol. Metab.* **86**, 4109–4114 (2001).
38. Roelfsema, F., Pijl, H., Keenan, D. M. & Veldhuis, J. D. Diminished adrenal sensitivity and ACTH efficacy in obese premenopausal women. *Eur. J. Endocrinol.* **167**, 633–642 (2012).
39. Watts, A. G. & Donovan, C. M. Sweet talk in the brain: glucosensing, neural networks, and hypoglycemic counterregulation. *Front. Neuroendocrinol.* **31**, 32–43 (2010).
40. McCrimmon, R. J. et al. Activation of ATP-sensitive K + channels in the ventromedial hypothalamus amplifies counterregulatory hormone responses to hypoglycemia in normal and recurrently hypoglycemic rats. *Diabetes* **54**, 3169–3174 (2005).
41. Routh, V. H. Glucosensing neurons in the ventromedial hypothalamic nucleus (VMN) and hypoglycemia-associated autonomic failure (HAAF). *Diabetes Metab. Res. Rev.* **19**, 348–356 (2003).
42. Popovic, V. et al. Blocked growth hormone-releasing peptide (GHRP-6)-induced GH secretion and absence of the synergic action of GHRP-6 plus GH-releasing hormone in patients with hypothalamopituitary disconnection: evidence that GHRP-6 main action is exerted at the hypothalamic level. *J. Clin. Endocrinol. Metab.* **80**, 942–947 (1995).

43. Popovic, V. et al. Ghrelin main action on the regulation of growth hormone release is exerted at hypothalamic level. *J. Clin. Endocrinol. Metab.* **88**, 3450–3453 (2003).
44. Cavagnini, F., Maraschini, C., Pinto, M., Dubini, A. & Polli, E. E. Impaired prolactin secretion in obese patients. *J. Endocrinol. Invest.* **4**, 149–153 (1981).

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Author contributions
Ma JH and Ding B designed the study; Huang R, Bao YJ and Xiong YL collected the data; Huang R and Bao YJ performed the analyses and wrote the first draft; and Ma JH and Ding B checked the manuscript and revised it. All authors approved the final submission.

Declarations

Competing interests
The authors declare no competing interests.

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