



Risk Factors of Ketosis in Obese Ketosis-Prone Diabetic Patients: A Case-Control Study

Li Shi · Liang Zhou · Juan Liu · Yang Ding · Xin-hua Ye ·
Jin-luo Cheng

Received: January 8, 2020 / Published online: March 13, 2020
© The Author(s) 2020

ABSTRACT

Introduction: Different types of ketosis-prone obese diabetic patients are seen in the clinic. At present, the mechanism responsible for ketosis onset in these patients remains unclear, and we do not know how these patients should be optimally treated to prevent recurrent ketosis. Therefore, this study aims to investigate risk factors of ketosis in obese ketosis-prone diabetic (OB-KPD) patients.

Methods: In an observational case-control study, primary OB-KPD patients [body mass index (BMI) ≥ 28 kg/m²] were selected as the

study group (OB-KPD group), and primary obese type 2 diabetes patients served as the control group (OB-T2DM group). Clinical diagnostic assessments of fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), blood lipid, area under curve of serum C-peptide (AUC_{C-P}) after steamed bread meal, insulin sensitivity index (ISI), β -hydroxybutyric acid (β -HB) and free fatty acid (FFA) values of the subjects were collected. Subjects in the OB-KPD group were followed up for 1 year to determine the likelihood of insulin therapy cessation and whether ketosis recurred by assessing clinical chemistry parameters at 1-year follow-up.

Results: Seventy-five subjects were screened, of which 15 were not included in the study for several identified clinical reasons. On enrollment, the OB-KPD group displayed significantly higher FPG, HbA1c and FFA levels than the OB-T2DM group ($p < 0.01$), while AUC_{C-P} and ISI values were significantly lower than in the OB-T2DM group ($p < 0.01$ and $p = 0.03$). Statistical analysis showed that increases in β -HB in the OB-KPD group were associated with increased blood glucose and FFA and decreased AUC_{C-P} and ISI values. Furthermore, decreases in AUC_{C-P} were closely associated with increased blood glucose values.

Conclusion: The occurrence of ketosis in ketosis-prone obese diabetic patients may be related to glucose and lipid metabolism disorders, increased insulin resistance and decreased β -cell secretory functions.

Enhanced Digital Features To view enhanced digital features for this article go to: <https://doi.org/10.6084/m9.figshare.11932020>.

L. Shi (✉) · L. Zhou · Y. Ding · X. Ye · J. Cheng (✉)
Department of Endocrinology, Changzhou No. 2
People's Hospital Affiliated to Nanjing Medical
University, Changzhou 213164, Jiangsu, China
e-mail: shili1204@sina.com

J. Cheng
e-mail: chengjinluo567@sina.com

L. Shi
Department of Clinical Nutrition, Changzhou No. 2
People's Hospital Affiliated to Nanjing Medical
University, Changzhou 213164, Jiangsu, China

J. Liu
Changzhou Diabetes Institute, Changzhou 213164,
Jiangsu, China

Trial Registration: This work was registered at the Chinese Clinical Trial Registry with trial registration identifier no. ChiCTR1900025909.

Keywords: Free fatty acids; Insulin sensitivity index; Ketosis-prone diabetes; Obesity

Key Summary Points

Why carry out this study?

Different types of ketosis-prone obese diabetic patients are seen in the clinic. At present, the mechanism responsible for ketosis onset in these patients remains unclear.

We do not know how these patients should be optimally treated to prevent recurrent ketosis.

What was learned from the study?

The occurrence of ketosis in ketosis-prone obese diabetic patients may be related to glucose and lipid metabolism disorders, increased insulin resistance and decreased islet β -cell secretory functions.

We recommend that treatment of obese ketosis-prone diabetes should be focused on the control of blood glucose, blood lipid and body weight values after correcting for ketosis, with the intention of preventing the recurrence of ketosis.

that of type 1 diabetes, which is manifested as ketosis or ketoacidosis, requiring insulin treatment.

Furthermore, most patients are relieved of symptomatology after insulin treatment and after achieving blood glucose control on discontinuation of insulin therapy, at which time their islet autoantibody levels are confirmed to be negative. According to the ADA diabetes diagnosis and classification criteria of 1997, these patients are obese and have negative islet autoantibody levels, being quite different from typical type 1 diabetes. They also have a tendency to present with spontaneous ketosis and have symptoms different from typical type 2 diabetes; therefore, some international investigators have named it atypical ketosis-prone diabetes (AKPD) or Flatbush diabetes [2].

Since these patients are experiencing diabetes for the first time, including ketosis requiring insulin treatment, these patients are worried that they may need long-term insulin treatment in the future, like their type 1 diabetic patient counterparts, so they are often afraid and anxious.

At present, the mechanism responsible for ketosis onset in OB-KPD patients remains unclear. Therefore, some important questions that concern us include the following: What are the differences in metabolic indices between obese ketosis-prone diabetic patients and patients with obese type 2 diabetes? What is the cause of severe impairment of their islet function? How can these patients be optimally treated?

At present, research in this field in China and by international centers abroad comprises mainly clinical cross-sectional and descriptive studies, and in most cases, the measurement of blood glucose, insulin, C-peptide and other clinical indices is used to evaluate the level of insulin resistance [3, 4]. However, the HOMA-IR index, which is calculated by fasting blood glucose and insulin levels, might better reflect insulin resistance of the liver, and these obese diabetic patients with ketosis are all treated with insulin. Thus, the HOMA-IR index may not accurately reflect the systemic insulin resistance level of OB-KPD patients. The recognized gold standard for the evaluation of insulin resistance

INTRODUCTION

Obese individuals are at increased risk of developing type 2 diabetes because of the accumulation of body fat, resulting in insulin resistance and hyperinsulinemia, and the decreased use of glucose by muscles and other tissues [1]. However, different types of obese diabetic patients have been seen in the clinics in recent years, mostly young and middle-aged men. Most have acute onset without obvious incentives, and their disease onset is similar to

in China and by international standards is the hyperinsulinemic euglycemic clamp technique, which has the advantages of accurate quantification and good repeatability. However, due to the complexity, high cost, time required and high demands placed on the operators of the method, there are few groups that have developed this technology in China.

Therefore, 40 obese diabetic patients of the Han nationality that first manifested as diabetes with spontaneous ketosis in our hospital from March 2016 to September 2018 were selected for this study. The secretory function of islet β -cells, level of insulin resistance, metabolism of glucose and lipids, and treatment with insulin in the early stages of the condition, and 12 months after treatment, were observed. At the same time, 20 newly presenting obese type 2 diabetic patients that visited our hospital over the same period were selected as controls with the aim of determining risk factors of ketosis onset and the related factors that affect the function of islet β -cells in OB-KPD patients of Han ethnicity.

METHODS

Study Participants

Patients with diabetes who were 18–70 years old and visited our hospital from March 2016 to September 2018 were recruited. All patients were recruited in concordance with the 1997 ADA diagnostic criteria for diabetes [5]. Our work was approved by the local ethics committee of the Changzhou No. 2 People's Hospital Affiliated with Nanjing Medical University with approval no. [2016] yk021-01. Our study was performed in accordance with the Helsinki Declaration of 1964 and its later amendments. All patients signed informed consents before enrollment.

Grouping and Inclusion Criteria

OB-KPD Group

Inclusion criteria: (1) diabetes at first onset; (2) urine ketone $2+$ (80 mg/dl) and/or an arterial

blood gas $\text{pH} \leq 7.35$; (3) $\text{BMI} \geq 28 \text{ kg/m}^2$; (4) measurements of glutamic acid decarboxylase antibodies (GAD-Ab), insulin autoantibodies (IAA) and islet cell antibodies (ICA) giving a negative result.

OB-T2DM Group

Inclusion criteria: (1) diabetes at first onset; (2) GAD Ab, IAA and ICA results all being negative; (3) fasting serum C peptide $\geq 1.1 \text{ ng/ml}$; (4) $\text{BMI} \geq 28 \text{ kg/m}^2$; (5) negative urine ketone levels. Exclusion criteria: (1) patients with stressful conditions such as infection, a surgical procedure and trauma, or pregnant patients; (2) patients taking medicine (such as glucocorticoids) that affects glucose and lipid metabolism within 1 month of initiating therapy; (3) patients presenting with ischemic cardio-cerebrovascular disease or acute/chronic cardiac insufficiency; (4) presentation of a special type of diabetes mellitus such as increased blood glucose resulting from exocrine pancreatic disease or other endocrine diseases; (5) serious liver and kidney diseases [glutamic-pyruvic transaminase (GPT) levels that were 2.5-fold higher than the upper limit of the normal reference value and a serum creatinine level $\geq 124 \mu\text{mol/l}$].

Research Methods

This study was an observational case-control study. The normal treatment of subjects was not disrupted or altered during the study. The clinical information of the selected subjects was collected, including medical history, height, weight and blood pressure. On the 2nd day post-admission, fasting venous blood was collected to measure the basic state levels of blood glucose, HbA1c, C peptide, liver and kidney function, blood lipids, FFA, β -HB, islet autoantibody GAD-Ab, IAA and ICA.

The standard steamed bread meal test was used to determine the secretory function of islet β -cells. The steps were as follows: patients fasted starting at 8:00 p.m. on the day before the test, fasting blood samples were collected from patients at 8:00 a.m. on the test day, and steamed bread made of 100 g flour (equivalent

to 75 g of glucose) was eaten. Starting with the first bite and finishing within 15 min, venous blood was collected at 30, 60, 120 and 180 min, respectively. The levels of blood glucose (GLU) and C-peptide (C-P) were detected. The test time of subjects in the OB-KPD group after the elimination of ketosis was confirmed.

$$\text{AUC}_{\text{GLU}} = (\text{GLU}_{0 \text{ min}} + \text{GLU}_{180 \text{ min}})/2 + \text{GLU}_{30 \text{ min}} + \text{GLU}_{60 \text{ min}} + \text{GLU}_{120 \text{ min}},$$

$$\text{AUC}_{\text{C-P}} = (\text{C-P}_{0 \text{ min}} + \text{C-P}_{180 \text{ min}})/2 + \text{C-P}_{30 \text{ min}} + \text{C-P}_{60 \text{ min}} + \text{C-P}_{120 \text{ min}}.$$

The criteria for the absence of islet β -cell function were: fasting serum C-peptide < 1 ng/ml and a peak serum C-peptide < 1.5 ng/ml [6].

The hyperinsulinemic euglycemic clamp test was performed to detect the insulin sensitivity of the recruited subjects. The test time was on the 2nd day of the standard steamed bread meal test. The clamp system software of the EKF Co. and a Biosen glucose analyzer were used for this experiment. The subjects in the OB-KPD and OB-T2DM groups were fasted for 10 h, height and weight were measured at 8:00 a.m., and then subjects were placed in the supine position after defecation. A catheter with 0.9% NaCl solution was placed in the cephalic vein or median vein of both forearms to maintain a venous channel for blood sampling and infusion of various test fluids. The forearm for sampling the blood was placed in the thermostat (set at 50 °C) to ensure the arterialization of venous blood. In the first 10 min of clamping, the infusion of human insulin solution (normal human insulin from the Novo Co., Denmark, at 40 U/ml) at a constant dose of 4 uIU/(kg⁻¹ · min⁻¹) was performed to rapidly increase the level of plasma insulin. The infusion was continued at a rate of 2 uIU/(kg⁻¹ · min⁻¹) for 140 min. During this period, the arterialization of venous blood glucose was measured every 5 min, and an infusion rate of 20% glucose was adjusted to make the clamp blood glucose value close to the normal fasting blood glucose value of studied subjects, which was usually set at 5.0 mmol/l. The adjusted time was recorded. Blood samples were collected

every 20 min to detect the plasma insulin concentration. The glucose utilization *M* value in the last 30 min was calculated.

$$\text{Insulin sensitivity index (ISI)} : [\text{M value} (\text{mg}/(\text{kg}^{-1} \cdot \text{min}^{-1}))/\text{insulin concentration} (\text{mU/L})] \times 100.$$

Treatment of hypoglycemia and lipid-lowering in the OB-KPD group took place during the follow-up period. Subjects in the OB-KPD group were hospitalized for 7–10 days and were treated with intravenous fluids, electrolytes and insulin therapy. At discharge, their treatment was a basal-bolus regimen (insulin glargine once daily and prandial pre-meal insulin aspart). If blood lipid levels were high, statins or fibrates were prescribed. After discharge, subjects were mainly treated by outpatient physicians from the Department of Endocrinology and community doctors within 1 year of being enrolled in the group. The insulin dose was adjusted according to the algorithm shown in Fig. 1. Three months after discharge, if the insulin stepwise reduction process could not be continued, an oral hypoglycemic agent (OHA) could be used simultaneously; metformin is recommended as the OHA of first choice. If blood glucose targets were still not met, other OHAs could also be used in combination with metformin, such as acarbose, dipeptidyl peptidase-4 (DPP-4) inhibitor, thiazolidinedione and sulfonylureas.

Subjects were asked to self-monitor their blood glucose (SMBG) for at least 3 days a week and four times per day (before three meals and before bed). Capillary whole-blood glucose measurements were performed with ONETOUGH® UltraVue (LifeScan).

The follow-up assessment of subjects after 1 year in the OB-KPD group comprised: 1 year after entering the group, the subjects in the OB-KPD group were sent to our hospital for further consultation. Their weight was measured. The fasting blood value was obtained to detect blood glucose, blood lipid and FFA levels. The standard steamed bread meal test and hyperinsulinemia normal glucose clamp test were also performed.

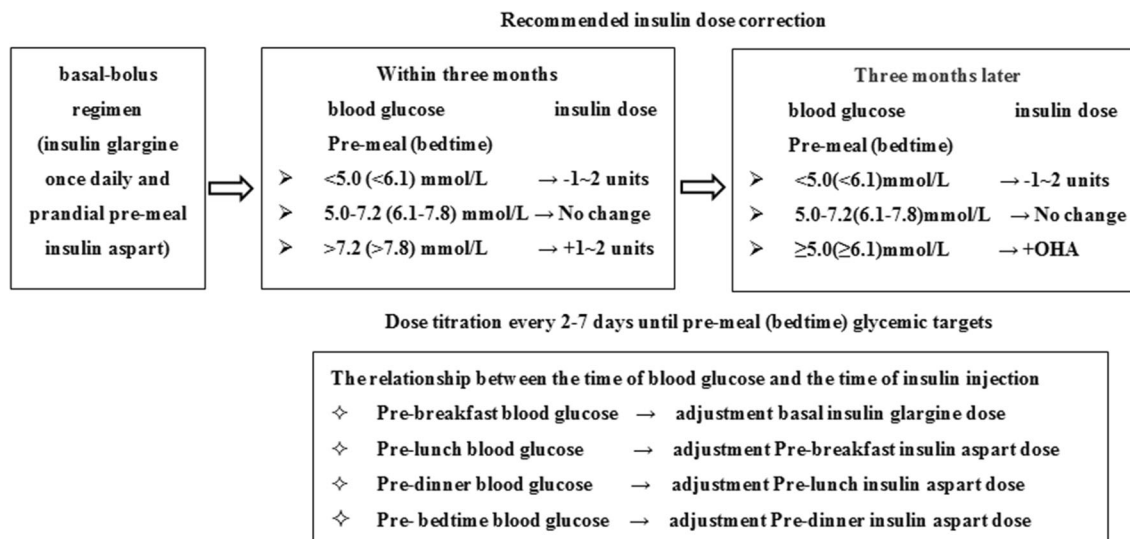


Fig. 1 A stepwise method for the introduction of basal-bolus insulin dose adjustment during follow-up. At discharge, the treatment is a basal bolus regimen (insulin glargine once daily and prandial pre-meal insulin aspart).

After discharge, the insulin dose is adjusted according to the SMBG results and the above algorithm

Standard for discontinuation of insulin [7]: If the blood glucose levels in the studied subjects remained acceptable, or if hypoglycemia occurred when the insulin treatment dose was very low (< 10 U/day), then under those circumstances insulin was discontinued and replaced by OHA or a simple diet treatment. In addition, no evidence of diabetic ketosis was seen 1 month after insulin was discontinued.

Statistical analysis: in this study, the data with a normal distribution were represented as mean ± standard deviation; an independent Student's *t* test was used to compare between groups; a paired Student's *t* test was used to compare the indicators at the time of enrollment with those 1 year later in the OB-KPD group. Non-normally distributed data were represented as the median and the upper and lower quartiles; the rank sum test of two independent samples was performed to compare differences between two groups; the rank sum test of paired comparisons was used for pre- and post-comparisons for intra-group differences. An alpha value of *p* < 0.05 was considered a statistical difference. Multiple Pearson's correlation and multiple stepwise analyses were used to analyze the correlation among variables. The

natural logarithm was calculated for data that were non-normally distributed, and a correlation regression analysis was performed after transformation into a normal distribution. The islet β-cell function and treatment effect were analyzed by the χ^2 test. All data were analyzed using SPSS statistical analysis software program, version 19.

RESULTS

Enrollment and follow-up of subjects

Fifty-two patients in the OB-KPD group and 23 patients in the OB-T2DM group were screened. Of them, 12 patients in the OB-KPD group were excluded (2 patients were positive for islet antibodies; 3 patients had a medical history of pancreatitis; 6 patients were in a state of infection or stress; 1 patient had a GPT level more than twice the upper limit of normal values). Forty patients in the OB-KPD group were finally selected. Three patients in the OB-T2DM group were excluded (one patient had taken drugs affecting glucose metabolism in the past 1 month, and two patients had GPT levels twice that of the upper limit of normal values).

Finally, 20 patients in the OB-T2DM group were selected.

During the 1-year follow-up period of the OB-KPD group, nine patients were lost to follow-up; two subjects continued insulin treatment and were reexamined by the standard steamed bread meal test, and the peak value never exceeded 1.5 ng/ml. Five patients had ketosis that recurred during the follow-up period after successful cessation of insulin therapy. Finally, we collected observation indices of the remaining 24 patients in the OB-KPD group 1 year later and compared them with indices at the time ketosis occurred 1 year before (Fig. 2).

Comparison of General Information Between the Two Groups

Comparison of general information between the two groups was completed. The results showed that the onset age for OB-KPD patients was significantly lower than that found for OB-T2DM patients, and there was no significant difference between the two groups in terms of

gender ratio, family history, blood pressure and BMI (Table 1).

Comparison of Blood Glucose, Blood Lipids and FFA Levels between the Two Groups

The levels of FPG, HbA1c, FFA and β -HB in the OB-KPD group were significantly higher than those found for the OB-T2DM group; there was no significant difference in the levels of total cholesterol (TCH), low density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) between the two groups (Table 2).

Comparison of Islet β -Cell Function and Insulin Sensitivity between the Two Groups at Enrollment

The area under the standard post-prandial blood glucose curve in the OB-KPD group was significantly higher than that found in the OB-

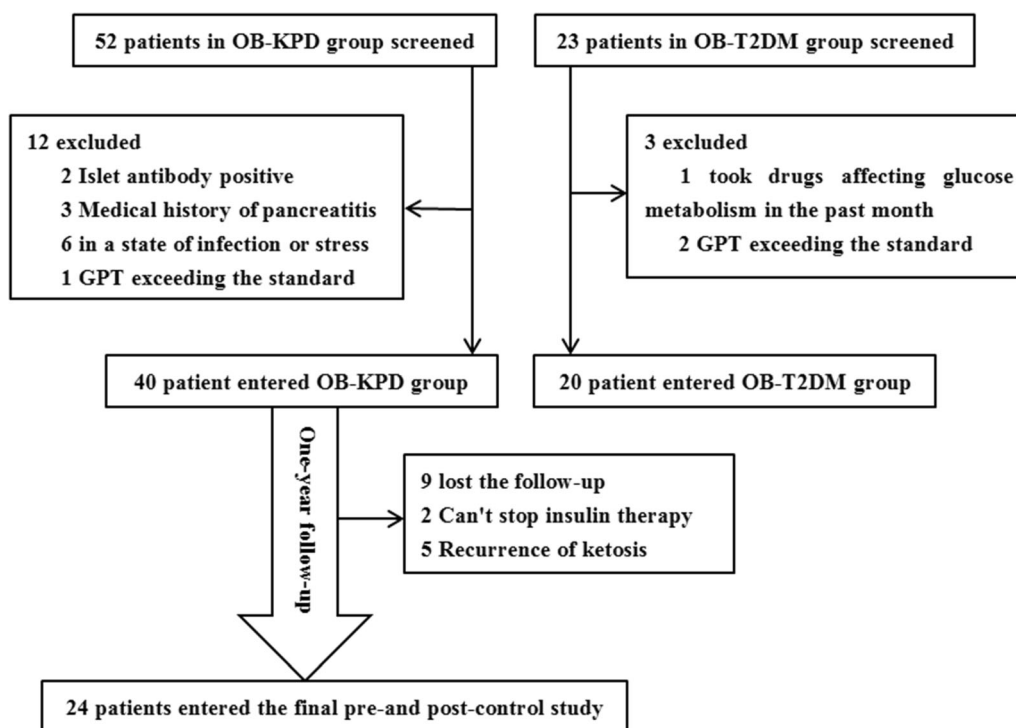


Fig. 2 Enrollment process and follow-up of study subjects

Table 1 Comparison of general information between the two groups

	OB-KPD	OB-T2DM	<i>T</i> or χ^2	<i>P</i>
Number of patients	40	20		
Age (years)	33.20 ± 9.12	40.50 ± 11.42	2.68	0.01
Gender (male/female)	34/6	13/7	3.14	0.10
Family history (yes/no)	23/17	14/6	0.88	0.41
Systolic pressure (mmHg)	134.82 ± 13.57	132.75 ± 18.69	0.49	0.63
Diastolic pressure (mmHg)	84.30 ± 11.36	86.65 ± 10.73	− 0.77	0.45
BMI(kg/m ²)	30.33 ± 2.00	31.02 ± 1.85	− 1.30	0.20

Table 2 Comparison of blood glucose, blood lipids and FFA between the two groups

	OB-KPD	OB-T2DM	<i>T</i> or <i>Z</i>	<i>P</i>
FPG (mmol/l)	12.05 ± 3.02	9.84 ± 1.65	3.67	0.00
HbA1c (%)	12.58 ± 1.95	9.33 ± 1.50	7.14	0.00
TCH (mmol/l)	5.22 ± 1.23	5.01 ± 0.72	0.84	0.40
TG (mmol/l)	2.64 (1.71, 3.69)	2.11(1.55, 4.61)	− 0.59	0.56
LDL-C (mmol/l)	2.97 ± 0.99	3.02 ± 0.64	− 0.17	0.86
HDL-C (mmol/l)	0.94 ± 0.25	1.05 ± 0.32	− 1.49	0.14
FFA (umol/l)	1156.18 ± 371.88	649.38 ± 149.92	7.49	0.00
β-HB (umol/l)	1700.58 ± 1379.52	250.65 ± 111.37	6.60	0.00

T2DM group ($P = 0.00$); AUC_{C-P} was significantly lower than that found in the OB-T2DM group ($P = 0.00$); the insulin sensitivity index was significantly lower than that found for the OB-T2DM group ($P = 0.03$; Table 3).

Analysis of Observational Indices in the OB-KPD Group 1 Year Post-Follow-Up

To determine the factors associated with the occurrence of ketosis in the OB-KPD group, at the 1-year follow-up period, we measured the levels of blood glucose, blood lipids, FFA, islet β-cell function and ISI of 24 subjects when insulin

Table 3 Comparison of islet β-cell function and insulin sensitivity between the two groups at enrollment

	OB-KPD	OB-T2DM	<i>T</i>	<i>P</i>
AUC _{GLU}	80.44 ± 14.72	72.35 ± 6.29	2.98	0.00
AUC _{C-P}	9.06 ± 4.09	20.99 ± 11.14	− 4.64	0.00
ISI	2.43 ± 0.68	2.86 ± 0.72	− 2.24	0.03

Table 4 Comparison of the relevant indicators in the OB-KPD group at the time of enrollment and 1 year later

	At enrollment	One year later	<i>T</i> (or <i>Z</i>)	<i>P</i>
FPG (mmol/l)	11.97 ± 3.01	6.88 ± 1.66	10.08	0.00
HbA1c (%)	12.75 ± 1.87	7.09 ± 1.04	13.16	0.00
AUC _{GLU}	81.14 ± 15.47	54.31 ± 10.77	8.55	0.00
TCH (mmol/l)	5.26 ± 1.36	4.47 ± 0.93	2.56	0.02
TG (mmol/l)	2.46 (1.75, 3.81)	1.99 (1.29, 2.34)	− 2.40	0.02
LDL-C (mmol/l)	2.86 ± 1.14	2.62 ± 0.73	0.90	0.38
HDL-C (mmol/l)	0.92 ± 0.22	1.01 ± 0.15	− 2.55	0.02
FFA (umol/l)	1171.08 ± 425.77	950.80 ± 261.13	3.36	0.00
AUC _{C-P}	7.95 ± 2.79	16.38 ± 7.65	− 5.43	0.00
ISI	2.39 ± 0.73	2.93 ± 0.55	− 4.30	0.00
Body weight (kg)	92.29 ± 10.02	90.46 ± 8.46	1.86	0.08

therapy was discontinued in the absence of recurrent ketosis.

As shown in Table 4, after a 1-year follow-up period, the levels of FPG, HbA1c, AUC_{GLU}, TCH, TG and FFA for the studied subjects were significantly lower than was determined at enrollment; moreover, HDL-C, AUC_{C-P} and ISI values were also significantly higher than had been determined at enrollment. There were no significant differences related to LDL-C levels and body weight.

Analysis of Factors Related to Ketosis Occurrence in the OB-KPD Group

To explore the reasons for the occurrence of ketosis in the OB-KPD group, correlation analysis between β-HB levels at enrollment and

levels of HbA1c, TCH, TG, HDL-C, FFA, AUC_{C-P} and ISI, which changed significantly in the time between enrollment and follow-up 1 year later, were analyzed. TG was converted to a normal distribution by taking the natural logarithm after pulsing 1. Results are shown in Table 5. It was found that β-HB was positively and statistically significantly correlated with HbA1c, TG and FFA and negatively correlated with AUC_{C-P} and ISI. No significant correlation was found with TCH and HDL-C. Subsequently, we performed a stepwise regression analysis of HbA1c, TG, FFA, AUC_{C-P} and ISI as they were related to β-HB. The results showed that β-HB synthesis might be associated with an increase in blood glucose and FFA and a decrease in islet secretion and insulin sensitivity. The regression equation

Table 5 Correlation analysis of β-HB

	HbA1c	TCH	TG	HDL-C	FFA	AUC _{C-P}	ISI
β-HB							
<i>r</i>	0.71	0.28	0.62	0.1	0.69	− 0.64	− 0.60
<i>p</i>	0	0.08	0	0.54	0	0	0

Table 6 Correlation analysis of AUC_{C-P} with HbA1c, TG, FFA and ISI

	HbA1c	TG	FFA	ISI
AUC_{C-P}				
<i>r</i>	− 0.61	− 0.37	− 0.48	− 0.26
<i>p</i>	0	0.02	0	0.11

$$\text{was } \beta\text{-HB} = 258.66 + 167.14 \text{ HbA1c} + 1.26 \text{ FFA} - 538.52 \text{ ISI} - 88.87 \text{ AUC}_{C-P}.$$

Furthermore, we conducted multiple correlation and stepwise regression analyses on AUC_{C-P} (Table 6). AUC_{C-P} was negatively correlated with HbA1c, TG and FFA, which also showed a statistical significance. Stepwise regression analysis showed that the decrease of islet secretion in the OB-KPD group might be related to the increase of blood glucose. The regression equation was $AUC_{C-P} = 25.01 - 1.27 \text{ HbA1c}$.

Effect of Islet β -Cell Function on a Curative Effect in OB-KPD Group Subjects

To investigate whether islet β -cell function of subjects in the OB-KPD group affected the curative effect, 31 subjects who were followed up for 1 year were divided into two groups according to the presence or absence of islet β -cell function at the onset of ketosis, the presence and absence groups, and both were observed to determine whether insulin

Table 7 Effect of islet β -cell function on whether insulin discontinuation could be performed after improving the condition in the OB-KPD group

	Whether insulin treatment can be discontinued after ketosis is improved		<i>P</i>
	No	Yes	
Islet β -cell function			
Absence group	2	3	0.02
Presence group	0	26	

discontinuation could be performed. As shown in Table 7, insulin discontinuation could be successfully performed on subjects with the presence of islet β -cell function at the time of onset. The results showed that patients with the presence of islet β -cell function were more likely to stop insulin treatment after improvement of ketosis ($P = 0.02$).

Influence of Altered Body Weight and Recurrence of Ketosis in the OB-KPD Group

Among the 31 subjects in the OB-KPD group that completed 1-year follow-up, 29 subjects successfully performed insulin discontinuation after short-term insulin treatment. According to whether the 29 subjects had a recurrence of ketosis during follow-up, they were divided into two groups: a non-recurrent group (24 patients) and a recurrent group (5 patients). Body weight changes before and after follow-up were observed. Observations showed that the body weight of subjects in the non-recurrent group did not significantly change before or after follow-up, while after 1 year the body weight of subjects in the recurrent group was significantly higher than that determined prior to enrollment (Table 8).

DISCUSSION

To determine the reasons for ketosis in this type of obese diabetic patient presenting with ketosis at first onset, we selected and recruited 40 Han Chinese primary obese diabetic patients with ketosis in this region. The BMI of these patients was $30.33 \pm 2.00 \text{ kg/m}^2$. Different from prior reports in China [7, 8], this study mainly focused on obese diabetic patients, but not overweight AKPD patients. At the same time, 20 obese type 2 diabetes patients were also selected as the control group.

In this study, the clinical characteristics of the OB-KPD group were essentially consistent with relevant reports of AKPD that were studied both in China and at international medical centers/institutes. It was found that young and middle-aged men were more commonly

Table 8 Body weight changes of subjects in the OB-KPD group during 1-year follow-up

	Non-recurrent group (24 patients)				Recurrent group (5 patients)			
	Before enrollment	After enrollment	<i>T</i>	<i>P</i>	Before enrollment	After enrollment	<i>T</i>	<i>P</i>
Body weight (kg)	92.29 ± 10.02	90.46 ± 8.47	1.86	0.08	88.00 ± 6.82	91.80 ± 8.64	− 3.41	0.03

affected, and the age of onset was lower than that found for type 2 diabetes; most patients had a family history of diabetes; and there was evidence of serious glucose and lipid metabolism disorders at the time of onset. In addition, our study found that the blood glucose and FFA levels of OB-KPD patients were significantly higher than those of OB-T2DM patients, suggesting that the presence of significantly increased blood glucose and FFA levels might be associated with the occurrence of ketosis.

We followed up with 31 patients in the OB-KPD group for 1 year following recovery from ketosis. Two patients (6%) failed to stop insulin treatment. The standard steamed bread meal test was reexamined with the fasting serum C-peptide < 1 ng/ml, and the peak value was always < 1.5 ng/ml. As a lack of islet β -cell function was evidenced, it was demonstrated that this was A⁻ β ⁻-ketosis-prone diabetes. This proportion was lower than that of a previously published study of African AKPD patients by Mauvais-Jarvis et al. [9], who found that 24% of African patients with ketosis tended to have type 2 diabetes that required continuous insulin treatment—an observation that might be associated with the small number of patients in this study and an observation that confirmed all patients included in this study were indeed obese. However, any contributory confounding by racial differences cannot be excluded.

Our study also found that for OB-KPD patients, islet β -cell function at the onset of ketosis was very important for future treatment planning in these patients. Patients with residual β -cell secretory function at ketosis onset were likely to cease insulin therapy after relieving ketosis.

Ketones include acetone, acetoacetic acid and β -HB, among which β -HB is the main factor

that accounts for 78% of cases. The results of this study showed that the level of β -HB in patients with ketosis in the OB-KPD group increased significantly. In type 1 diabetic patients, due to the serious lack of circulating insulin levels, the number of islet β -cells was < 10% of normal levels. Under normal conditions of peripheral insulin sensitivity, blood sugar levels cannot be reliably used in these patients, which leads to an increase in fat metabolism to provide cellular energy. A large amount of fat was metabolized to produce FFAs, which were condensed into ketones in the liver after β -oxidation. The results of this study also showed that the FFA level of the OB-KPD group was significantly higher than that of the OB-T2DM group. It is suggested that patients in the OB-KPD group also have increased fat metabolism during a ketosis episode.

The results of this study showed that the function of β -cell secretion by islets was impaired in patients with an active ketosis episode in the OB-KPD group, and the degree of impairment was worse than that seen in patients in the OB-T2DM group; however, it was unlike type 1 diabetes patients with a nearly absent secretory function. After improving blood glucose in most patients, the secretory function of islet β -cells improved significantly. Previous studies also found that the peak C-peptide levels of AKPD patients during fasting and after oral glucose loading were higher than those of type 1 diabetic patients [10, 11].

Multiple correlation and stepwise regression analyses have found that impaired islet β -cell secretion in the OB-KPD group was mainly related to hyperglycemia, suggesting that high glucose toxicity induced by hyperglycemia might account for impaired islet β -cell secretion in the OB-KPD group. Mauvais-Jarvis et al. [9]

also found that AKPD patients were more sensitive to glucotoxicity, which might serve as an initiating factor of ketosis. Rong et al. [12] found that persistent hyperglycemia and high FFA levels in obese diabetic patients could significantly reduce insulin secretion and increase apoptosis of β -cells in the islets. Glycolipid co-toxicity might be related to the onset of ketosis in AKPD patients. However, the studies of Umpierrez and Patel [13, 14] showed that the insulin secretory function of AKPD patients was not affected by fat emulsion infusion and increased non-esterified fatty acid levels. Therefore, the β -cells in patients in the OB-KPD group might be susceptible to high glucose toxicity, but the detailed mechanism remains unclear.

Since the extent of impaired insulin secretory function in the OB-KPD group was not as serious as that seen in type 1 diabetes, the unprovoked ketosis in the OB-KPD group cannot be fully explained by impaired insulin secretion alone. To further clarify the influencing factors of unprovoked ketosis in the OB-KPD group, 24 patients that stopped insulin treatment 1 year after ketosis recovery and showed no recurrence in the OB-KPD group were selected. We found that the blood glucose levels, TCH, TG, HDL-C and FFA levels of these patients were significantly lower, while that of AUC_{C-P} and ISI were significantly higher than those found at enrollment, which suggested that these indicators might be related to ketosis. Through multiple correlation and stepwise regression analyses, we found that the increases in blood glucose levels as well as increases in FFA production and decreases in the islet secretory function and insulin sensitivity were all significantly related to an occurrence of ketosis in the OB-KPD group. Thus, it was speculated that insulin resistance was involved in ketosis occurrence in the OB-KPD group.

In this study, the hyperinsulinemic euglycemic clamp test showed that patients in the OB-KPD group had severe insulin resistance when they were enrolled. This was significantly higher than that found in BMI-matched OB-T2DM patients without ketosis. This observation suggested that with the exception of the obesity-induced insulin resistance that was seen

in patients in the OB-KPD group, the FFA levels in the OB-KPD group were higher than those found in the OB-T2DM group, which might further aggravate insulin resistance. Previous studies have found that an increase in FFA can alter the insulin signaling pathway and induce or aggravate insulin resistance in the liver and muscle [15].

Thus, our research leads to the speculation that patients with ketosis-prone obese diabetes have insulin resistance and hyperglycemia due to obesity and an abnormal lipid metabolism, and the islet β -cells of these patients are susceptible to hyperglycemia toxicity [9]. When the blood glucose level is high, at least to a certain extent, the secretory function of islet β -cells obviously decreases, and the insulin secretion is insufficient. Moreover, due to insulin deficiency and resistance, higher blood glucose, increased lipodieresis and increased FFA levels further aggravate insulin resistance. Due to such a vicious cycle, too many FFAs enter the liver, leading to ketosis.

In addition, insulin discontinuation was performed in 29 patients in the OB-KPD group within 3–6 months after ketosis correction, and 5 of them recurred within 1 year. Compared with 24 patients without recurrence, these 5 patients gained significant weight. Mauvais-Jarvis et al. [9] also found that the recurrence of ketosis in AKPD patients was related to weight gain, which might be a key reason accounting for an increase in blood glucose levels. Thus, for OB-KPD patients, weight control might be beneficial in preventing recurrent ketosis.

Study Limitations

There are some shortcomings in our research. First, due to the influence of a single-center, morbidity, enrollment conditions and patient compliance, especially with regard the few cases enrolled and the subsequent follow-up, the sample size of this study is small. Second, the follow-up period was only 1 year, and the time was too short to accurately assess long-term changes of the secretory function of islet β -cells. Third, the control group had no initial type 1 diabetic ketoacidosis patients and only

compared the differences in islet β -cell secretory function between AKPD patients and type 1 diabetic patients by reference to published literature. Finally, there are no animal studies or molecular biologic level research investigations in this study. Thus, our report cannot draw a clear conclusion regarding causality, but can only draw conclusions from the correlation analyses and outcomes.

CONCLUSION

In conclusion, the clinical features, insulin resistance levels and significant recovery of islet β -cell secretory function after ketosis correction shown in this study indicate that the classification of OB-KPD patients is likely to be type 2 diabetes. The pathophysiologic mechanism of spontaneous ketosis in OB-KPD patients is made on the basis of glucose and lipid metabolic disorders, insulin resistance, blood glucose increases and high glucose toxicity, which impair the secretory function of islet β -cells. This results in increased lipodieresis and FFA production, which further aggravate insulin resistance and hyperglycemia, forming a vicious circle and thus ketosis. Most patients with the presence of islet β -cell function at the time of ketosis onset do not need long-term insulin-dependence treatment. We recommend that treatment of OB-KPD patients should be focused on the control of blood glucose, blood lipid and body weight values after correcting for ketosis, with the intention of preventing the recurrence of ketosis.

ACKNOWLEDGEMENTS

We thank the participants of the study.

Funding. This project was partly funded by the Provincial innovation team Discipline Construction Project. The Rapid Service Fee was funded by the authors.

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this

article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Authorship Contributions. LS, LZ, XHY and JLC contributed to the conception and design of the study. All authors contributed to the analysis or interpretation of data. LS wrote the initial draft of the manuscript. All authors reviewed and revised the manuscript. All authors approved the final version of the manuscript.

Disclosures. Li Shi, Liang Zhou, Juan Liu, Yang Ding, Xin-hua Ye and Jin-luo Cheng have nothing to disclose.

Compliance with Ethics Guidelines. Our work was approved by the local ethics committee of the Changzhou No. 2 People's Hospital Affiliated to Nanjing Medical University with approval no. [2016] yk021-01. Our study was performed in accordance with the Helsinki Declaration of 1964 and its later amendments. All patients signed informed consent forms before enrollment.

Data Availability. The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Open Access. This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License, which permits any non-commercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence,

visit <http://creativecommons.org/licenses/by-nc/4.0/>.

REFERENCES

1. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 2006;444(7121):840–6.
2. Lebovitz HE, Banerji MA. Ketosis-prone diabetes (Flatbush diabetes): an emerging worldwide clinically important entity. *Curr Diab Rep*. 2018;18(11):120.
3. Valabhji J, Watson M, Cox J, Poulter C, Elwig C, Elkeles RS. Type 2 diabetes presenting as diabetic ketoacidosis in adolescence. *Diabet Med*. 2003;20(5):416–7.
4. Yang YL. Clinical characteristics in anti-islet autoantibody-negative ketosis-prone patients with metabolic syndrome [D]. Changsha: The Second Xiangya Hospital, Central South University, 2008. pp. 1–52.
5. The expert committee on the diagnosis and classification of Diabetes Mellitus. *Diabetes Care*. 1997;20(7):1183–97.
6. Balasubramanyam A, Garza G, Rodriguez L, et al. Accuracy and predictive value of classification schemes for ketosis-prone diabetes. *Diabetes Care*. 2006;29(12):2575–9.
7. Zhang DM, Zhou ZG, Hu BY, Wei JL, Huang G, Wang JP. Clinical characteristics and classification of obese ketosis-prone diabetic patients. *Chin J Endocrinol Metab*. 2003;19(3):221–4.
8. Tan HW, Wang C, Yu YR, Yu HL, Zhang XX. Study on clinical characteristics and heterogeneity of ketosis-prone diabetic patients. *Chin J Endocrinol Metab*. 2013;29(12):1026–30.
9. Mauvais-Jarvis F, Sobngwi E, Porcher R, et al. Ketosis-prone type 2 diabetes in patients of sub-Saharan African origin: clinical pathophysiology and natural history of beta-cell dysfunction and insulin resistance. *Diabetes*. 2004;53(3):645–53.
10. Piñero-Piloña A, Litonjua P, Aviles-Santa L, Raskin P. Idiopathic type 1 diabetes in Dallas, Texas: a 5-year experience. *Diabetes Care*. 2001;24(6):1014–8.
11. Ramos-Román MA, Piñero-Piloña A, Adams-Huet B, Raskin P. Comparison of type 1, type 2, and atypical ketosis-prone diabetes at 4 years of diabetes duration. *J Diabetes Complicat*. 2006;20(3):137–44.
12. Yu YR, Tan HW, Zhao NQ, Yu HL, Geng LJ, Wang C. Study on the pathogenesis of obese ketosis-prone diabetes. *Int J Endocrinol Metab*. 2008;28(4):226–9.
13. Umpierrez GE, Smiley D, Robalino G, Peng L, Gosmanov AR, Kitabchi AE. Lack of lipotoxicity effect on β -cell dysfunction in ketosis-prone type 2 diabetes. *Diabetes Care*. 2010;33(3):626–31.
14. Patel SG, Hsu JW, Jahoor F, et al. Pathogenesis of $A\beta^+$ ketosis-prone diabetes. *Diabetes*. 2013;62(3):912–22.
15. Delarue J, Magnan C. Free fatty acids and insulin resistance. *Curr Opin Clin Nutr Metab Care*. 2007;10(2):142–8.