ORIGINAL

Diagnosis of insulinoma using the ratios of serum concentrations of insulin and C-peptide to glucose during a 5-hour oral glucose tolerance test

Xu Li *, Feng Zhang *, Haibing Chen, Haoyong Yu, Jian Zhou, Ming Li, Qing Li, Lianxi Li, Jun Yin, Fang Liu, Yuqian Bao, Junfeng Han and Weiping Jia

Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Key Clinical Center for Metabolic Disease, 600 Yishan road, Shanghai 200233, China

Abstract. The 72-hour fast test is the current standard for the diagnosis of insulinoma. However, to conduct this test patients require hospitalization due to the chance of severe hypoglycemic episodes. Thus, it is costly and stressful for the patient. An out-patient test would serve the patient better and be more economical. Our aim was to evaluate the value of insulin to glucose and C-peptide to glucose ratios during a prolonged 5-hour oral glucose tolerance test (5-hour OGTT) in qualitative diagnosis of insulinoma, and to identify the optimal threshold for clinical screening. Initially, 15 subjects with pathological insulinoma and 12 control subjects with reactive hypoglycemia were enrolled in the study. A further 75 subjects with symptoms of hypoglycemia as a chief complaint at their initial clinic visit were subsequently screened. Serum insulin, C-peptide levels and blood glucose were quantified after a 5-hour OGTT in all participants and the ratios of serum concentrations of insulin and C-peptide to glucose were calculated. Subjects with insulinoma had significantly different insulin-to-glucose and C-peptide-to-glucose ratios from reactive hypoglycemia at the times of fasting, 4-hour post glucose load and 5-hour post glucose load. Higher specificity (73.08%) and sensitivity (82.67%) were achieved with the combined insulin-to-glucose ratio at the 5-hour post load and the C-peptide-to-glucose ratio at fasting. In combination, ratios of insulin and C-peptide release relative to blood glucose levels, measured during a 5-hour OGTT, may have important clinical value in the diagnosis of insulinoma.

Key words: Insulinoma, 5-hour OGTT, Reactive hypoglycemia, Insulin, C-peptide

INSULINOMA is characterized by endogenous hyperinsulinism and repeated hypoglycemic episodes. It is the most common functional neuroendocrine tumor of the pancreas, with an estimated incidence of 4 cases per 1 million person-years [1]. Because of the rarity and diversity of symptoms of insulinoma [2-4], its diagnosis has often been overlooked. Therefore, before committing to surgical exploration or even determining tumor localization, it is vital to establish an absolute qualitative

diagnosis. This would facilitate distinguishing cases of insulinoma from the more common condition of reactive hypoglycemia. In this regard, the 72-hour fast test is the current gold standard to establish a qualitative diagnosis of insulinoma [5]. Although the efficacy of this test in establishing endogenous hyperinsulinism is high, it requires hospitalization for at least one week, carries inherent risks and is stressful for some patients. Therefore, developing alternative tests that could be performed in an outpatient setting with less discomfort would be advantageous.

A recent case report showed that the 72-hour fast was normal in two patients that required surgical removal of insulinomas, whereas a prolonged OGTT clearly demonstrated the induction of severe hyperinsulinemia followed by significant hypoglycemia [6]. A prolonged five-hour OGTT has a higher chance

Submitted Jun. 8, 2016; Accepted Aug. 24, 2016 as EJ16-0292
Released online in J-STAGE as advance publication Oct. 1, 2016
Correspondence to: Junfeng Han, Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Key Clinical Center for Metabolic Disease, 600 Yishan road, Shanghai 200233 China. E-mail: tjhjf@163.com
* These authors contributed equally to this work.

than ordinary three-hour OGTT in revealing an episode of hypoglycemia for the diagnosis of insulinoma [7]. Furthermore, the detection of elevated C-peptide during an OGTT also yields increased diagnostic reliability for insulinoma [8, 9]. More importantly, due to wide variations in individual fasting serum insulin, the ratio of serum insulin to blood glucose has been recommended by several studies as a more reliable parameter for the diagnosis of insulinoma [10-13]. Therefore, in the present study, our aim was to test the hypothesis that the ratio of insulin-to-glucose or C-peptide-to-glucose, measured during the course of a 5-hour OGTT, is a valid diagnostic approach in detecting insulinoma. Validating this approach would allow it to become an alternative screening index for Chinese insulinoma patients.

Materials and Methods

Study subjects

A total of 23 subjects with insulinoma were enrolled in this study between December 2009 and December 2014. Of these patients, 15 were a positive result during a 72-hour fast test (ratio of insulin-to-glucose greater than 0.4), and 8 patients were excluded from the initial analysis for the following reasons: a spontaneous episode of hypoglycemia in the outpatient setting and no available 72-hour fast test data ($n = 5$), hypoglycemia was observed in the fasting and postprandial state ($n = 2$), and exclusively presented with post-prandial hypoglycemia episodes and the 72-hour fast test were negative ($n = 1$). Each case pursued the tumor localization by different tools including CT, MRI and Intra-arterial calcium-stimulated venous sampling (ASVS). CT scan was usually our first choice for location and imaging of 20 subjects were positive. If negative, MRI would be used, and one in 23 subjects was confirmed. If imaging of both tools were negative, ASVS would be final choice before surgery. Luckily, other two subjects were located. Eventually, surgical pathology confirmed the diagnosis of insulinoma in each case. We initially studied 15 subjects with pathological insulinoma, and 12 patients diagnosed with reactive hypoglycemia over the same time period were used as the control group. A further 75 subjects with symptoms of hypoglycemia as a chief complaint at their initial clinic visit were subsequently screened. This study was preapproved by the Ethics Committee of Shanghai Jiaotong University Affiliated

Sixth People's Hospital, and all subjects provided written informed consent prior to study participation. All patients had a complete history and physical examination, routine blood examination, urine chemistries and 5-hour OGTT.

Clinical diagnosis of insulinoma and reactive hypoglycemia

Insulinoma was diagnosed by clinical hypoglycemia symptoms, and the following four criteria: 1) documented blood glucose levels near or below 50mg/dL ($<3.0\text{mmol/L}$); 2) concomitant insulin levels equal or greater than 3mU/L ($>18\text{pmol/L}$); 3) elevated C-peptide levels ($>0.6\text{ng/mL}$ or $>0.2\text{nmol/L}$); 4) absence of sulfonylurea in the plasma. In addition, we used computed tomography/positron emission tomography imaging of patients and pathological diagnosis of the surgically removed tumor [14]. Diagnosis of reactive hypoglycemia excluded alimentary hypoglycemia, diabetes mellitus, hormonal hypoglycemia, deficient early hepatic gluconeogenesis and other causes. Reactive hypoglycemia was characterized by postprandial onset, adrenergic mediated symptoms and relatively benign causes. Patients will often fail their 72-hour fast test, never developing hypoglycemia [15].

Anthropometric and biochemical measurements

All subjects were evaluated after an overnight fast of at least ten hours. Anthropometric assessments included height, weight and resting blood pressure (BP) measurements. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Biochemical variables were analyzed according to methods previously reported [16]. Hemoglobin A1c (HbA1c) was measured by high performance liquid chromatography with HLC-73G7 automated glycohemoglobin analyzer (Tosoh, Tokyo, Japan). The other biochemical indexes were measured on a Hitachi 7600 analyzer (Hitachi, Tokyo, Japan). Serum insulin and C-peptide were assayed using radioimmunoassay (Linco Research, St Charles, Missouri, USA). All laboratory measurements met the Shanghai center for Clinical Laboratory criteria.

All patients underwent 75g 5-hour OGTT after 10-hour fasting. Blood glucose levels, insulin and C-peptide were determined in the blood samples collected during the prolonged OGTT. Insulin-to-glucose

ratio was calculated as insulin (pmol/L)/glucose (mmol/L), while C-peptide-to-glucose ratio was calculated as C-peptide (nmol/L)/glucose (mmol/L) at different time points.

Statistical analysis

All statistical analyses were performed with SPSS Version 21.0 (IBM SPSS, Chicago, IL, USA). Normally distributed data were expressed as mean $\pm$ SEM. Data that were not normally distributed, as determined using Kolmogorov-Smirnov test, were expressed as median with interquartile range. Student's unpaired *t* test and nonparametric test were used for comparison between the insulinoma and reactive hypoglycemia groups. The accuracy of the models was evaluated using area under the receiver-operating characteristic (ROC) curve with 95% confidence interval (CI). We calculated the sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios, as well as their 95% CIs, using MedCalc Software (Mariakerke, Belgium).

Results

Characteristics of the insulinoma and reactive hypoglycemia patients

The general and biochemical characteristics of the patients with insulinoma (7 males and 8 females) and reactive hypoglycemia (6 males and 6 females) are shown in Table 1. Compared with reactive hypoglycemia subjects (median age = 60 years, 30 - 80 years), patients with insulinoma had a median age of 55 (22 - 79) years ($P>0.05$). By contrast, we observed that subjects with insulinoma had higher BMI compared to the reactive hypoglycemia group (25.24 ± 1.01 vs. 21.66 ± 0.85 kg/m²; $P=0.013$). A significant decrease in the total bilirubin and high-density lipoprotein cholesterol (HDL) was observed in the insulinoma group compared to the reactive hypoglycemia group (both $P<0.05$). Moreover, glycated albumin (GA) (11.53 ± 0.87 vs. $14.10\pm 0.44\%$; $P=0.036$) and HbA1c (4.65 ± 0.18 vs. $5.25\pm 0.11\%$; $P=0.009$) were significantly lower in the insulinoma group.

Table 1 Baseline clinical and laboratory characteristics of the study population

	Reactive hypoglycemia (N=12)	Insulinoma (N=15)	P-value
Male : Female	6 : 6	7 : 8	0.863
Age (years)	56.67 $\pm$ 4.47	46.53 $\pm$ 5.09	0.158
BMI (kg/m ²)	21.66 $\pm$ 0.85	25.24 $\pm$ 1.01	0.013 *
Haemoglobin (g/L)	138.46 $\pm$ 5.21	137.33 $\pm$ 3.39	0.852
ALT (U/L)	23.31 $\pm$ 2.94	30.79 $\pm$ 4.74	0.261
AST (U/L)	23.00 (18.00-25.00)	25.00 (21.00-37.00)	0.529
γ -GT (U/L)	23.89 $\pm$ 4.69	34.64 $\pm$ 5.70	0.197
TBL (μ mol/L)	14.98 $\pm$ 2.05	10.54 $\pm$ 0.77	0.037 *
TC (mmol/L)	4.42 $\pm$ 0.31	4.47 $\pm$ 0.25	0.915
TG (mmol/L)	1.10 $\pm$ 0.16	1.47 $\pm$ 0.25	0.241
HDL (mmol/L)	1.57 $\pm$ 0.12	1.12 $\pm$ 0.04	0.004 **
LDL (mmol/L)	2.31 $\pm$ 0.23	2.82 $\pm$ 0.22	0.125
Cr (μ mol/L)	80.00 $\pm$ 5.60	69.57 $\pm$ 4.64	0.164
Uric acid (μ mol/L)	279.80 $\pm$ 27.71	318.64 $\pm$ 20.06	0.256
TSH (mIU/L)	1.69 (1.01-2.69)	2.63 (2.47-3.32)	0.054
GA (%)	14.10 $\pm$ 0.44	11.53 $\pm$ 0.87	0.036 *
HbA1c (%)	5.25 $\pm$ 0.11	4.65 $\pm$ 0.18	0.009 **
AFP (ng/mL)	2.69 $\pm$ 0.39	2.70 $\pm$ 0.46	0.992
CEA (ng/mL)	2.11 $\pm$ 0.36	1.99 $\pm$ 0.36	0.820
CA125 (U/mL)	9.66 $\pm$ 1.01	15.88 $\pm$ 2.65	0.069
CA199 (U/mL)	9.13 $\pm$ 2.54	11.47 $\pm$ 2.90	0.583
ACTH (ng/L)	18.63 $\pm$ 4.04	28.11 $\pm$ 4.35	0.130
GH (μ g/L)	1.52 (0.56-2.59)	2.58 (0.53-3.98)	0.808

* $P<0.05$, ** $P<0.01$. Abbreviations: BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ -GT, γ -glutamyltranspeptidase; TBL, total bilirubin; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; Cr, creatinine; TSH, thyrotropic-stimulating hormone; HbA1c, glycatedhaemoglobin; GA, glycated albumin; AFP, alphafetoprotein; CEA, carcino-embryonic antigen; CA125, Carbohydrate antigen 125; CA199, Carbohydrate antigen 199; ACTH, adrenocorticotrop(h)ic hormone; GH, growth hormone.

Comparison of 5-hour OGTT in the insulinoma and reactive hypoglycemia groups

The results of 5-hour OGTT are shown in Fig. 1. In the insulinoma group, the blood glucose rose from the fasting level of 3.43 ± 0.16 mmol/L to the peak of 7.65 ± 0.49 mmol/L at 1-hour post load, followed by a slow decrease to 3.12 ± 0.33 mmol/L at 5-hour post load time point. The serum level of insulin in the subjects with insulinoma increased from the initial level of 163.14 ± 37.0 pmol/L and reached the peak of 725.85 ± 185.38 pmol/L at 30-min post load, then gradually declined to its nadir of 139.45 ± 20.31 pmol/L at 5-hour post load. Fasting serum C-peptide in patients with insulinoma was 1.17 ± 0.16 nmol/L, slowly reached its peak 2.51 ± 0.28 nmol/L at 1-hour post load, and then gradually decreased to its nadir 1.10 ± 0.14 nmol/L at 5-hour post load. However, in comparison to the reactive hypoglycemia group, only fasting and 5-hour post load time points for blood glucose (fasting: 3.43 ± 0.16 vs. 4.58 ± 0.29 mmol/L; 5-hour post load: 3.12 ± 0.33 vs. 4.22 ± 0.31 mmol/L), insulin (163.14 ± 37.00 vs. 65.56 ± 13.21 and 139.45 ± 20.31 vs. 43.78 ± 9.22 pmol/L), C-peptide (1.17 ± 0.16 vs. 0.70 ± 0.06 and 1.10 ± 0.14 vs. 0.72 ± 0.12 nmol/L) showed a significant difference with the insulinoma group (all $P < 0.05$).

To eliminate patient-to-patient variability in the measurements of blood glucose, insulin or C-peptide, the ratios of insulin to glucose or C-peptide to glucose were also calculated for each time point during the 5-hour OGTT. Although not all insulin-to-glucose ratios during 5-hour OGTT in insulinoma group were higher than 53.6 [13], all values were significantly different from the reactive hypoglycemia group at fasting (48.84 ± 11.11 vs. 15.24 ± 3.24), 4-hour post load (68.52 ± 22.38 vs. 16.32 ± 4.47), 5-hour post load (53.24 ± 10.12 vs. 11.16 ± 2.48) (all $P < 0.05$). Further, the C-peptide-to-glucose ratios during 5-hour OGTT were significantly higher in the insulinoma group at fasting (0.36 ± 0.06 vs. 0.16 ± 0.02), 4-hour post load (0.43 ± 0.08 vs. 0.25 ± 0.03) and 5-hour post load (0.40 ± 0.06 vs. 0.18 ± 0.03) (all $P < 0.05$). Therefore, the ratios were screened to evaluate if they could be used as a method to identify the two groups.

Predicting accuracy of new models in 5-hour OGTT

Through the above analysis, we found that 5-hour OGTT may have important clinical value in the diagnosis of insulinoma. Therefore, the receiver operating characteristic (ROC) curves were conducted

between the 5-hour OGTT and 72-hour fast test (as the gold standard) to show the predicting accuracy for qualitative diagnosis (Fig. 2). For blood glucose, the area under the curve (AUC) was 0.88 (95%CI 0.69 to 0.97) at fasting condition alone and 0.84 (95%CI, 0.61 to 0.96) for HbA1c alone. The AUC of insulin-to-glucose ratio at fasting, 5-hour post load during 5-hour OGTT were 0.87 (95%CI, 0.68 to 0.97), 0.91 (95%CI, 0.74 to 0.99), respectively. In addition, the AUC was 0.84 (95%CI, 0.65 to 0.95) for C-peptide-to-glucose ratio at fasting alone and 0.82 (95%CI, 0.62 to 0.94) for C-peptide-to-glucose ratio at 5-hour post load. However, it is interesting to note that the AUC of insulin-to-glucose ratio at 5-hour post load combined with insulin-to-glucose ratio at fasting (0.94; 95%CI, 0.78 to 1.00) was the same as insulin-to-glucose ratio at 5-hour post load combined with C-peptide-to-glucose ratio at fasting (0.94; 95%CI, 0.78 to 1.00). This AUC was highest of all models. The cut-off values selected by the closest distance to the left upper corner of the ROC curve were 20.45 pmol/mmol for insulin-to-glucose ratio at 5-hour post load, 13.54 pmol/mmol for insulin-to-glucose ratio at fasting and 0.19 nmol/mmol for C-peptide-to-glucose ratio at fasting, respectively.

The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio for insulinoma prediction are shown in Table 2. Insulin-to-glucose ratio at 5-hour post load combined with C-peptide-to-glucose ratio at 0 min proved to be the best model (sensitivity = 100.00%, specificity = 83.30%, positive predictive value = 88.20%, negative predictive value = 100.00%, positive likelihood ratio = 6.00, negative likelihood ratio = 0.00).

The cut-off of new model during 5-hour OGTT for predicting of insulinoma

To test the diagnostic value of the 5-hour OGTT, we further screened 75 patients with hypoglycemia symptoms as their chief complaint at their initial clinic visit. All patients performed 5-hour OGTT and twenty-three of them had a pathological diagnosis of insulinoma. The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio for predicting insulinoma of two models were shown in Table 3. Higher specificity (73.08% vs. 55.77%) was achieved in insulin-to-glucose ratio at 5-hour post load combined

Fig. 1 The profile of blood glucose, serum insulin, C-peptide during 5-hour OGTT in insulinoma and reactive hypoglycemia group (control)

(A), the comparison of profile of glucose between insulinoma and reactive hypoglycemia group. (B), the comparison of profile of insulin between insulinoma and reactive hypoglycemia group. (C), the comparison of profile of C-peptide between insulinoma and reactive hypoglycemia group. (D), the comparison of profile of insulin-to-glucose ratio during 5-hour OGTT between insulinoma and reactive hypoglycemia group. (E), the comparison of profile of C-peptide-to-glucose ratio during 5-hour OGTT between insulinoma and reactive hypoglycemia group. Black squares represent control (reactive hypoglycemia) group (N = 12); black diamonds represent insulinoma group (N = 15). Data are shown as mean±SEM.

Fig. 2 ROC curve for predicting insulinoma

Insulin-to-glucose ratio at 300 min + HbA1c, AUC = 0.92 (95% CI, 0.71 to 1.00); Insulin-to-glucose ratio at 300 min + C-peptide-to-glucose ratio at 0 min, AUC = 0.94 (95% CI, 0.78 to 1.00); Insulin-to-glucose ratio at 300 min + C-peptide-to-glucose ratio at 300 min, AUC = 0.92 (95% CI, 0.75 to 0.99); Insulin-to-glucose ratio at 300 min + BG 0 min, AUC = 0.92 (95% CI, 0.75 to 0.99); Insulin-to-glucose ratio at 300 min + Insulin-to-glucose ratio at 0 min, AUC = 0.94 (95% CI, 0.78 to 1.00), BG 0 min alone, AUC = 0.88 (95% CI, 0.69 to 0.97). AUC, the area under the curve; 95% CI, 95% confidence interval; BG, blood glucose.

Table 2 Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio for insulinoma prediction with insulin-to-glucose ratio and C-peptide-to-glucose ratio during 5-hour OGTT

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Positive likelihood ratio	Negative likelihood ratio
C-peptide-to-glucose ratio (0 min)	73.33 (44.90 to 92.00)	83.33 (51.60 to 97.40)	84.60 (54.50 to 97.60)	71.40 (41.90 to 91.40)	4.40 (3.00 to 6.50)	0.32 (0.07 to 1.50)
C-peptide-to-glucose ratio (300 min)	86.67 (59.50 to 98.00)	75.00 (42.80 to 94.20)	81.20 (54.30 to 95.70)	81.80 (48.20 to 97.20)	3.47 (2.40 to 5.10)	0.18 (0.04 to 0.90)
Insulin-to-glucose ratio (0 min)	93.33 (68.00 to 98.90)	75.00 (42.80 to 94.20)	82.40 (56.60 to 96.00)	90.00 (55.50 to 98.30)	3.73 (2.60 to 5.30)	0.09 (0.01 to 0.70)
Insulin-to-glucose ratio (300min)	80.00 (51.90 to 95.40)	91.67 (61.50 to 98.60)	92.30 (63.90 to 98.70)	78.60 (49.20 to 95.10)	9.60 (7.10 to 13.00)	0.22 (0.03 to 1.80)
Insulin-to-glucose ratio (300 min) + Insulin-to-glucose ratio (0 min)	93.33 (68.00 to 98.90)	83.33 (51.60 to 97.40)	87.50 (61.60 to 98.10)	90.90 (58.70 to 98.50)	5.60 (4.20 to 7.50)	0.08 (0.01 to 0.80)
Insulin-to-glucose ratio (300 min) + C-peptide-to-glucose ratio (0 min)	100.00 (78.00 to 100.00)	83.30 (51.60 to 97.40)	88.20 (63.50 to 98.20)	100.00 (69.00 to 100.00)	6.00 (4.70 to 7.70)	0.00 (NA)

NA = not applicable. All values were measured during 5-hour OGTT. To convert glucose values from mmol/L to mg/dL, divide by 0.0555. To convert insulin values from pmol/L to mU/L, divide by 7.175. To convert C-peptide values from nmol/L to ng/mL, divide by 0.331. Insulin-to-glucose ratio = insulin (pmol/L)/glucose (mmol/L). Insulin-glucose ratio at 0 min is the fasted insulin level at the start of the 5-hour OGTT and 300 min is the insulin level at the end of the OGTT. C-peptide-to-glucose ratio = C-peptide (nmol/L)/glucose (mmol/L). C-peptide-glucose ratio at 0 min is the fasted C-peptide level at the start of the 5-hour OGTT and 300 min is the C-peptide level at the end of the OGTT.

Table 3 Sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio for insulinoma prediction with the cut-off of insulin-to-glucose ratio at 300 min, 0 min and C-peptide-to-glucose ratio at 0 min during 5-hour OGTT (n = 75)

	Model	
	Insulin-to-glucose ratio at 300 min + Insulin-to-glucose ratio at 0 min	Insulin-to-glucose ratio at 300 min + C-peptide-to-glucose ratio at 0 min
Sensitivity (%)	86.96	82.67
Specificity (%)	55.77	73.08
Positive predictive value (%)	46.51	57.58
Negative predictive value (%)	90.63	90.48
Positive likelihood ratio	1.97	3.07
Negative likelihood ratio	0.23	0.24

with C-peptide-to-glucose ratio at 0 min model when compared to insulin-to-glucose ratio at 5-hour post load combined with insulin-to-glucose ratio at 0 min model. However, the corresponding sensitivity was 82.67%, slightly lower than insulin-to-glucose ratio at 5-hour post load combined with insulin-glucose ratio at 0 min model (86.96%). Negative predictive value, positive likelihood ratio and negative likelihood ratio were 90.48%, 3.07, 0.24 respectively.

Discussion

In this study, we provide the first evidence demonstrating that 5-hour OGTT has important clinical significance in qualitative diagnosis of insulinoma. Specifically, we established that the model of the insulin-to-glucose ratio at 5-hour post load over than 20.45 pmol/mmol combined with C-peptide-to-glucose ratio at 0 min with threshold of 0.19 nmol/mmol had high specificity and sensitivity for predicting insulinoma. The ROC-AUC of this test was 0.94, much higher than other parameters tested. Since 5-hour OGTT is easily carried out in clinics, this model could possibly be used as a novel and accurate baseline screening method for insulinoma with hypoglycemia symptoms. It may also be used for identifying those who are at high risk of developing this disease.

Although the 72-hour fast test is the current gold standard to establish a qualitative diagnosis of insulinoma, different tests either suppressing insulin secretion or stimulating insulin release and consequently changing plasma glucose concentrations have been used previously. However, they do not significantly improve diagnosis in routine clinical practice and remain utilized mainly only for research purposes, such as C-peptide suppressive test, tolbutamide test,

calcium test, glucagon test [17-19]. We now provide a new model calculated using insulin-to-glucose ratio at 5-hour post load and C-peptide-to-glucose ratio at 0 min to predict the insulinoma. The model significantly increased the ROC-AUC to 0.94, much better than fasting hypoglycemia alone (ROC-AUC value of 0.88) and offered high diagnostic sensitivity and specificity in a larger population of 75 patients with hypoglycemia symptoms. The insulin-to-glucose ratio at 5-hour post load combined with C-peptide-to-glucose ratio at 0 min also may be better than insulin-to-glucose ratio at 5-hour post load combined with insulin-to-glucose ratio at 0 min. Reasons for this may include the fact that C-peptide and insulin antibodies have no cross-reaction [20] and that it does not undergo significant hepatic extraction [21, 22]. Note, the liver is the major site of insulin metabolism, variably extracting ~50% of insulin delivered to it [23-25]. It has therefore been suggested that peripheral C-peptide levels more accurately reflect pancreatic insulin secretion rates than do peripheral insulin levels.

Furthermore, in our study we also report the patterns of blood glucose, serum insulin, serum C-peptide during 5-hour OGTT in patients with insulinoma. Although the overall blood glucose concentrations of glucose tolerance curve were lower than reactive hypoglycemia, it was not a low flat curve. The peak of glucose tolerance curves was reached at 60 min and declined to their nadir at 300 min. The secreted insulin concentrations at each time point of 5-hour OGTT were higher than reactive hypoglycemia group, especially at 0, 300 min, which completely reflected the autonomous secretion pattern in insulinoma [26]. However, the peak of insulin secretion did not delay, which is different from early type 2 diabetes, and

may contributed to differential diagnosis. Although the C-peptide concentrations reached a peak at 60 min, it rapidly reached a high value at 30 min and a plateau remained for 90 min from 30min to 120min. C-peptide is a by-product of proinsulin digestion, and insulin and C-peptide are secreted from the pancreatic β -cell in equimolar amounts [27-29], which means measurable C-peptide in the plasma of a hypoglycemic patient implies an endogenous source of insulin. Additionally, C-peptide is not extracted by liver [30, 31], and its half-life is longer than that of insulin [32-35]. Thus, we infer that the unsynchronized result of insulin and C-peptide in patients with insulinoma during 5-hour OGTT was attributed to the immature forms of insulin.

Although we are the first to evaluate the diagnostic value of insulin-to-glucose ratio and C-peptide-to-glucose ratio for insulinoma during 5-hour OGTT, we should bear in mind the limited number of patients, the cross-sectional nature of the study and the limitation that no proinsulin concentrations were available. We suggest that a more meaningful outcome may arise from the evaluation of a larger series of insulinoma patients. Nevertheless, it is important to regard that although the 72-hour fast test has diagnostic value for insulinoma, the major draw-back of the 72-hour fast test is it requires hospitalization and close monitoring of patients against severe hypoglycemic episodes. It is also time-consuming, uncomfortable and stressful for the patient. However, the 5-hour OGTT can be performed in one out-patient clinical visit. Therefore,

instead of the 72-hour fast test we recommend, for an initial diagnosis, calculating insulin-to-glucose ratio and C-peptide-to-glucose ratio during 5-hour OGTT in every patient for whom a hypoglycemic disorder is suspected. Nevertheless, the final diagnosis of insulinoma should be made by integrating all available results, including tumor localization.

Conclusion

In our study, we found insulin-to-glucose ratio at 5-hour post load combined with C-peptide-to-glucose ratio at 0 min during 5-hour OGTT may have important clinical value in the diagnosis of insulinoma.

Acknowledgments

The authors are grateful for support from the Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated Sixth People's Hospital. We are also extremely appreciative of all of the participants for their dedication in data collection and laboratory measurements. This work was supported by grants from the National Natural Sciences Foundation of China (81200564) to Junfeng Han.

Disclosure

The authors declare that they have no conflict of interest associated with this manuscript.

References

1. Service FJ, McMahon MM, O'Brien PC, Ballard DJ (1991) Functioning insulinoma—incidence, recurrence, and long-term survival of patients: a 60-year study. *Mayo Clin Proc* 66: 711-719.
2. Renca S, Santos G, Cerejeira J (2013) An insulinoma presenting with hypochondriac delusions and food refusal. *Int Psychogeriatr* 25: 1909-1911.
3. Connor H, Scarpello JH (1979) An insulinoma presenting with reactive hypoglycaemia. *Postgrad Med J* 55: 735-738.
4. Shreenivas AV, Leung V (2014) A rare case of insulinoma presenting with postprandial hypoglycemia. *Am J Case Rep* 15: 488-491.
5. Service FJ (1995) Hypoglycemic disorders. *N Engl J Med* 332: 1144-1152.
6. Kar P, Price P, Sawers S, Bhattacharya S, Reznick RH, et al. (2006) Insulinomas may present with normoglycemia after prolonged fasting but glucose-stimulated hypoglycemia. *J Clin Endocrinol Metab* 91: 4733-4736.
7. Konng YG (1990) Value of 5-hour glucose tolerance test in the diagnosis of insulinoma. *Zhonghua Wai Ke Za Zhi* 28: 331-332 (In Chinese).
8. Ahn CH, Kim LK, Lee JE, Jung CH, Min SH, et al. (2014) Clinical implications of various criteria for the biochemical diagnosis of insulinoma. *Endocrinol Metab (Seoul)* 29: 498-504.
9. Arzamendi AE, Rajamani U, Jialal I (2014) Pseudoinulinoma in a white man with autoimmune hypoglycemia due to anti-insulin antibodies: value of the free C-Peptide assay. *Am J Clin Pathol* 142: 689-693.
10. Finlayson E, Clark OH (2004) Surgical treatment of insulinomas. *Surg Clin North Am* 84: 775-785.

11. Pasiëka JL, McLeod MK, Thompson NW, Burney RE (1992) Surgical approach to insulinomas. Assessing the need for preoperative localization. *Arch Surg* 127: 442-447.
12. Grunt JA, Pallotta JA, Soeldner JS (1970) Blood sugar, serum insulin and free fatty acid interrelationships during intravenous tolbutamide testing in normal young adults and in patients with insulinoma. *Diabetes* 19: 122-126.
13. Nauck MA, Meier JJ (2012) Diagnostic accuracy of an "amended" insulin-glucose ratio for the biochemical diagnosis of insulinomas. *Ann Intern Med* 157: 767-775.
14. Cryer PE, Axelrod L, Grossman AB, Heller SR, Montori VM, *et al.* (2009) Evaluation and management of adult hypoglycemic disorders: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 94: 709-728.
15. Hofeldt FD (1975) Reactive hypoglycemia. *Metabolism* 24: 1193-1208.
16. Bao Y, Ma X, Yang R, Wang F, Hao Y, *et al.* (2013) Inverse relationship between serum osteocalcin levels and visceral fat area in Chinese men. *J Clin Endocrinol Metab* 98: 345-351.
17. Fajans SS, Vinik AI (1989) Insulin-producing islet cell tumors. *Endocrinol Metab Clin North Am* 18: 45-74.
18. Service FJ, O'Brien PC, Kao PC, Young WF Jr (1992) C-peptide suppression test: effects of gender, age, and body mass index; implications for the diagnosis of insulinoma. *J Clin Endocrinol Metab* 74: 204-210.
19. Oneda A, Maruhama Y, Itabashi H, Horigome K, Yanbe A (1975) Diagnostic value of intravenous glucagon test in insulinoma. *Tohoku J Exp Med* 116: 205-211.
20. Rubenstein AH, Welbourne WP, Mako M, Melani F, Steiner DF (1970) Comparative immunology of bovine, porcine and human proinsulins and C-peptides. *Diabetes* 19: 546-553.
21. Waldhäusl W, Bratusch-Marrain P, Gasic S, Korn A, Nowotny P (1979) Insulin production rate following glucose ingestion estimated by splanchnic C-peptide output in normal man. *Diabetologia* 17: 221-227.
22. Bratusch-Marrain PR, Waldhäusl WK, Gasic S, Hofer A (1984) Hepatic disposal of biosynthetic human insulin and porcine C-peptide in humans. *Metabolism* 33: 151-157.
23. Rubenstein AH, Pottenger LA, Mako M, Getz GS, Steiner DF (1972) The metabolism of proinsulin and insulin by the liver. *J Clin Invest* 51: 912-921.
24. Jaspan J, Polonsky K (1982) Glucose ingestion in dogs alters the hepatic extraction of insulin. In vivo evidence for a relationship between biologic action and extraction of insulin. *J Clin Invest* 69: 516-525.
25. Kaden M, Harding P, Field JB (1973) Effect of intraduodenal glucose administration on hepatic extraction of insulin in the anesthetized dog. *J Clin Invest* 52: 2016-2028.
26. Saddig C, Goretzki PE, Starke AA (2008) Differentiation of insulin secretion patterns in insulinoma. *World J Surg* 32: 918-929.
27. Steiner DF, Oyer PE (1967) The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. *Proc Natl Acad Sci U S A* 57: 473-480.
28. Steiner DF, Cunningham D, Spigelman L, Aten B (1967) Insulin biosynthesis: evidence for a precursor. *Science* 157: 697-700.
29. Steiner DF, Clark JL, Nolan C, Rubenstein AH, Margoliash E, *et al.* (1969) Proinsulin and the biosynthesis of insulin. *Recent Prog Horm Res* 25: 207-282.
30. Waldhäusl W, Bratusch-Marrain P, Gasic S, Korn A, Nowotny P (1979) Insulin production rate following glucose ingestion estimated by splanchnic C-peptide output in normal man. *Diabetologia* 17: 221-227.
31. Bratusch-Marrain PR, Waldhäusl WK, Hofer A (1984) Hepatic disposal of biosynthetic human insulin and porcine C-peptide in humans. *Metabolism* 33: 151-157.
32. Home PD, Massi-Benedetti M, Shepherd GA, Hanning I, Alberti KG, *et al.* (1982) A comparison of the activity and disposal of semi-synthetic human insulin and porcine insulin in normal man by the glucose clamp technique. *Diabetologia* 22: 41-45.
33. Sönksen PH, Tompkins CV, Srivastava MC, Nabarro JD (1973) A comparative study on the metabolism of human insulin and porcine proinsulin in man. *Clin Sci Mol Med* 45: 633-654.
34. Turner RC, Grayburn JA, Newman GB, Nabarro JD (1971) Measurement of the insulin delivery rate in man. *J Clin Endocrinol Metab* 33: 279-286.
35. Horwitz DL, Starr JI, Mako ME, Blackard WG, Rubenstein AH (1975) Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. *J Clin Invest* 55: 1278-1283.