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ORIGINAL ARTICLE

Pathophysiological characteristics in patients with latent autoimmune diabetes in adults using clamp tests: evidence of a continuous disease spectrum of diabetes

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Abstract

Aims This study aimed to assess islet insulin secretion and insulin resistance in Chinese patients with latent autoimmune diabetes in adults (LADA).

Methods Twelve patients with LADA, 10 with type 1 diabetes mellitus (T1DM), 10 with type 2 diabetes mellitus (T2DM), and 10 nondiabetic healthy controls (HCs) were included. Patients with LADA were subtyped according to the glutamic acid decarboxylase antibody (GADA) titer (LADA1, GADA titer ≥ 180 U/mL; LADA2, GADA titer 18–180 U/mL). Insulin secretion and sensitivity were assessed using hyperglycemic and hyperinsulinemic–euglycemic clamp tests, respectively.

Results The first-phase insulin secretion gradually increased in patients with T1DM, LADA1, LADA2, and T2DM to HCs (29.32 ± 6.00 mU/L vs. 68.71 ± 4.50 mU/L vs. 87.60 ± 11.60 mU/L vs. 138.27 ± 13.18 mU/L vs. 248.49 ± 21.97 mU/L; $P < 0.05$). The second-phase insulin secretion (2 ph) and maximum insulin secretion (MIS) were significantly lower in patients with LADA2 and T2DM than in HCs, but higher in those with LADA1 and T1DM. No significant differences in 2 ph and MIS were observed between patients with LADA1 and T1DM, and between those with LADA2 and T2DM. The levels of insulin sensitivity index (ISI) during hyperinsulinemic–euglycemic clamps were lower in patients with LADA and T2DM than in those with T1DM. Patients with T1DM displayed lower ISI compared with HCs.

Conclusions Chinese patients with LADA and T1DM had impaired insulin sensitivity and β-cell function. Furthermore, the hypothesis that diabetes is a continuous spectrum from T1DM, LADA1, LADA2 to T2DM was confirmed in this study.

Keywords Hyperglycemic clamp test · Hyperinsulinemic–euglycemic clamp test · Insulin resistance · Islet β-cell function · Latent autoimmune diabetes in adults

Introduction

Latent autoimmune diabetes in adults (LADA) is a T cell-mediated islet autoimmune disease and etiologically belongs to type 1A diabetes [1]. However, LADA is manifested as an intermediate phenotype between type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), and characterized by positive islet autoantibodies. Although the mechanism of LADA remains unclear, autoimmunity, islet β-cell function, and insulin sensitivity are all important factors involved in it. Classical T1DM is generally featured as an intensive autoimmune process, causing destruction of β-cell function, and with relatively mild insulin resistance (IR). Classical T2DM is characterized by nonautoimmunity, preserved β-cell function, and a high IR. Most studies on LADA have focused on β-cell function but rarely on IR, which may help one

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understand the pathogenesis of LADA and provide more opportunities for the intervention of LADA. Interestingly, LADA can be further divided into two subtypes: LADA1 and LADA2 based on high and low titers of glutamic acid decarboxylase antibody (GADA), respectively [2]. Besides the GADA titer, the combination of ICA can also help one discriminate LADA1 from LADA2 [3, 4]. The clinical characteristics of LADA1 and LADA2 are close to those of T1DM and T2DM, respectively [3–8]. Therefore, the immunological and clinical features of the disease present a continuous spectrum from T1DM, LADA1, LADA2 to T2DM. This study aimed to explore whether a continuous spectrum exists for their pathological characteristics. Consequently, the β-cell function and IR were assessed in different groups using hyperglycemic and hyperinsulinemic–euglycemic clamp tests, which are the golden standard methods and rarely used in clinical studies.

Methods

Participants

This was a cross-sectional study conducted in the Department of Endocrinology, the Second Xiangya Hospital in Changsha, China, according to the principles of the 1975 Declaration of Helsinki, which was revised in 1983. Diabetes was diagnosed in accordance with the report by the World Health Organization (WHO) in 1999 [9]. The inclusion criteria of patients with T1DM were as follows: (1) insulin-dependent acute-onset ketosis or insulin-dependent ketoacidosis and (2) GADA positivity.

The inclusion criteria of patients with LADA were as follows: (1) diabetes diagnosed according to the WHO report in 1999; (2) aged 25–70 years; (3) GADA positivity; (4) insulin independence for at least 6 months after diagnosis; and (5) no ketosis or ketoacidosis [5, 10]. Patients with high-titer GADA ($\geq$180 U/mL) were defined as LADA1, whereas those with low-titer GADA (between 18 and 180 U/mL) were defined as LADA2 [5, 6].

The diagnostic criteria of T2DM were as follows: (1) diabetes diagnosed according to the WHO report in 1999, (2) GADA negative, and (3) no insulin dependence.

The healthy control (HC) group showed normal glucose tolerance in a 75-g oral glucose tolerance test (OGTT). The exclusion criteria for the HC group were as follows: secondary diabetes, infectious disease or other autoimmune disease, pregnancy, history of immunosuppressive drugs or steroids, and malignant disease.

A total of 42 participants (12 with LADA, 10 with classic T1DM, 10 with T2DM, and 10 HCs) were included in this study.

Study protocol

The patients were admitted to the Department of Endocrinology and Metabolism, the Second Xiangya Hospital, Central South University. All the patients received insulin intensive therapy for 1 week before the study. Slow-release sulfonylureas were discontinued for 48 h and all the other anti-diabetic medication for 24 h before the research program. In patients treated with long-acting insulin, short-acting insulin was used to replace long-acting insulin one night before the clamp test. Patients treated with insulin secretagogue stopped taking this drug for 1 day. On the first day, the hyperglycemic clamp was performed after fasting for 12 h, and fasting blood glucose (BG), C-peptide (CP), and blood lipid were sampled. On day 2, the hyperinsulinemic–euglycemic glucose clamp test was carried out.

GADA assay

The GADA level was tested using the radioligand assay [11–13]. To test the specificity of antibody binding to GAD, when the RLA of GADA was firstly established in our laboratory, competition inhibition analysis with unlabeled purified recombinant human cold GAD (Diamyd, Sweden) was applied to verify the intermediate and low GADA titers in some patients. It was found that in the sera negative for GADA in the absence of cold competitor, adding cold GAD did not increase nonspecific binding, while in all the sera positive for GADA in the absence of cold competitor, adding cold GAD resulted in disappearance of binding completely [12, 13]. GADA positivity was defined as 18 U/mL or higher (WHO units) [5, 6]. The assay has been certified by the Diabetes Antibody Standardization Program (2012) and sponsored by the Immunology of Diabetes Society. The inter- and intra-assay variation coefficients were 7.1–10.8% and 4.9–8.3%, respectively. The sensitivity and specificity of the GADA assay were 82% and 98%, respectively.

Assessment of serum glucose, insulin, CP, and hemoglobin A1c

Blood glucose concentrations during clamp studies were measured using the glucose oxidase method (Glucose and Lactate Analyzer 2300 Stat Plus, Yellow Springs Instrument Co. Inc., OH, USA). The serum insulin was measured by radioimmunoassay (Boehringer Mannheim, Mannheim, Germany). The inter- and intra-assay variation coefficients of insulin were 3.2–4.6% and 2.6–5.9%, respectively. CP was determined by chemiluminescence (ADVIA Centaur,

Siemens, Munich, Germany). The inter- and intra-assay coefficients of the variation were 3.7–4.1% and 1.0–3.3%, respectively. The hemoglobin A1c (HbA1c) levels were measured by automated liquid chromatography (HLC-723G8, Tosoh). The inter- and intra-assay variation coefficients were less than 3% and 1%, respectively.

Hyperglycemic clamp test

A hyperglycemic clamp was carried out 1–2 weeks after the OGTT. At the start of the intervention and time points of follow-up visits, participants underwent a hyperglycemic clamp test after fasting for at least 12 h. Subsequently, normal saline (0.9%) and glucose (20%) were administered in the supine position to initiate perfusion. The blood was drawn through a cannula inserted into the vein on the back of the hand of the other arm. The patient's hand was placed in a 45 °C thermostat to arterialize venous blood. After infusing a bolus of glucose (150 mg/kg), blood glucose was measured using a glucose analyzer (glucose/lactic acid analyzer, Biotin, Inc., EKF, Germany) for every 2 min. Blood glucose was maintained at the level of 13.9 mmol/L for 150 min. Insulin samples were taken at 2-min intervals for the first 10 min and at every 10 min for the remaining 140 min.

The β-cell function was evaluated by calculating the first-phase insulin secretion (1 ph) in the first 10 min of the clamp, which is the area under curve of insulin for the first 10 min of the clamp (using the trapezoid rule). The second-phase insulin secretion (2 ph) was also calculated. Under stable conditions of constant hyperglycemia, the mean plasma insulin concentration for the remaining 140 min was regarded as 2 ph. In addition, the maximum insulin secretion (MIS) was calculated, which was considered as the average insulin values during the 120- to 150-min interval of the clamp test.

Euglycemic–hyperinsulinemic clamp test

The euglycemic–hyperinsulinemic clamp test reported by DeFronzo et al. [14] was used in this study to measure insulin sensitivity. Briefly, on the morning of the trial, after 12-h fasting, a 20-g catheter fitted with a blood sampling connector was retrogradely inserted into a precubital vein to prevent blockage by a slow, continuous saline infusion. Another 20-g catheter was inserted for the infusion of insulin and glucose. When the patient was resting, humulin insulin (Eli Lilly, IN, USA) was infused constantly at a rate of 20 μU/(m^2 · min) [120 pmol/(m^2 · min)] for 2.5 h. Blood glucose was measured by taking blood samples from the opposite side veins every 5 min, and the infusion rate of 20% glucose was adjusted. After insulin administration, the blood glucose was maintained at 5 mmol/L (90 mg/dL) for 150 min. For analysis, steady-state insulin concentrations were defined as the values at the time interval of 120–150 min during clamping tests. Insulin sensitivity, expressed as the glucose disposal rate [GDR; mg/(kg · min)], was determined during the stable state of the euglycemic–hyperinsulinemic clamp test. The ISI [mg · mL/(kg · min · μU)] was calculated by dividing the steady state for the mean glucose infusion rate (mg/kg body weight/min) by the mean insulin concentration (μU/mL).

Statistical analysis

Kolmogorov–Smirnov (KS) test was used to evaluate the normality of continuous data. Continuous variables of normal distribution were represented by mean ± standard deviation. Categorical variables were performed as the number of cases. Differences between groups were evaluated using the analysis of variance test for continuous data, and the Chi-square test and Yates' correction or Fisher's exact test for categorical variables. Bonferroni test for post hoc analysis was used to find where the significant differences actually occurred between groups. All statistical tests were bilateral, and a significance level of $P < 0.05$ was used in all analyses. IBM SPSS version 23 (IBM Corporation, New York, NY, USA) was used for statistical analyses.

Results

Clinical characteristics of various diabetes subgroups of patients and controls

The characteristics of patient are shown in Table 1. No differences in gender and mean duration were found among the four groups. Moreover, the age and body mass index (BMI) were comparable between patients with LADA and T2DM; however, they were lower in patients with T1DM than in those with T2DM, LADA, and HCs. In terms of glucose parameters, no significant differences in fasting BG (FBG), postprandial BG (PBG), and HbA1C were found between LADA and T2DM groups; however, they were all higher in patients with T1DM than in those with LADA. To be specific, the fasting CP (FCP) levels were significantly higher in patients with LADA (326.6 ± 163.0 pmol/L) and T2DM (381.1 ± 209.9 pmol/L) than in those with T1DM (119.5 ± 83.3 pmol/L, both $P < 0.05$), but no difference was found in patients with LADA and T2DM ($P > 0.05$). The blood lipid profile also differed among the three diabetic groups. The serum total cholesterol and triglyceride levels were significantly higher, and the high-density lipoprotein cholesterol concentration was significantly lower in patients with T2DM than in those with T1DM and LADA. No

Table 1 Clinical characteristics of all the study subjects

	NC ($N=10$)	T1DM ($N=10$)	LADA ($N=12$)			T2DM ($N=10$)	Overall p value
			LADA1 ($N=8$)	LADA2 ($N=4$)	Total ($N=12$)		
Age (years)	32.9 ± 6.7	22.0 ± 7.7$^{*\#\#\#}$	33.1 ± 9.5	38.3 ± 7.5	34.8 ± 8.9$^{**\&\&\&}$	39.0 ± 14.3	0.000
Sex (M/F)	5/5	4/6	4/4	2/2	6/6	5/5	0.959
Duration (years)	N/A	2.3 ± 1.0	1.7 ± 1.0	2.0 ± 0.9	1.8 ± 1.1	2.2 ± 1.2	0.935
BMI (kg/m^2)	23.4 ± 1.0	20.5 ± 3.6$^{*\#\#\#}$	21.6 ± 2.2	25.8 ± 2.8	23.0 ± 2.4$^{\#\#\#}$	25.9 ± 2.9$^{\&\&\&}$	0.000
HbA1C (%)	N/A	7.9 ± 1.3	6.5 ± 1.7	6.0 ± 1.7	6.3 ± 1.7$^{\#\&}$	7.8 ± 2.2	0.000
FBS (mmol/L)	N/A	8.61 ± 2.06	7.25 ± 2.19	8.67 ± 2.72	7.72 ± 2.38	8.37 ± 2.30	0.015
2 h BS (mmol/L)	N/A	12.50 ± 2.97	14.77 ± 4.87	16.15 ± 5.48	15.23 ± 5.08	13.05 ± 4.88	0.000
FCP (pmol/L)	N/A	119.5 ± 83.3$^{\#\#\#}$	230.8 ± 87.5	518.2 ± 253.8	326.6 ± 163.0$^{\#\#\&\&\&}$	381.1 ± 209.9	0.000
2 h CP (pmol/L)	N/A	156.0 ± 63.4$^{\#\#\#}$	266.4 ± 156.7	734.2 ± 121.9	667.1 ± 268.2$^{\#\&\&\&}$	1350.5 ± 639.2	0.000
TC (mmol/L)	4.98 ± 0.33	3.09 ± 0.69$^{**\#\#\#}$	4.77 ± 0.61	4.74 ± 0.36	4.76 ± 0.54$^{\#\#\&\&}$	5.28 ± 0.55$^{\&\&\&}$	0.000
TG (mmol/L)	1.27 ± 0.24	1.17 ± 0.27$^{\#\#\#}$	1.12 ± 0.37	1.09 ± 0.40	1.11 ± 0.38$^{\#\#\#}$	2.17 ± 0.42$^{**\&\&\&}$	0.000
HDL (mmol/L)	1.80 ± 0.26	2.22 ± 0.53	2.44 ± 1.23	2.21 ± 0.82	2.36 ± 1.11	1.45 ± 0.41	0.232
LDL (mmol/L)	2.97 ± 0.73	2.44 ± 0.44$^{*\#}$	2.68 ± 1.08	2.98 ± 1.28	2.78 ± 1.15$^{\&}$	2.97 ± 0.73$^{\&}$	0.004

Data are presented as mean ± SD or number of cases. ANOVA for continuous variables and Pearson χ^2 tests for categorical variables.

$^{*}P<0.05$ compared with NC. $^{**}P<0.001$ compared with NC. $^{\#}P<0.05$ compared with T2DM. $^{\#\#}P<0.01$ compared with T2DM. $^{\#\#\#}P<0.001$ compared with T2DM. $^{\&}P<0.05$ compared with T1DM. $^{\&\&}P<0.01$ compared with T1DM. $^{\&\&\&}P<0.001$ compared with T1DM

significant difference was found between T1DM and LADA groups.

Parameters of β-cell function during hyperglycemic clamp test among the four groups

Significant differences in the mean 1 ph, 2 ph, and MIS were found during the hyperglycemic clamp test among the four groups ($P<0.01$; Fig. 1). In descending order, the mean values of 1 ph were 248.5 ± 22.0 mU/L (controls), 138.3 ± 13.2 mU/L (T2DM), 75.0 ± 11.7 mU/L (LADA), and 29.3 ± 6.0 mU/L (T1DM). The patients with T1DM, LADA, and T2DM all showed significantly lower 1 ph, 2 ph, and MIS compared with the HCs (all $P<0.05$). The 1 ph, 2 ph, and MIS were all higher in patients with LADA than in those with T1DM (all $P<0.05$), but lower than in those with T2DM. The 2 ph and MIS were comparable between patients with LADA2 and T2DM, but significantly greater in patients with LADA1 and T1DM (both $P<0.05$). Moreover, the 1 ph, 2 ph, and MIS were the highest in the HCs, followed by the patients with T2DM, LADA1, LADA2, and classic T1DM.

Insulin sensitivity assessed by the euglycemic–hyperinsulinemic clamp test among the four groups

Significant differences in the mean GDRs and ISI were found during the euglycemic–hyperinsulinemic clamp test among the four groups ($P<0.01$, Fig. 2). The GDRs and ISI were both lower in patients with LADA than in those with T1DM [GDR, 7.70 ± 1.78 in LADA and 11.02 ± 0.72 mg/(kg · min) in T1DM; ISI, 4.35 ± 0.89 in LADA vs. 7.00 ± 1.35 mg · mL/(kg · min · μU) in T1DM; all $P<0.05$)]. Moreover, patients with LADA and T2DM had similar GDRs and ISI [GDR, 8.03 ± 1.70 mg/(kg · min); ISI, 4.65 ± 0.98 mg · mL/(kg · min · μU), both $P<0.05$]. The patients with T1DM showed lower ISI compared with HCs [7.00 ± 1.45 vs. 11.80 ± 2.71 mg mL/ (kg · min · μU), $P<0.05$]. Therefore, the GDRs and ISI were comparable between patients with LADA and T2DM but were significantly greater in those with T1DM.

Discussion

LADA is a specific subtype of T1DM and is characterized by gradual dysfunction of islet β cells. More importantly, LADA shares the features of both T1DM and T2DM and is in the middle of the diabetes spectrum, which is called the type 1.5 diabetes [1, 4, 15–19]. LADA is identified from phenotypic T2DM patients by islet autoantibodies including GADA, phosphatase protein tyrosine antibody (IA2-A), zinc transporter 8 antibody (ZnT8A) and insulin autoantibody (IAA) in clinic [20–22], but GADA has been demonstrated as the most important one for the diagnosis of LADA [5, 10, 23]. Many researchers have confirmed that GADA titers can identify two subgroups of patients with adult-onset autoimmune diabetes with distinct clinical, autoimmune, and genetic features named LADA1 and LADA2. Compared with LADA2 patients,

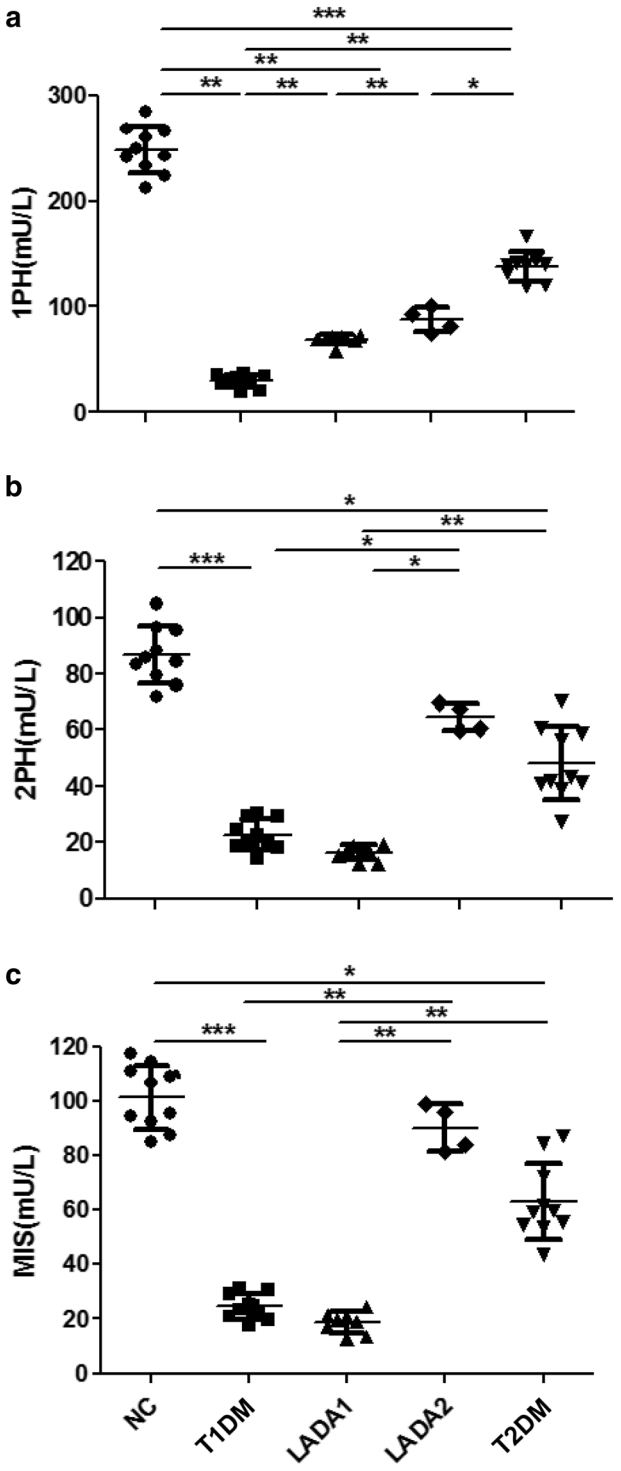

Fig. 1 Derived parameters of β-cell function during hyperglycemic clamp test in the four groups. **a** The first-phase insulin secretions in patients with T1DM, LADA, and T2DM during the hyperglycemic clamp test. **b** The second-phase insulin secretions derived from the hyperglycemic clamp test in each group. **c** Maximum insulin secretion in the four groups during the hyperglycemic clamp test. Data are dot plots with indications of mean values and SD. $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$

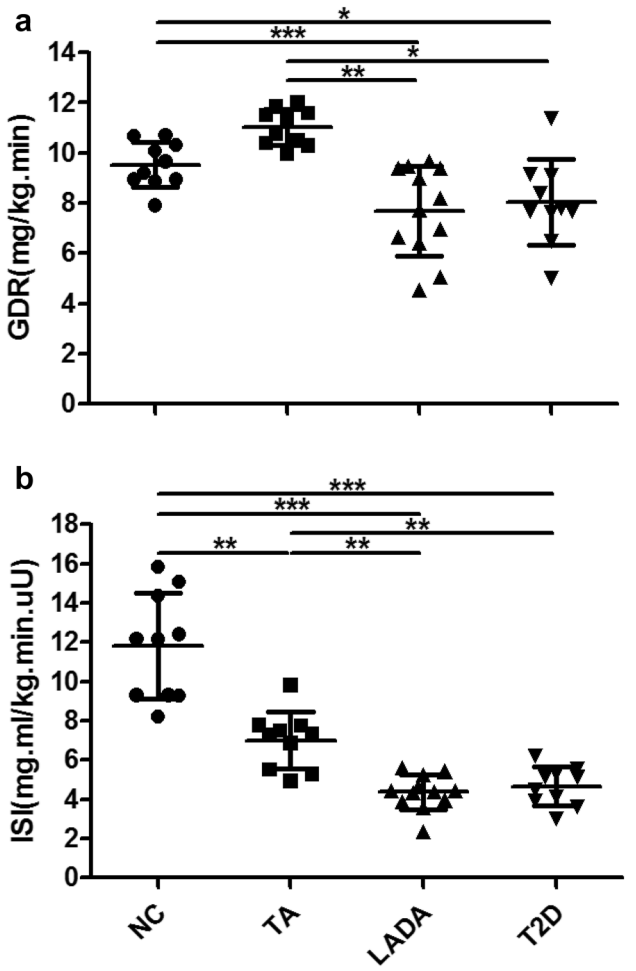

Fig. 2 Derived variables of hyperinsulinemic–euglycemic clamp test in the four groups. **a** Glucose disposal rate (GDR) at baseline during the hyperinsulinemic–euglycemic clamp test in each group. **b** Insulin secretion index (ISI) derived from the hyperinsulinemic–euglycemic clamp test in study groups. Data are dot plots with indications of mean values and SD. $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$

LADA1 presented as much leaner, with worse insulin deficiency, lower prevalence of metabolic syndrome and its components [3–7]. Zampetti et al. [2] demonstrated some different anthropometric and biochemical features in the two groups that high GADA titer was associated with phenotype of more severe autoimmunity and, in male gender, predisposed to thyroid autoimmunity. Consistent with this, we also reported that thyroid-antibody-positive LADA patients had higher GADA titre and lower C-peptide levels, and they were associated with the development of thyroid autoimmunity [23]. Maybe high GADA titer was associated with a profile of more severe other organ-specific autoimmunity which may further help us to differentiate LADA1 and LADA2 patients in clinic to some extent [2, 23]. In this study, we only enrolled GADA-positive LADA patients because we would like to observe

the subtle differences in islet function and IR between LADA1 and LADA2 subtypes which is differentiated by GADA titer [4–7].

The assessment of β-cell function in patients with LADA in earlier studies was more based on crude measurements of insulin secretion, including FCP [24, 25] and glucose-/glucagon-/arginine-stimulating CPs [26], or the homeostasis model assessment (HOMA) model [27]. These tests would hardly stress the β cells to their limits to allow the detection of more subtle defects in the β-cell capacity. One important advantage of the assessment of islet function using the golden standard test, which is the hyperglycemic clamp test, was that it quantified β-cell function in response to glucose and examined the early- and late-phase insulin secretion independent of IR. This study was novel in using the hyperglycemic and hyperinsulinemic–euglycemic clamp tests simultaneously to examine the islet β-cell function and IR in Chinese patients with LADA and further verify the hypothesis that diabetes is a continuous spectrum from T1DM, LADA to T2DM. Although LADA belongs to T1DM, IR may also exist in patients with LADA; however, relevant studies are rare [4, 28]. Carlsson et al. observed the response of β cells to glucose and arginine in 11 patients with LADA at 3 blood glucose levels (5.6/14/28 mmol/L) and used half of the glucose levels at peak insulin secretion when stimulated by arginine to reflect insulin sensitivity. They found no difference between LADA and T2DM [29]. Another study showed an equivalent impairment in insulin sensitivity in LADA and T2DM compared with HCs [30]. Recently, a European study, ACTION LADA8, found no difference in insulin secretion or insulin sensitivity between LADA and T2DM after adjusting for BMI [31]. Except the ACTION LADA 8 study, which used the modified hyperinsulinemic–euglycemic clamp test, most studies used HOMA or arginine test to assess IR. First, the aforementioned outcomes were validated using the golden standard hyperinsulinemic–euglycemic clamp test in Chinese patients with LADA. IR existed in patients with LADA, but was less severe than in those with T2DM when using HOMA, as evaluated in 2005; similar results were found from other studies [5, 32].

The mechanism of IR in LADA is unclear. It may be related to metabolic disorders such as sugar toxicity, lipid toxicity, and obesity [33–35]. Another study on Chinese patients with LADA found that the patients also had metabolic syndrome (MS) [5, 15]. The clinical manifestations of patients with LADA without MS were not statistically different from those of the nondiabetic control group in terms of BMI and blood lipid level. However, the ISI was significantly lower in these patients compared with the HCs, suggesting that IR might still exist in LADA even without MS. Therefore, patients with LADA can benefit from insulin sensitizers, which confirms the aforementioned findings of IR in LADA [36, 37].

Also, a few studies evaluated the insulin sensitivity in LADA and compared the result with that in T1DM and T2DM. In this study, the LADA group exhibited more severe IR compared with the T1DM group. Although β-cell dysfunction was the main pathological feature involved in the pathogenesis of classic T1DM, Chinese patients with T1DM were found to have impaired insulin sensitivity and β-cell function using the clamp test. The underlying molecular mechanism deserves further carefully designed investigation.

This study demonstrated that IR was found in both LADA and T2DM at a similar magnitude, although it was severe in LADA and T2DM compared with T1DM. Moreover, the study validated that the islet β-cell function gradually increased from classic T1DM to LADA and finally to T2DM using the hyperglycemic clamp test. Thus, the hypothesis that diabetes is a continuous spectrum from T1DM, LADA1, LADA2 to T2DM was confirmed in this study using the clamp test. The findings might lead to the development of novel immunologic therapies to protect the β-cell function and improve insulin sensitivity in patients with LADA.

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Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethical committee of the Second Xiangya Hospital of Central South University (#2010-S(001)) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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