

## **Effects of insulin therapy on glucagon in patients with newly diagnosed type 2 diabetes.**

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### **Abstract**

The aim of this study was to investigate the effects of insulin therapy on glucagon in patients with newly diagnosed Type 2 Diabetes (nd-T2DM patients). We recruited 93 nd-T2DM patients, including 45 non-obese patients and 48 obese patients. A 100 g bread meal test was performed before and after insulin therapy, and glucagon levels were measured before and after the experiment. Compared with the control group, the serum glucagon levels before treatment in both the obese and non-obese nd-T2DM patients were significantly higher ( $P=0.001$ ). After treatment, the serum glucagon levels in the nd-T2DM patients had significantly decreased ( $P=0.001$ ), but they were still higher than those in the control group ( $P=0.001$ ). Additionally, the area under the curve of serum glucagon and postprandial glucagon levels in the non-obese nd-T2DM patients decreased significantly ( $P=0.001$  and  $P<0.01$ , respectively). The serum glucagon level in the obese nd-T2DM patients decreased non-significantly ( $P>0.05$ ). Insulin therapy improved serum glucagon levels in nd-T2DM patients. The serum glucagon level in the non-obese nd-T2DM patients improved significantly, but that in the obese nd-T2DM patients did not. The CP levels in the non-obese T2DM group at 30 min ( $P=0.003$ ), 60 min ( $P=0.001$ ), 120 min ( $P=0.001$ ), and 180 min ( $P=0.001$ ) after treatment had significantly increased compared to that before treatment. The CP levels in the obese T2DM group at 120 min ( $P=0.001$ ) and 180 min ( $P=0.001$ ) after treatment had significantly increased compared to that before treatment. This improvement might be related with a potential association between T2DM and obesity.

**Keywords:** Type 2 diabetes, Obesity, Glucagon, C peptide.

*Accepted on September 29, 2016*

### **Introduction**

Type 2 Diabetes Mellitus (T2DM) is a common metabolic disease characterized by a decline in insulin sensitivity and relative insulin insufficiency-induced hyperglycaemia [1]. Pancreatic  $\beta$ -cells secrete insulin, which is dependent on blood glucose levels. Insulin secretion is also influenced by paracrine interactions with surrounding cells, mainly  $\alpha$ -cells and  $\gamma$ -cells. In normal physiological processes, stable fasting (basal) insulin and glucagon levels are maintained within a certain range, and insulin and glucagon work together to maintain stable blood glucose levels after an oral bread meal test. Patients with T2DM have defects in the secretion of both insulin and glucagon; they have low or high basal insulin levels, but their basal glucagon levels are increased or remain unchanged [2,3]. After the bread meal test, insulin is insufficiently secreted, and peak secretion is delayed; however, glucagon levels inappropriately decrease or increase [4-7]. Patients with T2DM and high basal insulin levels are often obese [8], but those with

low basal insulin levels are usually non-obese. In addition to the abnormal secretion of insulin, the abnormal secretion of glucagon plays an important role in the occurrence and development of T2DM [9-15]. Abnormal glucagon secretion has different effects in non-obese and obese patients with T2DM. In non-obese patients with T2DM, basal glucagon levels are high or relatively normal [16,17], but postprandial glucagon levels are high [17]. Glucagon levels are believed to increase because fasting hyperglycaemia impairs glucagon secretion suppression, which is mediated by blood glucose levels [18]. Additionally, some studies reported that high blood glucose directly stimulates the secretion of glucagon [19]. Postprandial glucagon secretion is mainly regulated by insulin [20]. The main evidence for this is that, after consuming mixed meals, glucagon secretion is inhibited in individuals with normal insulin secretion, while the glucagon level increases in individuals whose postprandial insulin level does not increase, such as patients with type 1 diabetes mellitus [21]. In obese patients with T2DM, basal glucagon levels are higher [17], and

the increase in bread meal-stimulated glucagon levels are more significant [17] than in non-obese patients with T2DM. Furthermore, the basal and postprandial glucagon levels are higher than in non-obese patients with T2DM [16,17,22,23]. Other studies suggested that the combination of T2DM and obesity is not related with the basal and postprandial glucagon levels observed in non-obese patients with T2DM [10]. During the treatment of patients with newly diagnosed T2DM (nd-T2DM), a variety of drugs can improve insulin secretion and basal and postprandial insulin levels [24,25]. Direct insulin therapy for patients with T2DM can not only directly improve insulin deficiency but also decrease glucagon levels; however, it cannot restore glucagon levels to normal levels [26-28]. It is still unclear whether insulin affects glucagon levels in non-obese and obese nd-T2DM patients. Therefore, this study investigated the effects of insulin therapy on glucagon levels in non-obese and obese nd-T2DM patients.

## Materials and Methods

### General data

A total of 93 nd-T2DM patients hospitalized in the Department of Endocrinology in the Third Affiliated Hospital of Anhui Medical University between February 2012 and November 2015 were selected; they had not received any medications. T2DM was confirmed for all patients according to the diagnostic and typing criteria for diabetes proposed by the World Health Organization (WHO) in 1999. Body Mass Index (BMI) was defined as weight (kg)/height (m<sup>2</sup>). According to the standards of obesity defined by the WHO guidelines for the Asian Pacific population (WHO/IASO/IOTF, 2000), these patients were divided into two groups: (1) non-obese T2DM group (BMI < 25 kg/m<sup>2</sup>), which included 45 patients aged 37-75 years, and (2) obese T2DM group (BMI ≥ 25 kg/m<sup>2</sup>), which included 48 patients aged 21-77 years. This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Anhui Medical University. Written informed consent was obtained from all participants. None of the patients had stress; cancer; autoimmune diseases; diabetic ketoacidosis; a hyperosmolar hyperglycaemic state; severe heart, liver, or lung diseases; or positive serum insulin antibodies and/or glutamic acid decarboxylase antibodies. Twelve age- and sex-matched healthy subjects were also included. General information about the study subjects is shown in Table 1.

### Treatment of patients

Height and body weight were measured in the morning for all subjects. The serum Total Cholesterol (TC), Triglyceride (TG), and Haemoglobin A1 (HbA1C) levels were determined after sampling venous blood. A 100 g bread meal test was then performed, after which venous blood was sampled to measure blood glucose, C-Peptide (CP), and glucagon levels at different times (after fasting (T0) and 30 min (T1), 60 min (T2), 120 min (T3), and 180 min (T4) after the bread meal test). After admission, Novo Rapid 30 (insulin as part 30 injection) or

glargine plus lispro insulin therapy was administered, and the dose was adjusted according to the blood glucose monitoring results, which were obtained using fingertip capillary blood samples. After glycaemic control was achieved (average treatment time, 13.5 ± 3.6 days), the 100 g bread meal test was performed after an overnight fast, and the indexes were measured again. This study applied individual glucose reducing programs; therefore, if some patients presented with symptoms of hypoglycaemia after achieving good blood glucose control, the blood glucose control range could be widened appropriately (≤ 8 mmol/l). Blood glucose was measured using the glucose oxidase method using venous blood samples and monitored using the Sure Step blood glucose meter (Johnson and Johnson, USA) for fingertip trace glucose. CP levels were detected using chemiluminescent immunoassay kits (Weifang 3V Bioengineering Group Co., Ltd., China). To measure glucagon, 2 ml venous blood was placed into a special anti-coagulated tube and stored at -18°C after the plasma separated. Then, plasma glucagon levels were measured using radioimmunoassay kits (Atom Hi-tech Co. Ltd., China).

### Statistical analysis

All data are expressed as mean ± Standard Deviation (SD). The Area Under the Curve (AUC) was calculated using the trapezoidal method, the independent samples t-test was used for intergroup comparisons, and the paired t-test was used for intragroup comparisons. P > 0.05 was considered statistically significant. We used SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) for statistical analysis.

## Results

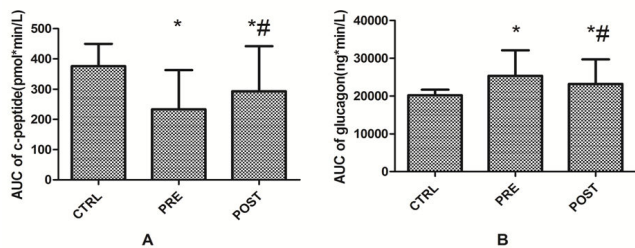
### Comparison of general conditions and related indexes before treatment

Except for age (P=0.936), the values for the remaining indexes in the T2DM group were all significantly higher than those in the control group (P=0.001). When comparing the non-obese T2DM group and control group, there were significant differences in HbA1C levels (P=0.001) and BMI (P=0.001), but no significant difference in age (P=0.585). The differences between the obese T2DM group and control group were also significant for HbA1C levels (P=0.001) and BMI (P=0.001), but not for age (P=0.718). When comparing the obese and non-obese T2DM groups, there were no significant differences in age (P=0.527) or HbA1C levels (P=0.432), but a significant difference in BMI (P=0.001) was observed. TC and TG levels in the obese and non-obese T2DM groups were significantly higher than in the control group (P=0.001, Table 1).

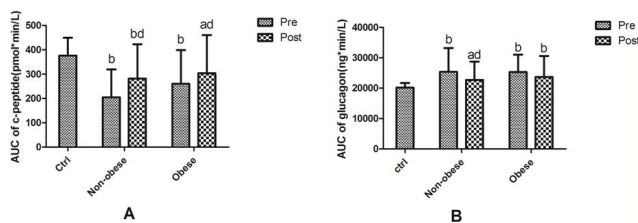
### Comparison of the area under the curve (AUC) of C-peptide and glucagon before and after treatment

Before treatment, the AUC<sub>Sum</sub> (Sum of the Area Under the Curve) of CP in T2DM patients was significantly lower than that in the control group (P=0.001). After treatment, the

AUC<sub>Sum</sub> of CP in T2DM patients was lower than that in the control group (P=0.004), but significantly higher than that before treatment (P=0.001, Figure 1A). Before treatment, the AUC<sub>Sum</sub> of glucagon in T2DM patients was higher than that in the control group (P=0.001). After treatment, the AUC<sub>Sum</sub> of glucagon in T2DM patients was significantly higher than that in the control group (P=0.001) and significantly lower than that before treatment (P=0.001, Figure 1B). The AUC<sub>Sum</sub> of CP before treatment in the non-obese T2DM group was significantly lower than that in the control group (P=0.001). After treatment, the AUC<sub>Sum</sub> of CP was lower than that in the control group (P=0.003), but significantly higher than that before treatment (P=0.001). Pre-treatment, the AUC<sub>Sum</sub> of CP in the obese T2DM group was significantly lower than that in the control group (P=0.007). Post-treatment, the AUC<sub>Sum</sub> of CP was still lower than that in the control group (P=0.025), but significantly higher than that pre-treatment (P=0.001, Figure 2A). Before treatment, the AUC<sub>Sum</sub> of glucagon in the non-obese (P=0.001) and obese (P=0.001) T2DM groups were significantly higher than that in the control group. After treatment, the AUC<sub>Sum</sub> of glucagon in the non-obese T2DM group remained significantly higher than that in the control group (P=0.016), but decreased significantly compared to that before treatment (P=0.004). After treatment, the AUC<sub>Sum</sub> of glucagon in the obese T2DM group remained significantly higher than that in the control group (P=0.002) and was not significantly different compared to that before treatment (P=0.05, Figure 2B).



**Figure 1.** AUC<sub>Sum</sub> of CP and glucagon in T2DM patients before and after the treatment, vs. control group \*P<0.01 vs. before the treatment #P<0.01.



**Figure 2.** AUC<sub>Sum</sub> of CP and glucagon in T2DM patients after bread meal test before and after the treatment. Compared with the control group before and after the treatment, a: P<0.05, b: P<0.01; comparison within the experimental groups before and after the treatment, c: P<0.05, d: P<0.01.

### Comparison of blood glucose, C-peptide, and glucagon levels at different times before and after treatment

Before treatment, the blood glucose levels in the non-obese and obese T2DM groups were significantly different from that in the control group at different times (P=0.001), but the differences between the non-obese and obese T2DM groups were not significant (P (T0)=0.713, P (T1)=0.968, P (T2)=0.580, P (T3)=0.197, P (T4)=0.263; Table 2). Before treatment, the CP levels in the non-obese T2DM group showed significant differences when compared with the control group at T1 (P=0.001), T2 (P=0.001), and T3 (P=0.001). The CP levels in the obese T2DM group were also significantly different from those in the control group at T1 (P=0.001), T2 (P=0.001), and T3 (P=0.040, Table 3). The glucagon levels in the non-obese and obese T2DM groups before treatment showed significant differences when compared with the control group at T1 (P=0.001, 0.001, respectively), T2 (P=0.001, 0.001, respectively), T3 (P=0.001, 0.001, respectively), and T4 (P=0.004, 0.002, respectively), but not at T0 (P=0.613, 0.087, respectively); however, the differences between the non-obese and obese T2DM groups were not significant (P (T0)=0.382 P (T1)=0.861, P (T2)=0.642, P (T3)=0.575, and P (T4)=0.680; Table 4).

After treatment, the blood glucose levels in the non-obese and obese T2DM groups were still significantly higher than that in the control group (P=0.001), but had significantly decreased compared to that before treatment (P=0.001, Table 2). The CP levels after treatment in the non-obese T2DM group were significantly lower than those in the control group at T1 (P=0.001), T2 (P=0.001), and T4 (P=0.006), but had significantly increased at T1 (P=0.003), T2 (P=0.001), T3 (P=0.001), and T4 (P=0.001) compared with that before treatment. The CP levels after treatment in the obese T2DM group were significantly lower than those in the control group at T1 (P=0.001), T2 (P=0.001), and T4 (P=0.007), but had significantly increased at T3 (P=0.001) and T4 (P=0.001, Table 3) compared with that before treatment. The glucagon levels after treatment in the non-obese T2DM group were significantly higher than those in the control group at T1 (P=0.031), T2 (P=0.001), and T3 (P=0.016). The glucagon levels after treatment in the non-obese T2DM group had significantly decreased at T1 (P=0.007), T2 (P=0.009), T3 (P=0.010), and T4 (P=0.024) compared with that before treatment. The glucagon levels after treatment in the obese T2DM group were significantly higher than those in the control group at T1 (P=0.001), T2 (P=0.001), and T3 (P=0.009). The glucagon levels in the obese T2DM group after treatment also had significantly decreased at T4 (P=0.022) compared with that before treatment (Table 4).

**Table 1.** Comparison of general conditions and biochemical indexes ( $\bar{x} \pm s$ ).

Index	Control	T2DM	P	Obese T2DM	P	Non-obese T2DM	P
Age (years)	54.85 ± 11.62	54.95 ± 11.53	0.936a	54.45 ± 12.24	0.718 <sup>a</sup>	55.50 ± 10.78	0.585 <sup>a</sup> 0.527 <sup>b</sup>
Gender (M/F)	12 (7/5)	93 (59/34)	-	48 (32/16)	-	45 (27/18)	-
BMI (kg/m <sup>2</sup> )	22.25 ± 1.65	24.45 ± 3.08 <sup>a</sup>	0.001 <sup>a</sup>	26.82 ± 1.78 <sup>a</sup>	0.001 <sup>a</sup>	21.80 ± 1.77 <sup>b</sup>	0.001 <sup>a</sup> 0.001 <sup>b</sup>
TG (mmol/l)	1.23 ± 0.56	2.47 ± 2.11 <sup>a</sup>	0.001 <sup>a</sup>	2.57 ± 1.80 <sup>a</sup>	0.001 <sup>a</sup>	2.28 ± 2.37 <sup>a</sup>	0.001 <sup>a</sup> 0.381 <sup>b</sup>
TC (mmol/l)	4.6 ± 1.03	5.06 ± 1.30 <sup>a</sup>	0.001 <sup>a</sup>	5.21 ± 1.44 <sup>a</sup>	0.001 <sup>a</sup>	4.93 ± 1.13 <sup>a</sup>	0.001 <sup>a</sup> 0.355 <sup>b</sup>
HbA1C (%)	4.95 ± 0.67	10.07 ± 2.57 <sup>a</sup>	0.001 <sup>a</sup>	10.30 ± 2.57 <sup>a</sup>	0.001 <sup>a</sup>	10.44 ± 2.55 <sup>a</sup>	0.001 <sup>a</sup> 0.432 <sup>b</sup>

Note: Comparison between the obese T2DM group and the control group: <sup>a</sup>P<0.01, comparison between the obese T2DM group and the non-obese T2DM group: <sup>b</sup>P<0.01.

**Table 2.** Blood glucose at different time points before and after the treatment.

		Control	Non-obese T2DM		Obese T2DM	
			Before	After	Before	After
Blood glucose	0	4.71 ± 0.30	9.91 ± 2.58 <sup>b</sup>	7.31 ± 1.03 <sup>bd</sup>	9.72 ± 2.50 <sup>b</sup>	7.10 ± 1.18 <sup>bd</sup>
			P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.001	P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.001
	30	5.85 ± 0.28	13.27 ± 3.00 <sup>b</sup>	10.13 ± 1.83 <sup>bd</sup>	13.25 ± 3.04 <sup>b</sup>	9.80 ± 2.08 <sup>bd</sup>
			P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.001	P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.001
	60	7.07 ± 0.22	17.45 ± 3.77 <sup>b</sup>	13.59 ± 2.48 <sup>bd</sup>	17.03 ± 3.59 <sup>b</sup>	12.89 ± 2.58 <sup>bd</sup>
			P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.001	P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.001
120	6.00 ± 0.31	19.85 ± 4.55 <sup>b</sup>	15.44 ± 3.19 <sup>bd</sup>	18.64 ± 4.44 <sup>b</sup>	14.14 ± 3.21 <sup>bd</sup>	
		P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.001	P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.001	
180	4.87 ± 0.32	18.03 ± 5.22 <sup>b</sup>	14.34 ± 3.76 <sup>bd</sup>	16.84 ± 4.99 <sup>b</sup>	12.01 ± 3.66 <sup>bd</sup>	
		P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.001	P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.001	

Note: Compared with the control group before and after the treatment, <sup>a</sup>P<0.05, <sup>b</sup>P<0.01; comparison within the experimental groups before and after the treatment, <sup>c</sup>P<0.05, <sup>d</sup>P<0.01.

**Table 3.** CP at different time points before and after the treatment.

		Control	Non-obese T2DM		Obese T2DM	
			Before	After	Before	After
CP	0	0.61 ± 0.12	0.52 ± 0.27	0.66 ± 0.30	0.68 ± 0.33	0.56 ± 0.31
	30	1.80 ± 0.32	0.73 ± 0.37 <sup>b</sup>	1.00 ± 0.47 <sup>bd</sup>	0.95 ± 0.47 <sup>b</sup>	0.87 ± 0.46 <sup>b</sup>
			P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.003	P <sup>b</sup> =0.001	P <sup>b</sup> =0.001
	60	2.66 ± 0.59	1.01 ± 0.54 <sup>b</sup>	1.46 ± 0.70 <sup>bd</sup>	1.33 ± 0.78 <sup>b</sup>	1.37 ± 0.71 <sup>b</sup>
		P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.001	P <sup>b</sup> =0.001	P <sup>b</sup> =0.001	
120	2.42 ± 0.58	1.46 ± 0.91 <sup>b</sup>	2.22 ± 1.30 <sup>d</sup>	1.78 ± 1.00 <sup>a</sup>	2.02 ± 1.10 <sup>d</sup>	
		P <sup>b</sup> =0.001	P <sup>d</sup> =0.001	P <sup>a</sup> =0.040	P <sup>d</sup> =0.001	

180	1.60 ± 0.36	1.42 ± 0.82	2.18 ± 1.24 <sup>bd</sup>	1.82 ± 1.42	2.14 ± 1.06 <sup>bd</sup>
		N.S.	P <sup>b</sup> =0.006, P <sup>d</sup> =0.001	N.S.	P <sup>b</sup> =0.007, P <sup>d</sup> =0.001

Note: Compared with the control group before and after the treatment, <sup>a</sup>P<0.05, <sup>b</sup>P<0.01; comparison within the experimental groups before and after the treatment, <sup>c</sup>P<0.05, <sup>d</sup>P<0.01. N.S: No Significance

**Table 4.** Glucagon at different time points before and after the treatment.

		Control	Non-obese T2DM		Obese T2DM	
			Before	After	Before	After
Glucagon	0	108.52 ± 9.19	111.46 ± 34.49	101.48 ± 28.90	117.33 ± 29.90	110.60 ± 36.22
	30	118.97 ± 9.72	151.88 ± 54.65 <sup>b</sup>	131.93 ± 34.53 <sup>ad</sup>	153.61 ± 40.02 <sup>b</sup>	142.36 ± 39.82 <sup>b</sup>
			P <sup>b</sup> =0.001	P <sup>a</sup> =0.031, P <sup>d</sup> =0.007	P <sup>b</sup> =0.001	P <sup>b</sup> =0.001
	60	118.46 ± 9.43	155.89 ± 47.62 <sup>b</sup>	140.52 ± 37.16 <sup>bd</sup>	160.09 ± 39.22 <sup>b</sup>	150.89 ± 49.78 <sup>b</sup>
			P <sup>b</sup> =0.001	P <sup>b</sup> =0.001, P <sup>d</sup> =0.009	P <sup>b</sup> =0.001	P <sup>b</sup> =0.001
	120	110.32 ± 7.68	141.58 ± 50.67 <sup>b</sup>	126.34 ± 40.53 <sup>ac</sup>	136.39 ± 37.61 <sup>b</sup>	128.67 ± 44.22 <sup>b</sup>
			P <sup>b</sup> =0.004	P <sup>a</sup> =0.016, P <sup>c</sup> =0.010	P <sup>b</sup> =0.001	P <sup>b</sup> =0.009
	180	101.63 ± 7.33	120.54 ± 39.32 <sup>b</sup>	109.43 ± 32.91 <sup>c</sup>	117.55 ± 29.86 <sup>b</sup>	107.51 ± 32.34 <sup>c</sup>
			P <sup>b</sup> =0.001	P <sup>c</sup> =0.024	P <sup>b</sup> =0.002	P <sup>c</sup> =0.022

Note: Compared with the control group before and after the treatment, <sup>a</sup>P<0.05, <sup>b</sup>P<0.01; comparison within the experimental groups before and after the treatment, <sup>c</sup>P<0.05, <sup>d</sup>P<0.01.

## Discussion

This study mainly investigated the effects of insulin therapy on glucagon levels in non-obese and obese T2DM patients. In most patients with T2DM, two metabolic defects can occur: insulin resistance and/or insulin secretion deficiency. Insulin deficiency can reduce the inhibition of glucagon. Previous studies [29-31] showed that endogenous insulin may act directly on the insulin receptors of  $\alpha$ -cell membranes and inhibit the secretion of glucagon through the PI 3-kinase/Akt signalling pathway. Alternatively, it may indirectly reduce the sensitivity of the  $\alpha$ -cell KATP channels [32] and strengthen the  $\gamma$ -amino butyric acid pathway [30] to inhibit glucagon secretion. In the present study, the overall glucagon levels in the T2DM group were higher than those in the control group, which was consistent with the results of previous studies [33]. However, there were no differences in basal glucagon levels among the patients with T2DM. This may be related with the lack of significant differences in the basal insulin level, compared with the control group. Patients with T2DM who have better blood glucose control have small variations in fasting glucagon levels, but those with poor blood glucose control or ketosis have significantly higher basal glucagon levels than healthy people [7,34]. The postprandial glucagon levels in the patients with T2DM were higher than that in the control group. Postprandial glucagon levels tend to increase throughout the mixed meal test [16] or oral glucose tolerance test [4]. The increase in postprandial glucagon could be related with impaired insulin secretion, and the decrease in postprandial insulin secretion impairs the inhibitory effects on

glucagon [35-38]. In the present study, patients with T2DM had a deficiency in postprandial insulin secretion, supporting the findings of previous studies.

Insulin therapy is a shared method for treating T2DM; as observed in previous studies, the present study found that the glucagon levels decreased after insulin treatment in patients with T2DM [26,27]. The results suggest that insulin treatment could reduce glucagon levels in patients with T2DM. This phenomenon is related with the direct and indirect actions of endogenous insulin on the islet  $\alpha$ -cells. The administration of exogenous insulin prevents the inhibitory action of endogenous insulin on glucagon secretion by  $\alpha$ -cells. Similar to previous studies, in the present study, after treatment using exogenous insulin, endogenous insulin secretion was restored to some extent, and this recovery was reflected by the elevated level of CP [39,40]. The recovery of endogenous insulin may enhance the inhibition of glucagon secretion via the PI 3-kinase/Akt and  $\gamma$ -amino butyric acid pathways, as already discussed. Certain studies [32,41] also reported that after treatment using exogenous insulin, insulin secreted by *in vivo*  $\beta$ -cells increased, and this increase may cause the secretion of Zn<sup>2+</sup>, which has inhibitory effects on glucagon.

The relationship between glucagon and obesity is a popular research topic. However, consensus regarding this relationship has not yet been reached. An increase in glucagon levels might be associated with obesity-related insulin resistance [42,43]. In addition, obese people with normal glucose tolerance have higher glucagon levels [22,44]. Changes in levels of leptin [45,46] and inflammatory cytokines [22] associated with

obesity-related insulin resistance could elevate glucagon levels. The increase in gastric inhibitory polypeptide levels in obese people with normal glucose tolerance and in patients with T2DM after an oral glucose tolerance test might be related with increased postprandial glucagon levels [17,34]. In obese patients with T2DM, findings regarding glucagon levels differ. In one study, the overall glucagon levels in obese patients with T2DM were higher than those in non-obese patients with T2DM [16]. In another study, there were no differences in the basal and post-mixed meal test glucagon levels between obese and non-obese individuals with T2DM [10]. In the present study, both non-obese and obese patients with T2DM had higher glucagon levels than the control group. The absolute basal and postprandial glucagon levels in the obese patients with T2DM at all the time intervals were slightly higher than those in the non-obese patients with T2DM, but the differences were not statistically significant. The results of the present study may be related with the similar degrees of obesity (BMI of 26.82 kg/m<sup>2</sup> vs. 21.80 kg/m<sup>2</sup>).

The present study further analysed the effects of insulin therapy on glucagon levels in non-obese and obese patients with T2DM. After treatment, the glucagon levels overall and at different postprandial time intervals in non-obese patients with T2DM decreased significantly; however, in the obese T2DM group, these levels did not decrease as much and were only significantly different at T4. This decrease in the non-obese patients with T2DM may be associated with the noticeable recovery of endogenous insulin in non-obese patients with T2DM. CP levels reflect endogenous insulin levels. After treatment, CP levels in non-obese patients with T2DM increased at all of the postprandial time intervals. The increased CP levels suggest that the functions of *in vivo*  $\beta$ -cells recovered, and glucose toxicity decreased to some extent. However, insulin therapy did not significantly reduce the glucagon level in obese patients with T2DM, which may be related with insufficient recovery of the endogenous insulin secretion [47]. In the present study, simple insulin therapy did not significantly improve endogenous insulin secretion, and the postprandial CP levels after treatment only showed statistically significant improvements at T3 and T4.

In conclusion, non-obese and obese nd-T2DM patients have lower insulin levels and higher glucagon levels than people without T2DM. Insulin therapy can decrease glucagon levels. A greater decrease in glucagon levels was observed in non-obese patients with T2DM after treatment than in obese patients with T2DM; the decrease was not statistically significant for the obese patients with T2DM. After treatment, CP levels increased. This increase mainly occurred in non-obese patients with T2DM, and the changes for obese patients with T2DM were not statistically significant. New treatments or programs that decrease glucagon levels and increase CP levels in obese patients with T2DM need to be investigated further.

## Acknowledgements

Special thanks should be given to the Third Affiliated Hospital of Anhui Medical University.

## Conflicts of Interest

All of the authors declare that they have no conflicts of interest regarding this paper.

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