Islet function and insulin sensitivity in latent autoimmune diabetes in adults taking sitagliptin: A randomized trial

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Abstract:

**Context:** The long-term effects of dipeptidyl peptidase-4 inhibitors on β-cell function and insulin sensitivity in latent autoimmune diabetes in adults (LADA) are unclear.

**Objective:** To investigate the effects of sitagliptin on β-cell function and insulin sensitivity in LADA patients receiving insulin.

**Design and Setting:** A randomized controlled trial at the Second Xiangya Hospital.

**Patients and Interventions:** Fifty-one patients with LADA were randomized to sitagliptin + insulin group (SITA group) or insulin alone group (CONT group) for 24 months.

**Main Outcome Measures:** Fasting C-peptide (FCP), 2-hour postprandial C-peptide (2hCP) during mixed-meal tolerance test, ΔCP (2hCP - FCP) and updated homeostatic model assessment of β-cell function (HOMA2-B) were determined every 6 months. In 12 subjects, hyperglycemic clamp and hyperinsulinemic euglycemic clamp (HEC) tests were further conducted at 12-month intervals.

**Results:** During the 24-month follow-up, there were no significant changes in β-cell function in SITA group, whereas the levels of 2hCP and ΔCP in CONT group were reduced at 24 months. Meanwhile, the changes in HOMA2-B from baseline were larger in SITA group than in CONT group. At 24 months, first-phase insulin secretion was improved in SITA group by hyperglycemia clamp, which was higher than in CONT group (P<0.001), while glucose metabolized(M), insulin sensitivity index and M over logarithmical insulin ratio in HEC were increased in SITA group (all P<0.01 vs. baseline), which were higher than in CONT group.

**Conclusions:** Compared with insulin intervention alone, sitagliptin plus insulin treatment appeared to maintain β-cell function and improve insulin sensitivity in LADA to some extent.

**Key words:** Sitagliptin; islet β-cell function; insulin sensitivity; latent autoimmune diabetes in adults
Latent autoimmune diabetes in adults (LADA) is a subtype of autoimmune type 1 diabetes, accounting for approximately 6-10% of patients with phenotypic type 2 diabetes (1,2). Importantly, the decreasing rate of islet β cell function in LADA is three times that in type 2 diabetes, as we have reported(3). Thus, the therapeutic goal for LADA is to suppress autoimmune islet destruction, preserve β-cell function and prevent complications. However, the optimal intervention strategy for LADA is currently not yet clear. Since insulin therapy had been shown to have a beneficial effect in LADA(4,5), we tried to explore promising agents plus insulin as a therapeutic strategy for LADA.

Dipeptidyl peptidase-4 (DPP4) inhibitors, a class of oral antidiabetic agents, seem to have the potential to preserve β-cell function in LADA in our pilot study(6). However, due to the small sample size, relatively short follow-up period and the rough assessment of β-cell function, there are few clinical trials to date that have evaluated the potential long-term effects of DPP-4 inhibitors on islet β-cell function accurately in patients with LADA.

Here, we carried out a 2-year randomized controlled parallel study to investigate the potential long-term effects of the DPP-4 inhibitor sitagliptin on islet β-cell function in patients with LADA receiving insulin. The hyperglycemic clamp test was used to quantify islet β-cell function in some participants.

In addition to islet β-cell function, insulin sensitivity is another important target. Some data regarding DPP-4 inhibitors have implied potential benefits to improve insulin sensitivity in patients with type 2 diabetes, but are still inconclusive at present(7,8). In this study, we further explored the possible effects of sitagliptin on insulin sensitivity in patients with LADA by hyperinsulinemic euglycemic clamp (HEC) technique.
Materials and Methods

Participants

This was an open-label randomized controlled clinical trial conducted in the Department of Metabolism and Endocrinology at the Second Xiangya Hospital of Central South University. Recruitment occurred between December 2014 and December 2017. The inclusion criteria were as follows: (1) diagnosis of diabetes (World Health Organization 1999 criteria); (2) age 25-70 years; (3) duration of diabetes ≤ 3 years; (4) insulin-independence for at least 6 months postdiagnosis; (5) glutamic acid decarboxylase autoantibody (GADA) positivity; and (6) fasting C-peptide (FCP) ≥ 0.2 nmol/L. The exclusion criteria included (1) insulin requirements > 0.8 U/kg/day; (2) chronic or acute infection within 4 weeks prior to visit 1; (3) history of any malignancy; (4) pregnancy, lactation or planned pregnancy within 2 years; (5) specific types of diabetes; (6) congestive heart failure; (7) serum creatinine ≥ 1.5 mg/dL for males and ≥ 1.4 mg/dL for females; (8) history of pancreatitis; or (9) other severe diseases.

All procedures followed were in accordance with the ethical standards of Ethics Committee of the Second Xiangya Hospital of Central South University and with the Helsinki Declaration. The study protocol was available at www.clinicaltrials.gov (identifier: NCT01159847). Informed consent was obtained from all participants included in the study.

Study protocol

Briefly, patients who met the inclusion and exclusion criteria entered a screening period for at least 4 weeks. During this period, the subjects used single insulin therapy to achieve the glycemic target of glycosylated hemoglobin A1c (HbA1c) ≤ 7.5%. After the screening period, 51 eligible patients entered a 24-month treatment period and were randomized 1:1 to receive insulin therapy with sitagliptin 100mg daily (SITA group, n=25) or without sitagliptin (CONT group, n=26) according to simple randomization procedures (computer-generated random
numbers) and were followed at baseline, 6, 12, 18 and 24 months. The allocation sequence was concealed from the researchers and assessing patients in sequentially numbered envelopes. Only after the enrolled participants completed all baseline assessments, corresponding envelopes were opened, and it was time to allocate the intervention. In our initial protocol, every participant in the study was suggested to receive hyperglycemic clamp and HEC tests at baseline, 12 months and 24 months. Due to the large volume of blood requirement, frequent blood collection and time-consuming procedures of the clamp technique, research patients who refused the glucose clamp tests were allowed to participate in the study for better feasibility. Eventually, 12 subjects (6 patients in each group) with excellent compliance finished all clamp tests.

During the study, other antidiabetic agents were not allowed except insulin and sitagliptin. Basal insulin analogues (glargine), premixed insulin (aspart 30 or lispro 25) or basal (glargine) + bolus insulin (aspart) regimens were assigned to patients based on their glycemic profile(9). The insulin doses were adjusted by physicians to reach the glycemic goal without hypoglycemia based on the American Association of Clinical Endocrinologists (AACE) Comprehensive Diabetes Management Algorithm 2013(9).

Two prespecified primary outcomes were chosen: (1) the change in β-cell function by the standardized mixed-meal tolerance test (MMTT) at 6, 12, 18 and 24-month of the study; and (2) the change in β-cell function with hyperglycemic clamp and insulin sensitivity with HEC in 12 LADA patients at 12 and 24 months. Secondary parameters included glycemic control measured by HbA1c and possible immunomodulatory effects on T cell subsets and transcription factors, which have been published(10).

To detect a 2hCP difference between two groups of 0.56 nmol/L (standard deviation, SD, 0.62 nmol/L) according to our previous data for 12 months, with a two-sided 5% significance level and a power of 90%, a sample size of 25 patients per group was necessary given an
anticipated dropout rate of 10% per year.

**GADA assay**

GADA was analyzed by a radioligand assay in duplicate as previously described(11). GADA positivity was defined as a GADA titer ≥ 18 U/mL, and high-titer GADA was defined as ≥ 180 U/mL(12). In the Islet Autoantibody Standardization Program (IASP) 2012, the sensitivity and specificity for the GADA assay were 78.0% and 96.7%, respectively.

**Mixed-meal tolerance test**

Serum glucose and C-peptide levels were measured before and 120 minutes after a standard 543.6 kilocalories MMTT (44.4% of calories as carbohydrate, 47.7% as fat, and 7.9% as protein). Serum C-peptide levels were detected by a chemiluminescence method using the Adiva Centaur XP immunoassay system (Siemens, Germany). The inter- and intra-assay variation coefficients were 3.7–4.1% and 1.0–3.3%, respectively. Sitagliptin was held for 7 days (> 10 half-lives). Long-acting insulin was withheld the night before visits, and morning doses of insulin were withheld on the day of MMTT, hyperglycemic clamp test (the second day) and HEC test (the third day). The updated homeostasis model assessment (HOMA2)(13) was used to measure β-cell function (HOMA2-B) and insulin resistance (HOMA2-IR) based on FCP and fasting blood glucose (FBG) according to the calculator available at https://www.dtu.ox.ac.uk/homacalculator/index.php.

**Assessment of insulin and HbA1c**

Serum insulin was measured by a radioimmunoassay (Boehringer Mannheim, Mannheim, Germany), and HbA1c was measured by automated liquid chromatography (VARIANT-II Hemoglobin Testing System; Bio-Rad Laboratories, Hercules, CA).
**Hyperglycaemic clamp test**

At baseline, 12 and 24 months, 12 subjects completed hyperglycaemic clamp tests after fasting for at least 12 hours as described before (14). A rapid infusion of glucose solution (20%) was administered intravenously to raise the blood glucose level to reach the plateau of 13.9 mmol/L. Blood was sampled through an intravenous catheter needle inserted into the median cubital vein of the other arm. The venous blood was arterialized by placing the hand in a 45°C thermostat (15). After infusion of a bolus of glucose (150 mg/kg), blood glucose was measured with a glucose analyser (Glucose/lactic acid analyzer, Biotin, Inc., EKF, Germany) at 2-min intervals. Thereafter, blood glucose was maintained for 150 min at the level of 13.9 mmol/L by adjusting the glucose infusion rate (GIR) according to blood glucose level assessed every 5 min. Blood samples were collected at 2-min intervals during the first 10 minutes and at 10-min intervals for the remaining 140 min for measurement of serum insulin.

To estimate acute β-cell function, the first-phase insulin secretion (1PH) was analyzed as the area under the curve of the insulin levels for the first 10 min of the clamp, using the trapezoidal rule. Moreover, the second-phase insulin secretion (2PH) was regarded as the mean insulin concentration for the remaining 140 min. In addition, the maximum insulin secretion (MIS) was calculated as the average serum insulin levels between 120 and 150 min of the clamp.

**Hyperinsulinemic euglycemic clamp test**

The HEC test was performed according to the protocol based on DeFronzo et al. (16) and slightly adjusted according to Parvanova et al. (17). Briefly, after an overnight fast, a catheter was inserted into one antecubital vein for the insulin and glucose infusion. Regular human insulin (Humulin R, Eli Lilly, Indianapolis, Ind) was infused. A priming insulin dose was administered at a rate of 4 mU/(kg body weight·min) for the first 10 min, followed by a
constant insulin infusion at a rate of 2 mU/(kg body weight·min) for the remaining 140 min of clamping to achieve insulin concentration ~200 mU/L with total suppression of hepatic glucose production(17,18). Another catheter connected to a T-branch pipe for blood sampling was inserted into the median cubital vein of the other arm. The blood glucose level was maintained with a target of 5 mmol/L for 150 min by adjusting the GIR of 20% glucose solution according to blood glucose measured every 5 min. Blood samples were drawn for measurement of serum insulin every 10 min during 120-150 min of the clamp test.

The insulin sensitivity, expressed in terms of glucose metabolized (M), was calculated as average value of GIR in steady state of clamp (120-150 min) minus space correction as there was no need to correct for urinary loss of glucose in HEC. The insulin sensitivity index (ISI, \(M/I \times 100\)) was determined as the ratio of the M value in steady state divided by the average serum insulin concentration (I) in steady state multiplied by 100. In addition, \(M/\log I\) ratio (i.e. the ratio of M divided by the natural logarithmical insulin concentration in steady state) was also included to assess insulin sensitivity since the effects of insulin on glucose uptake increase in a logarithmical manner(19,20).

**Safety assessments**

Safety and tolerability were evaluated based on adverse events, vital signs, physical and laboratory examinations throughout the study. Adverse events were assessed by physicians for the frequency, intensity and relationship with drugs. Hypoglycemia was confirmed as a blood glucose level \(\leq 3.9 \text{ mmol/L}\) with related symptoms.
Statistics

Statistical analysis was performed with IBM SPSS Statistics 20 (IBM Corporation, USA). The normal distribution of data was tested by the one-sample Kolmogorov-Smirnov test. Continuous data with a normal distribution are presented as mean ± SD or as indicated. Categorical variables are expressed as the number of cases, percentages or as indicated. Comparisons between groups were performed using independent Student’s t-test for variables with a normal distribution. Covariance analysis was adopted to adjust the effects of FBG and low-density lipoprotein (LDL) cholesterol on islet function. A Chi-square test was used to compare categorical variables between groups. The changes in parameters during follow-up were analyzed by repeated measures ANOVA, and the degree of freedom for F value was adjusted if spherical symmetry was rejected. Multivariate analysis of variance (MANOVA) was used to compare the variables between groups at each follow-up time point. Due to the exploratory analysis of intra-group and inter-group treatment effects, the P value of multiple comparison was not adjusted. A two-sided P value of <0.05 was considered significant.

Results

Baseline characteristics and demographics

The analysis excluded patients who could not meet the HbA1c control targets in scheduled and extra visits to eliminate the effect of glucose toxicity on islet function. Among 51 randomized cases, 22 subjects in SITA group and 25 patients in CONT group were analyzed at 6 and 12 months, while 18 in SITA group and 22 in CONT group were included for analysis at 24 months (Figure 1). Twenty subjects (ten cases in each group) received premixed insulin regimens.

Among the patients who finished 1-year follow-up, the baseline FBG level in SITA group was higher than that in CONT group (Table 1, P=0.024). Moreover, the baseline level of LDL cholesterol in SITA group was higher too (P=0.036). There were no significant differences in
the baseline FCP, 2-hour postprandial C-peptide (2hCP) and \( \triangle CP \) (\( \triangle CP = 2hCP - FCP \)) between two groups after adjusting for FBG and LDL cholesterol by covariance analysis. No significant differences were observed in other baseline parameters between these two groups.

**Glycaemic control**

There were no significant differences in HbA1c levels between two groups at each follow-up time point. Compared with the baseline, there were no significant differences in HbA1c levels at different follow-up time points in both groups (Table 2, all \( P > 0.05 \)).

**Body mass index**

No significant differences were observed in body mass index (BMI) between two groups at each follow-up time point and there were no significant differences in BMI at each follow-up time point in both groups compared with the baseline (Table 2, all \( P > 0.05 \)). Changes in BMI at 24 months from baseline in two groups seemed different, but no statistical significance was found (\( P = 0.051 \)).

**Daily insulin dose**

There were no significant differences in the insulin requirements (U/kg/day) between two groups at each follow-up time point. Moreover, compared with those at baseline, no significant differences in insulin requirements were found at different follow-up visits in both groups (Table S1 in the repository) (21).

**Evaluation of islet \( \beta \)-cell function by MMTT**

Among the patients who completed the 1-year follow-up, changes in islet \( \beta \)-cell function in SITA group (n=22) were not noted, while the levels of 2hCP in CONT group (n=25) significantly decreased at 6 months [Table 2, mean (95% confidence interval, CI) change from baseline: -0.28 (-0.56, -0.01) nmol/L, \( P = 0.043 \)] and 12 months [-0.49 (-0.73, -0.24) nmol/L, \( P < 0.001 \)]. Likewise, the levels of \( \triangle CP \) declined at 12 months [-0.43 (-0.65, -0.21) nmol/L, \( P = 0.001 \)]. Furthermore, changes in \( \triangle CP \) as well as HOMA2-B from baseline in
SITA group were significantly higher than those in CONT group [difference (95% CI) in changes of △CP from baseline: 0.30 (0.01, 0.57) nmol/L, \( P=0.042 \); difference (95% CI) in changes of HOMA2-B from baseline: 19.0 (0.1, 38.0) %, \( P=0.049 \)].

Based on the results from patients who completed 1.5-year follow-up, there were no notable changes in islet β-cell function in SITA group (n=19), while the HOMA2-B, 2hCP and △CP in CONT group (n=23) were significantly reduced from baseline at 18 months [mean (95% CI) change from baseline in HOMA2-B: -19 (-36.2, -1.8) %, \( P=0.032 \); 2hCP: -0.35 (-0.61, -0.09) nmol/L, \( P=0.010 \); △CP: -0.30 (-0.55, -0.06) nmol/L, \( P=0.019 \)]. In the meantime, the changes in HOMA2-B from baseline in SITA group were significantly higher than those in CONT group [difference (95% CI): 31.3 (9.9, 52.6) %, \( P=0.005 \)].

Among the patients who finished the 2-year follow-up, there was still no significant change in islet β-cell function in SITA group (n=18) from baseline; in CONT group (n=22), 2hCP and △CP decreased gradually at 24 months from baseline [mean (95% CI) change from baseline in 2hCP: -0.29 (-0.53, -0.06) nmol/L, \( P=0.018 \); △CP: -0.27 (-0.50, -0.04) nmol/L, \( P=0.024 \)]. In addition, the changes in HOMA2-B from baseline in SITA group were higher than those in CONT group at 24 months [difference (95% CI): 31.6 (4.6, 58.4) %, \( P=0.023 \)].

**Evaluation of islet β-cell function by hyperglycemic clamp**

At baseline, no significant difference in demographic and clinical characteristics were observed between two groups who completed the hyperglycemic clamp and HEC tests (6 cases in each group, data not shown). Among these 12 patients, one in SITA group and two cases in CONT group had premixed insulin therapy. The detail figures of insulin levels during hyperglycemic clamp at baseline, 12-month and 24-month for each group were presented in
the data repository(21). The levels of fasting insulin (FINS) in SITA group increased continually from 6.7±1.1 mU/L to 7.5±1.0 mU/L at 12 months [Fig. 2A, mean (95% CI) change from baseline: 0.8 (0, 1.6) mU/L, \( P=0.049 \)] and further to 8.8±1.0 mU/L at 24 months \([2.1 (0.6, 3.6) \text{ mU/L}, \ P=0.017]\). In contrast, the FINS levels in CONT group decreased significantly from baseline to 24 months \([4.4±1.1 \text{ vs. } 7.8±2.1 \text{ mU/L}, \ -3.4 (-5.1, -1.7) \text{ mU/L}, \ P=0.004]\). At 24 months, the FINS level in SITA group was significantly higher than that in CONT group [difference (95% CI): 4.4 (3.0, 5.7) mU/L, \( P<0.001 \)]. Similarly, the 1PH insulin response increased from baseline to 24 months in SITA group [Fig. 2B, 73.4±12.1 vs. 81.6±7.4 mU/L, 8.2 (0.5, 15.9) mU/L, \( P=0.040 \)], but declined significantly in CONT group (baseline, 76.6±12.2 vs. 24 months, 56.2±4.5 mU/L; \(-20.4 (-29.4, -11.4) \text{ mU/L}, \ P=0.002\)).

The levels of 1PH in SITA group were higher than those in CONT group at 24 months [difference (95% CI): 25.4 (17.6, 33.3) mU/L, \( P<0.001 \)]. Whereas there were no significant changes in the 2PH and MIS in two groups during follow-up (Fig. 2C and Fig. 2D, both \( P>0.05 \)).

**Evaluation of insulin sensitivity by HEC**

The detail figures of insulin levels and GIR during HEC at baseline, 12-month and 24-month for each group were shown in the data repository(21). In SITA group, M increased gradually from 7.0±2.1 mg/(kg·min) to 8.6±2.5 mg/(kg·min) at 12 months [Fig. 3A, mean (95% CI) change from baseline: 1.6 (0.7, 2.6) mg/(kg·min), \( P=0.007 \)] and to 10.4±1.6 mg/(kg·min) at 24 months [3.4 (1.4, 5.5) mg/(kg·min), \( P=0.007 \)], while M in CONT group did not change during follow-up. Moreover, M levels in SITA group were much higher than those in CONT group at 24 months \([10.4±1.6 \text{ vs. } 8.2±1.2 \text{ mg/(kg·min)}, \ 2.2 (0.4, 4.0) \text{ mg/(kg·min), } P=0.019] \). Likewise, the ISI levels in SITA group increased from baseline to 12 months [Fig. 3B, 5.0±1.8 vs. 3.5±1.1 (mg·mL)/(kg·min·μU), mean (95% CI) change from baseline: 1.5 (0.3, 2.7) (mg·mL)/(kg·min·μU), \( P=0.027 \)] and further to 5.8±1.7
(mg·mL)/(kg·min·μU) at 24 months [2.3 (0.9, 3.6) (mg·mL)/(kg·min·μU), \(P=0.008\)], but decreased significantly in CONT group from baseline to 24 months [3.8±0.8 vs. 4.5±0.6 (mg·mL)/(kg·min·μU), -0.7 (-1.1, -0.4) (mg·mL)/(kg·min·μU), \(P=0.003\)]. Moreover, the ISI levels in SITA group were much higher than those in CONT group at 24 months [difference (95% CI): 2.0 (0.3, 3.7) (mg·mL)/(kg·min·μU), \(P=0.024\)]. Similarly, the M/log I ratio in CONT group did not alter during follow-up while M/log I in SITA group increased from baseline to 12 months [Fig. 3C, 1.7±0.4 vs. 1.3±0.4 (mg·mL)/(kg·min·μU), mean (95% CI) change from baseline: 0.3 (0.1, 0.5) (mg·mL)/(kg·min·μU), \(P=0.011\)] and further to 24 months [0.7 (0.3, 1.0) (mg·mL)/(kg·min·μU), \(P=0.004\)]; it was higher than that in CONT group [2.0±0.2 vs. 1.5±0.2 (mg·mL)/(kg·min·μU), difference (95% CI): 0.5 (0.2, 0.7) (mg·mL)/(kg·min·μU), \(P=0.004\)].

**Safety and tolerability**

One patient in the SITA group dropped out at 18 months due to severe elevation of transaminase (hepatitis B virus related), which returned to a normal level after entecavir therapy. No other severe adverse event or serious adverse event was reported. During 24-month follow-up, two cases in SITA group (one at 18-month and the other case at 24-month follow-up) and one in CONT group (at 12-month) had detected hypoglycemia (blood glucose level < 3.9 mmol/L).

**Discussion**

This study suggested that sitagliptin combined with insulin therapy seemed to delay the decline of islet β-cell function and improve insulin sensitivity in patients with LADA compared with insulin treatment alone. To our knowledge, this is the first prospective study using the glucose clamp technique to evaluate the effects of DPP-4 inhibitors on insulin secretion and insulin resistance in patients with LADA.
In this study, the baseline islet \( \beta \)-cell function (FCP, HOMA2-B, 2hCP and \( \triangle \)CP) as well as insulin resistance (HOMA2-IR) were comparable between two groups. During the follow-up period, both groups were in good glycaemic control, eliminating the effect of glucose toxicity on islet function and the possibility of islet \( \beta \)-cell resistance to glucagon-like peptide-1 (GLP-1) under poor glycaemic control\(^{22}\). In addition, one-week sitagliptin washout (>10 half-lives) before the visits eliminated the potential bias in the assessment of islet function by acute stimulation of insulin secretion via incretin in SITA group\(^{23}\).

Currently, there are several methods to measure islet \( \beta \)-cell function. (1) Hyperglycemic clamp test, which is considered as the standard method to evaluate islet \( \beta \)-cell function, is only suitable for small-sample scientific research due to the labor-consuming, time-costing, high-cost technique; (2) Intravenous glucose tolerance test is not affected by gastrointestinal hormones and individual absorption but with complex procedures, frequent blood collection and poor repeatability; (3) Minimum model has similar disadvantages with hyperglycemic clamp; (4) Oral glucose tolerance test (OGTT) is simple and suitable for epidemiological studies. However, it is affected by glucose absorption, thus is not accurate enough, and it is difficult to reflect the first-phase insulin secretion. (5) Arginine stimulation test and glucagon stimulation test (GST) are simple methods but unable to measure the second-phase insulin secretion and to estimate the response of islet \( \beta \)-cells to glucose. (6) MMTT, containing more stimuli than OGTT, simulates insulin secretion under more physiological conditions. However, the load of MMTT is needed to be standardized. (7) HOMA is simple to evaluate \( \beta \)-cell function and insulin sensitivity, thus it is of great value in epidemiological studies. But sometimes it may overestimate the islet \( \beta \)-cell function. In a word, there is not any perfect method to quantify \( \beta \) cell function to date given that insulin secretion is a dynamic and nonlinear process in addition to its synthesis and storage processes. Herein, we attempted to evaluate \( \beta \) cell function by the combined application of several methods, such as FCP,
HOMA2-B, MMTT 2hCP, △CP as well as hyperglycemic clamp test. In general, the MMTT is the recommended test and C-peptide, not affected by exogenous insulin, is the appropriate outcome measure for the assessment of β cell function in intervention trials in type 1 diabetes(24-27). The 2hCP and △CP values during MMTT may reflect the secretory capacity in response to a mixed meal, and our group has utilized 2hCP, △CP as well as FCP to measure the effects of oral antidiabetic agents including rosiglitazone(28,29), sitagliptin(6) and saxagliptin or combined with vitamin D3(30) on β cell function in a series of clinical trials in patients with LADA. FCP is frequently used in clinical practice due to its good correlation with MMTT or glucagon stimulated C-peptide(31-34) and the ability to predict β cell function failure in autoimmune type 1 diabetes(35,36). Moreover, FCP is not affected by gastrointestinal status compared with 2hCP and △CP, but it is affected by fasting blood glucose. Matthews et al. updated the homeostasis model assessment in 1998(13), making it closely related with glucose clamp test, minimum model etc. In our study, although there was no significant difference in FCP between two groups during follow-up, the parameters of HOMA2-B, 2hCP and △CP consistently demonstrated the protective benefit of sitagliptin on islet β-cell function in patients with LADA. In addition, mathematical model could extract more useful information on overall β-cell function(37-40), but the outcomes varied. The inability to develop a mathematical model of insulin secretion was one of our limits. Herein, our results of the hyperglycemic clamp test displayed that sitagliptin combined with insulin therapy could improve the first-phase insulin secretion and the reserve function of islet β-cells. Some studies have revealed that GLP-1 analogue exenatide could improve the acute insulin response (AIR) in intravenous glucose tolerance test in patients with type 2 diabetes(41). However, it is not clear whether sitagliptin improves 1PH indirectly by increasing active GLP-1. Here, both MMTT and hyperglycemic clamp test showed that sitagliptin plus insulin treatment appeared to halt the decline of islet β-cell function in
patients with LADA compared with insulin alone. Similarly, in the few clinical trials of DPP-4 inhibitors in LADA (saxagliptin: 6 months(42), 12 months(30); sitagliptin: 12 months(6), 21 months(43) and 48 months(44); linagliptin(45): 24 months), except the Norway study(43) and our pilot study of saxagliptin(30) (further discussion below), these studies consistently revealed the protective effects of DPP-4 inhibitors on islet function in LADA. However, the above trials may have limited clinical significance because of short follow-up periods(6,30,42), small sample sizes(44,45), historical control(44), and significant different baseline FCP levels between groups(45), and so on. In our study, the longitudinal follow-up was up to 24 months, and a total of 40 patients with LADA were followed at 24 months.

A randomized controlled clinical trial conducted in Norway and published in 2019(43) (Norway study for short below) did not show an advantage on β-cell function in 32 LADA patients taking sitagliptin compared with 32 LADA patients receiving insulin, which was not completely consistent with our study. The reasons for this inconsistency may include the following: (1) distinct treatment protocols: the Norway study did a direct comparison between insulin and sitagliptin, and insulin was not permitted in sitagliptin group; (2) different methods for evaluating islet function: previous studies have indicated that glucagon stimulation test (GST) conducted in the Norway study appeared to be less sensitive and less reproducible than MMTT we carried out(24) in addition to the hyperglycemic clamp test applied in our study; and (3) different study population: the participants in the Norway study seemed to have larger BMIs, representing varying degrees of insulin resistance, which could affect the efficacy of DPP-4 inhibitors(46). Nevertheless, from another point of view, the Norway study only showed that the protective effects of single sitagliptin therapy on β-cell function in LADA may be comparable with insulin since the current evidence-based medicine has confirmed the benefits of insulin on β-cell function in LADA(4,5).
Interestingly, in our recent pilot study, compared with conventional therapy (metformin and/or insulin), vitamin D3 plus saxagliptin and conventional therapy could maintain β-cell function in LADA rather than saxagliptin plus conventional therapy (group B)(30). In addition to different study drugs, relatively higher levels of HbA1c in group B than those in our study implied that influences of glucose toxicity on islet function might account for this disparity and saxagliptin without Vitamin D3 probably could not totally reverse this glucotoxicity to maintain β-cell function.

Although sitagliptin seem to have the potential to preserve β-cell function in LADA, the benefit of sitagliptin may not great enough to make clinically relevant changes, e.g., the daily insulin requirements. We supposed, besides β-cell function and insulin sensitivity, daily insulin requirements were affected by many other factors, such as exercises and diets, both of which were difficult to be controlled in the long–term clinical trials. Larger-scale studies with restricting the potential confounding factors (exercises, diets, etc.) are required for confirmation in the future.

In our study, no statistical differences were observed in the proportions of patients receiving premixed insulin between SITA and CONT groups; or between the subgroups having clamp tests. In addition, there were at least 15 hours between the last injection of premixed insulin and the blood sampling. However, it is worth mentioning that our study didn’t eliminate the confounding effect of premixed insulin on the β-cell function related measurements due to their pharmacokinetic properties even though the potential influence may be minimal and similar between the two groups. Future studies with further restrictions of insulin are required, e.g., premixed insulin analogues are withheld the night before visits.

In addition, our study showed that the M and ISI levels in SITA group were significantly higher than those in CONT group at 24 months, suggesting the benefits of sitagliptin for
improving insulin sensitivity in LADA. Since the effect of sitagliptin combined with insulin on BMI was neutral (shown in Table 2), the changes in insulin sensitivity in SITA group should not be interpreted by weight loss. Previous studies have found that DPP-4 inhibitors can ameliorate insulin resistance in diabetic animal models (47,48) and patients with type 2 diabetes (7), which may be related to its anti-inflammatory action (49-51). However, the small sample size (12 subjects; 6 patients in each group) was one of the limitations of our study as a result of the complex procedure of glucose clamp technique and frequent blood collection. Moreover, it is worth mentioning that we followed the uncommon HEC protocol from Parvanova et al. (17), but not from DeFronzo et al. (16), which would also be one of the limitations. Larger-scale studies are needed for confirmation in the future.

DPP-4 inhibitors are known to be multitarget agents. At present, the specific mechanism of islet β-cell preservation and insulin sensitivity improvement in LADA has not been fully elucidated. DPP4 (also known as CD26) is widely distributed on the surface of endothelial cells, epithelial cells, NK cells, lymphocytes and monocytes. DPP4/CD26 not only has peptidase activity, but also plays an important role in immune regulation. As a cell surface antigen, CD26 has a costimulatory function on T cell activation and proliferation (52). Our group’s recent study implied that sitagliptin could modulate cellular immunity by increasing the proportion of T helper 2 cells, decreasing the proportion of T helper 17 cells in LADA patients and down-regulating the messenger RNA expression of T box expressed in T cells (TBET) and related orphan receptor C (RORC) in LADA patients (10).

In conclusion, compared with insulin alone, sitagliptin combined with insulin therapy appeared to delay the decline of islet β-cell function and improve insulin sensitivity in patients with LADA to some extent. Our findings provide clues for the treatment of LADA.
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Data Availability. The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.
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**Figure Legends**

**Figure 1** Flow diagram of randomized patients. FCP, fasting C-peptide.

**Figure 2** Islet β-cell function evaluated by hyperglycemic clamp. Black circles: SITA group (n = 6); white squares: CONT group (n = 6). Fasting insulin (FINS, A), the first-phase insulin secretion (1PH, B), the second-phase insulin secretion (2PH, C) and the maximum insulin secretion (MIS, D) were calculated at the indicated month. Data were expressed as mean and SEM. *P < 0.05, **P < 0.01, ***P < 0.001 vs. CONT group (MANOVA), △P < 0.05 vs. the baseline in SITA group and #P < 0.05, ##P < 0.01 vs. the baseline in CONT group (repeated measures ANOVA).

**Figure 3** Insulin sensitivity evaluated by hyperinsulinemic euglycemic clamp. Black circles: SITA group (n = 6); white squares: CONT group (n = 6). Glucose metabolized (M, A), insulin sensitivity index (ISI, B) and M/log I ratio were calculated at the indicated month. Data were presented as mean and SEM. *P < 0.05 vs. CONT group (MANOVA), △P < 0.05, △△P < 0.01 vs. the baseline in SITA group and #P < 0.05, ##P < 0.01 vs. the baseline in CONT group (repeated measures ANOVA).
|                      | SITA group (n=22) | CONT group (n=25) | P value |
|----------------------|-------------------|-------------------|---------|
| Men/women            | 13/9              | 15/10             | 0.985   |
| Age (years)          | 48.2±11.5         | 48.2±12.3         | 0.985   |
| Duration of diabetes (years) | 1.8±1.4         | 2.3±1.8           | 0.310   |
| Daily insulin does (U) | 10.9±10.6        | 13.4±9.3          | 0.384   |
| Insulin dose (U/kg/day) | 0.18±0.17        | 0.22±0.15         | 0.511   |
| BMI (kg/m²)          | 23.2±2.8          | 23.8±2.6          | 0.433   |
| Waist circumference (cm) | 81.8±10.3        | 84.1±6.8          | 0.361   |
| Waist circumference in men (cm) | 84.0±12.0       | 85.5±6.8          | 0.682   |
| Waist circumference in women (cm) | 78.6±6.6        | 82.1±6.6          | 0.273   |
| Hip circumference (cm) | 94.6±5.7         | 95.9±5.2          | 0.424   |
| Waist-to-hip ratio   | 0.86±0.07         | 0.88±0.06         | 0.435   |
| Waist-to-hip ratio in men | 0.87±0.08       | 0.88±0.07         | 0.667   |
| Waist-to-hip ratio in women | 0.85±0.06       | 0.87±0.05         | 0.468   |
| Systolic blood pressure (mmHg) | 120±12         | 119±17            | 0.697   |
| Diastolic blood pressure (mmHg) | 75±10           | 75±12             | 0.915   |
| GADA titer (U/mL)    | 469.5±374.9       | 435.8±399.8       | 0.768   |
| High-titer GADA (%)  | 63.6              | 56.0              | 0.595   |
| FBG (mmol/L)         | 6.8±1.3*          | 6.1±1.0           | 0.024   |
| 2hBG (mmol/L)        | 12.5±3.8          | 12.3±4.2          | 0.863   |
| HbA1c (%)            | 6.3±0.7           | 6.3±0.8           | 0.907   |
| FCP (nmol/L)         | 0.45±0.17         | 0.43±0.19         | 0.796†  |
| 2hCP (nmol/L)        | 1.60±0.62         | 1.65±0.70         | 0.991†  |
| ΔCP (nmol/L)         | 1.15±0.55         | 1.22±0.62         | 0.926†  |
| HOMA2-B (%)          | 55.0±22.7         | 68.4±36.0         | 0.139   |
| HOMA2-IR             | 1.10±0.42         | 1.00±0.45         | 0.425   |
| Triglyceride (mmol/L) | 1.38±0.82         | 1.34±0.84         | 0.848   |
| Total cholesterol (mmol/L) | 4.79±0.74        | 4.57±0.75         | 0.324   |
|                | Mean ± SD 1 | Mean ± SD 2 | p-value |
|----------------|-------------|-------------|---------|
| HDL cholesterol (mmol/L) | 1.28±0.27   | 1.39±0.31   | 0.227   |
| HDL cholesterol in men (mmol/L) | 1.19±0.26   | 1.32±0.35   | 0.280   |
| HDL cholesterol in women (mmol/L) | 1.42±0.24   | 1.49±0.21   | 0.500   |
| LDL cholesterol (mmol/L) | 3.00±0.71*  | 2.54±0.74   | 0.036   |

Data were shown as mean ± SD, frequency and percentage. *P<0.05 vs. CONT group (Student t-test for variables with normal distribution and Chi-square test for categorical variables). †Adjustment for FBG and LDL cholesterol (covariance analysis). BMI, body mass index; GADA, glutamic acid decarboxylase autoantibody; FBG, fasting blood glucose; 2hBG, 2-hour postprandial blood glucose; HbA1c, glycosylated hemoglobin A1c; FCP, fasting C-peptide; 2hCP, 2-hour postprandial C-peptide; ΔCP, 2hCP - FCP; HOMA2-B, updated homeostasis model assessment of β-cell function; HOMA2-IR, updated homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Table 2 Changes of glycaemic control, BMI and islet β-cell function evaluated by MMTT

|          | 6 months |          |          | 12 months |          |          | 18 months |          |          | 24 months |          |
|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|          | SITA group | CONT group | SITA group | CONT group | SITA group | CONT group | SITA group | CONT group | SITA group | CONT group |
| n        | 22       | 25       | 22       | 25       | 19       | 23       | 18       | 22       |
| HbA1c (%/mmol/mol) |          |          |          |          |          |          |          |          |          |          |
| Baseline | 6.3±0.7/45±8 | 6.3±0.8/45±9 | 6.3±0.7/45±8 | 6.3±0.8/45±9 | 6.2±0.7/44±8 | 6.2±0.8/44±9 | 6.1±0.7/43±8 | 6.2±0.8/44±9 |
| Follow-up| 6.3±0.7/45±8 | 6.5±0.8/48±9 | 6.3±0.9/45±10 | 6.4±0.8/46±9 | 6.1±0.8/43±9 | 6.5±0.8/48±9 | 6.4±0.7/46±8 | 6.4±0.7/46±8 |
| Change from baseline | 0.1±0.7/1±8 | 0.2±1.0/2±11 | 0.0±0.9/0±10 | 0.1±1.0/1±11 | -0.1±0.6/-1±7 | 0.3±0.9/3±10 | 0.2±0.6/2±7 | 0.2±0.8/2±9 |
| BMI (kg/m²) |          |          |          |          |          |          |          |          |          |          |
| Baseline | 23.2±2.8 | 23.8±2.6 | 23.2±2.8 | 23.8±2.6 | 23.4±2.9 | 23.9±2.6 | 23.4±3.0 | 23.8±2.6 |
| Follow-up| 23.0±2.6 | 24.0±2.8 | 22.9±2.7 | 24.1±2.5 | 23.3±2.8 | 24.3±2.7 | 23.2±2.6 | 24.4±2.9 |
| Change from baseline | -0.2±1.1 | 0.2±1.4 | -0.3±1.4 | 0.3±1.3 | -0.2±1.0 | 0.4±1.6 | -0.2±1.1 | 0.6±1.4 |
| FCP (nmol/L) |          |          |          |          |          |          |          |          |          |          |
| Baseline | 0.45±0.17 | 0.43±0.19 | 0.45±0.17 | 0.43±0.19 | 0.45±0.18 | 0.43±0.19 | 0.45±0.18 | 0.42±0.19 |
| Follow-up| 0.39±0.15 | 0.39±0.21 | 0.38±0.19 | 0.37±0.21 | 0.42±0.18 | 0.38±0.20 | 0.45±0.30 | 0.40±0.24 |
| Change from baseline | -0.05±0.16 | -0.04±0.19 | -0.07±0.15 | -0.06±0.21 | 0.02±0.42 | -0.02±0.46 | 0.00±0.25 | -0.02±0.25 |
Table 2 Changes of glycaemic control, BMI and islet β-cell function evaluated by MMTT (continued)

|                       | 6 months  | 12 months | 18 months | 24 months |
|-----------------------|-----------|-----------|-----------|-----------|
|                       | SITA group| CONT group| SITA group| CONT group| SITA group| CONT group| SITA group| CONT group|
| HOMA2-B (%)           |           |           |           |           |           |           |           |           |
| Baseline              | 55.0±22.7 | 68.4±36.0 | 55.0±22.7 | 68.4±36.0 | 55.9±23.9 | 70.7±36.4 | 57.1±24.0 | 70.8±37.3 |
| Follow-up             | 55.7±21.5 | 55.0±28.7 | 59.8±23.2 | 54.2±26.6 | 68.2±30.7 | 51.7±28.9# | 69.7±39.5 | 51.8±29.9 |
| Change from baseline  | 0.7±24.6  | -13.4±31.9| 4.8±23.1* | -14.2±38.5| 12.3±25.5**| -19.0±39.8| 12.6±41.7*| -19.0±41.8|
| 2hCP (nmol/L)         |           |           |           |           |           |           |           |           |
| Baseline              | 1.60±0.62 | 1.65±0.70 | 1.60±0.62 | 1.65±0.70 | 1.61±0.66 | 1.70±0.70 | 1.61±0.68 | 1.68±0.71 |
| Follow-up             | 1.43±0.57 | 1.37±0.73#| 1.40±0.71 | 1.16±0.66###| 1.55±0.81 | 1.35±0.68#| 1.38±0.64 | 1.39±0.71#|
| Change from baseline  | -0.17±0.50| -0.28±0.66| -0.21±0.44| -0.49±0.60| -0.06±0.55| -0.35±0.60| -0.23±0.50| -0.29±0.53|
| △CP (nmol/L)         |           |           |           |           |           |           |           |           |
| Baseline              | 1.15±0.55 | 1.22±0.62 | 1.15±0.55 | 1.22±0.62 | 1.16±0.58 | 1.27±0.63 | 1.16±0.60 | 1.25±0.64 |
| Follow-up             | 1.03±0.49 | 0.97±0.63 | 1.02±0.56 | 0.79±0.56###| 1.12±0.73 | 0.97±0.59#| 0.92±0.42 | 0.98±0.56#|
| Change from baseline  | -0.12±0.50| -0.25±0.62| -0.13±0.40*| -0.43±0.53| -0.04±0.53| -0.31±0.58| -0.24±0.50| -0.27±0.52|
Data were expressed as mean ± SD. *P < 0.05, **P < 0.01, vs. CONT group (MANOVA), #P < 0.05, ##P < 0.01 and ###P < 0.001 vs. the baseline in CONT group (repeated measures ANOVA). BMI, body mass index; HbA1c, glycosylated hemoglobin A1c; FCP, fasting C-peptide; HOMA2-B, updated homeostasis model assessment of β-cell function; 2hCP, 2-hour postprandial C-peptide; △CP, 2hCP – FCP.
Figure 1

Randomized (n=51)

Allocation

SITA group (n=25)

Month 6 Visit

Month 6 Visit (n=25)
Analysed (n=22)
• Excluded from analysis (Poor glycemic control, n=3)

Month 12 Visit

Month 12 Visit (n=25)
Analysed (n=22)
• Excluded from analysis (Poor glycemic control, n=3)

Month 18 Visit

Month 18 Visit (n=22)
• Discontinued (Severe adverse event, n=1; Withdrawn consent, n=2)
Analysed (n=19)
• Excluded from analysis (Poor glycemic control, n=3)

Month 24 Visit

Month 24 Visit (n=20)
• Discontinued (Withdrawn consent, n=2)
Analysed (n=18)
• Excluded from analysis (Poor glycemic control, n=2)

CONT group (n=26)

Month 6 Visit

Month 6 Visit (n=25)
• Discontinued (Poor compliance, n=1)
Analysed (n=25)

Month 12 Visit

Month 12 Visit (n=25)
Analysed (n=25)

Month 18 Visit

Month 18 Visit (n=23)
• Discontinued (Withdrawn consent, n=1; FCP < 0.05 nmol/L, n=1)
Analysed (n=23)

Month 24 Visit

Month 24 Visit (n=23)
• Excluded from analysis (Poor glycemic control, n=1)
Figure 2

(A) FINS (mU/L) over Months

(B) 1PH (mU/L) over Months

(C) 2PH (mU/L) over Months

(D) MIS (mU/L) over Months

- **A** and **B**: Comparison between SITA group (filled circle) and CONT group (empty square) with statistical significance indicated by Δ, **Δ**, and ##, ##

- **C** and **D**: Data points for each group with error bars
Figure 3

(A) M [mg/(kg·min)]

(B) ISI [(mg·mL)/(kg·min·μU)]

(C) M/log I [(mg·mL)/(kg·min·μU)]