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Original Research Paper

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THE COMPARISON OF THE SECOND PHASE INSULIN SECRETION DERIVED FROM ORAL GLUCOSE TOLERANCE TEST AND LOW DOSE GRADED GLUCOSE INFUSION TEST

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ABSTRACT

Background: the pathogeneses of type 2 diabetes (T2DM) are impaired insulin action and secretion, including the second phase insulin secretion (SPIS). However, SPIS is difficult to be measured. The study aimed to validate the SPIS derived from the simpler oral glucose tolerance test (OGTT) against the SPIS derived from the more complicated gold standard test, i.e. low dose graded glucose infusion test (LDGGI).

Methods: Fourteen participants (3 with normal glucose tolerance, 8 with pre-diabetes and 3 with T2DM) were enrolled. They received both a standard LDGGI and an OGTT. The mathematical method which is called deconvolution was applied in both tests. The slope of the insulin secretion rate (ISR) against glucose levels during the LDGGI was obtained and regarded as the gold standard (SPIS-L). At the same time, the SPIS calculated from OGTT with minimal model was also obtained (SPIS-O).

Results: Pearson correlation was used to assess the correlation between SPIS-L and SPIS-O. There was a significant correlation between SPIS-L and SPIS-O ($r = 0.843$, $p = 0.000$). At the same time, a good agreement between the SPIS-L and SPIS-O was also found from the Bland-Altman plot.

Conclusion: SPIS-O is highly correlated with the gold standard, i.e., the SPIS-L. Since it is easier to be performed, future researches focusing on SPIS by using OGTT might be expedited.

KEYWORDS : Second phase insulin secretion, low dose graded glucose infusion test, oral glucose tolerance test

INTRODUCTION :

It is well-established that increased insulin resistance (IR) and decreased insulin secretion are the most important underlying pathophysiologies in the development of type 2 diabetes (T2DM) [1]. Early in the course of diabetes, -cell tries hard to compensate the decline of insulin sensitivity (SI, i.e. the increased IR) to maintain normoglycemia. Thus, normal glucose tolerance (NGT) is kept during this stage. However, several years later, -cell becomes exhausted and eventually is unable to compensate. Hyperglycemia will occur and the frank T2DM consequently takes place [2].

Cerasi et al. first described that there are two phases of insulin secretion after -cell exposed to square wave of hyperglycemia [3,4]. The first phase insulin secretion (FPIS) is normally secreted by -cells within 5-10 minutes after the exposure to a prompt rise of plasma glucose concentrations due to the initial release of pre-docked insulin-contained granules. In the meanwhile, the second phase insulin secretion (SPIS), which represents the delayed release and the true "storage insulin" , will rise gradually and sustain for 2-3 hours [4]. Evidences have shown that that the FPIS usually deteriorates in the stage of pre-diabetes (PreDM) and nearly completely disappears in frank T2DM [5-7]. In contrast, SPIS is still partially maintained even after T2DM occurs which suggests that the tight glucose control with oral hypoglycemic agents in T2DM majorly relies on SPIS but not on FPIS [8]. However, in the past, most of the studies done to measure -cell function were only focusing on the FPIS [9-11].

Various methods were proposed to estimate SPIS such as clamp technique [12], oral glucose tolerance test (OGTT) [13] and low dose graded glucose infusion test (LDGGI) [14]. Among these methods, LDGGI, which calculates the insulin secretion in response to the gradual increment of plasma glucose levels, is considered as the gold standard measuring SPIS. However, LDGGI is time-consuming and labor-intensive, which limit the wide use of this method in clinical settings. On the contrary, OGTT is a relatively less complex method. By using minimal model, Breda et al. had demonstrated that SPIS could also be measured [15]. In the same time, this method had never been validated against the gold standard, i.e. the LDGGI method which limits the widely use of this method.

In this study, 14 subjects with different glucose tolerance test received both LDGGI and OGTT. By using deconvolution, SPIS was derived from both methods (SPIS-L and SPIS-O, respectively). The concordance of the SPIS-O with the SPIS-L was evaluated. Thus the SPIS-O could be validated.

MATERIALS AND METHODS:

Subjects:

We enrolled 14 subjects, including 3 normal glucose tolerance (NGT), 8 pre-diabetes (PreDM) and 3 T2DM from our out-patient clinic between 2011 to 2012 in Cardinal Tien Hospital. They were between 40-70 years old and other than diabetes, they did not have other significant medical diseases, history of diabetic ketoacidosis, nor had any changes of dose of oral hypoglycemic medications during the study period. The diagnostic criteria for diabetes were based on the 2012 American Diabetes Association criteria [16]. The study was reviewed and approved by the Institutional Review Board of the hospital (CTH-102-1-2A23) and all subjects provided written informed consent prior to participation. After 10 hours of fasting, the subjects visited the clinical research center and underwent complete physical examinations. The body mass index (BMI) was calculated as body weight/body height^2 (kg/m^2), while systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured on the right arm of seated subjects using standard mercury sphygmomanometers. Dietary education was given before the beginning of the study. The LDDGI and OGTT were performed in a random order.

Standard LDGGI:

This test was originally proposed by Polonsky et al [14]. After a 10-h overnight fast, the tests were done at 0800 with participants in the sitting position. An intravenous catheter was placed in each forearm, one for blood sampling and one for glucose infusion. The sampling catheters were kept patent by slow infusion of 0.9% saline. Stepped intravenous infusion of glucose (20% dextrose) was then started at a rate of 1, 4, 8,

16 and 24mg/kg/min. Each infusion rate was maintained for 40 min and blood samples were drawn at a 10-min interval for the measurement of plasma insulin, C-peptide and glucose levels. Deconvolution was done to quantify ISR. At each time point, there is a corresponding ISR. The slope of ISR (y-axis) against the plasma glucose level (x-axis) is regarded as the SPIS-L, gold standard of SPIS.

OGTT:

On the other day. The OGTT was performed. Again after a 10-hour overnight fast, a standard 75-g OGTT was performed at 8:00 AM. Blood was drawn before the glucose load and at 5, 10, 20, 30, 45, 60, 80, 100, 150 and 180 minutes after the glucose load for the measurements of plasma glucose, insulin and C-peptide. The C-peptide minimal model was used for measuring SPIS-O [17]. In brief, three different components were obtained by the C-peptide minimal model-first phase insulin secretion (FPIS-O) and second phase insulin secretion (SPIS-O) and total insulin secretion (TIS-O; composed of FPIS-O and SPIS-O).

The blood samples were centrifuged immediately and stored at –30°C until time of analysis. Plasma insulin was measured by a commercial solid phase radioimmunoassay kit (Coat-A-Count insulin kit, Diagnostic products Corporation, Los Angeles, CA, USA). Intra- and –inter-assay coefficients of variance for insulin were 3.3 and 2.5%, respectively. C-peptide Plasma glucose was measured by a glucose oxidase method (YSI 203 glucose analyzer, Scientific Division, Yellow Spring Instrument Company Inc., Yellow spring, OH, USA). The HbA1c was measured by the Bio-Rad Variant II automatic analyzer (Bio-Rad Diagnostic Group, Los Angeles, CA). Plasma C-peptide was measured by radioimmunoassay from RADIM S.P.A (Radium, Italia). The intra- and inter-assay CV were 8 and 15 %, respectively.

Statistical Analysis

Data was shown as mean standard deviation. The correlation between SPIS-L and SPIS-O was evaluated with Pearson correlation. A higher correlation coefficients (r) represents a better correlation. The Bland-Altman plot was also used to evaluate the agreement between them.

All statistical analyses were performed by using the SPSS software system, version 13.0 (SPSS Inc., Chicago, IL, USA). A *p* value less than 0.05 was considered to be statistically significant.

RESULTS:

Table 1 depicts the demographic data, FPG, FPI, FPIS and SPIS derived from the two methods. Figure 1 shows the glucose infusion rate at LDGGI. The corresponding plasma glucose, insulin, C-peptide levels and ISRs at each time point during the LDGGI (Panel A, B, C, D, respectively) are shown in Figure 2. In Figure 3, the plasma glucose, insulin, and C-peptide levels at different time points during the OGTT are demonstrated (Panel A, B, and C, respectively).

Table 1: The Demographic Data Of The Study Subjects

	Total
n	14
Gender (female/male)	7/7
Age (years)	51.0 ± 12.1
Body mass index (kg/m^2)	25.6 ± 2.9
Hemoglobin A1c (%)	6.5 ± 1.1
Waist circumference	89.5 ± 8.1
Systolic blood pressure (mmHg)	125.9 ± 14.5
Diastolic blood pressure (mmHg)	76.5 ± 7.8
Fasting plasma glucose (mmol/l)	5.5 ± 2.7
Fasting plasma insulin (pmol/l)	121.6 ± 124.2
SPIS-L	0.08 ± 0.03
SPIS-O	30.2 ± 20.8
FPIS-O	21.4 ± 54.5
TIS-O	30.3 ± 20.7

Data are shown as mean ± standard deviation

HDL-cholesterol: high-density lipoprotein cholesterol; SPIS-L: second phase insulin secretion derived from standard graded glucose infusion; SPIS-O: second phase insulin secretion derived from oral glucose tolerance test; FPIS-O: first phase insulin secretion derived from oral glucose tolerance test; TIS-O: total insulin secretion derived from oral glucose tolerance test.

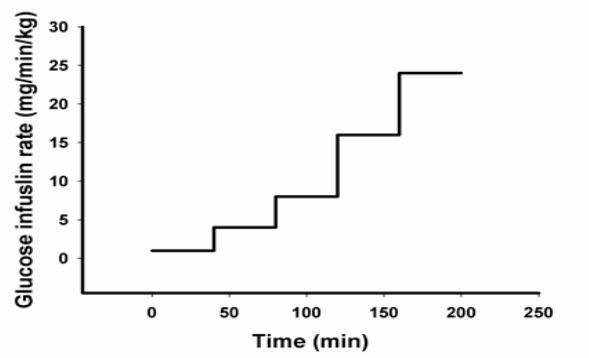

Figure.1- The Infusion Rate Of Glucose In Low Dose Graded Glucose Infusion Test

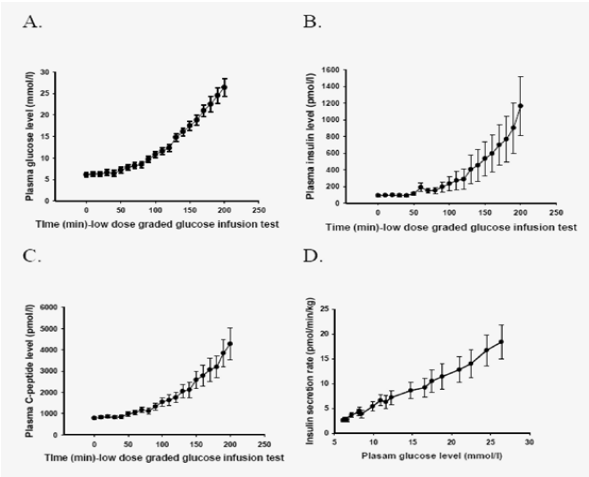

Figure.2- Plasma Glucose (panel A), Insulin (panel B), C-peptide Level (panel C) And Insulin Secretion Rate (d) At Each Time Point During Standard Low Dose Graded Glucose Infusion Test

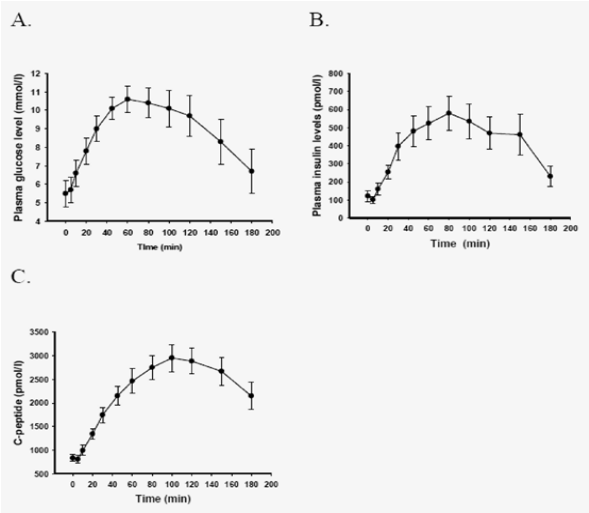

Figure3- Plasma Glucose (panel A) And Insulin Levels (panel

B) In Each Time Point During Oral Glucose Tolerance Test

The relationship between SPIS-L and SPIS-O was evaluated by Pearson's correlation and the result is shown in Figure 4. Significant correlation was found between them ($r = 0.867$, $p = 0.000$, Panel A). From the Bland-Altman plot, we also found good agreement between the SPIS-L and SPIS-O (Figure 4, Panel B).

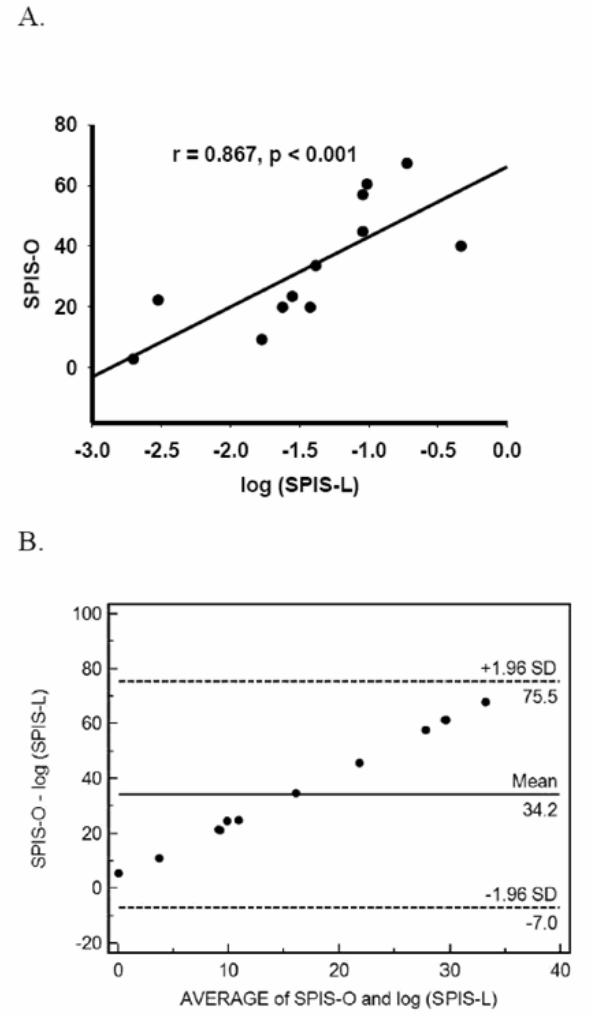

Figure 4: The correlation (Panel A) and the Bland-Altman plot (Panel B) between second phase insulin secretion derived from oral glucose tolerance test (SPIS-O) and from low dose graded glucose infusion test (SPIS-L)

DISCUSSION:

In this study, we compared the OGTT derived SPIS (SPIS-O) with the gold standard, i.e. SPIS-L to validate its accuracy in a group of subjects with various glucose tolerance. Our results showed that the concordance of the two SPIS was high and thus the simpler and routinely-used OGTT is a reliable method.

It is generally agreed the underlying pathophysiology of T2DM is majorly characterized by reduced insulin secretion and increased IR. Evidences have shown that compared to IR, the deterioration of -cell function plays the fundamental role in the development of T2DM, especially in Asian [18,19]. As aforementioned, the SPIS deteriorates much slower than the FPIS during the clinical course of diabetes. It could be noted that well glucose controlled could be obtained in many diabetic patients with just oral hypoglycemic drugs at least for several years before insulin treatment is started. This fact indirectly suggests that there must be reserved β-cell function during at this period of time which, in the present study, is referred to as the SPIS [5,8,9]. However, little is known about the role of SPIS in diabetes. It has been usually overlooked in clinical researchers and very few studies were focused on it.

One of the possible reason for this phenomenon is the difficulty to quantify SPIS. As mentioned in the introduction, both hyperglycemia clamp and LDDGI are the gold standards to quantify the SPIS. However, both methods are labor-intensive and expensive. Moreover, deconvolution method is needed for the calculation of SPIS in LDGGI which further decreases its usability. Therefore, simplifying the method to estimate SPIS has been an important issue for the researchers in this field.

For this purpose, several simplified model-based methods were proposed to estimate SPIS for the epidemiological and clinical research. Among them, Toffolo et al. has first proposed C-peptide minimal model to determine both phases of insulin secretion with FSIGT [20,21]. However, this method did not solve the fundamental problems of the LDGGI and Clamp which are the needs for frequent blood sampling and minimal model software. This would be the reason why simpler tests such as OGTT [15,22] and meal tolerance test [22-25] were further proposed. Among these methods, OGTT is not only the most physiological and but also the simplest method, which has been routinely done in many research centers and hospitals. From the Cobelli's original model, not only SPIS could be calculated but also the FPIS and the SI could also be estimated. However, until now, the SPIS derived from OGTT with C-peptide minimal model has not been validated even though it is important.

To our best knowledge, our study is the first one done to solve this problem. However, there are still some limitations. First, because there might be different -cell function remained in subjects with different glucose tolerance, the results might more persuasive if the SPIS-O is validated in NGT, pre-T2DM, and T2DM separately. However, as our study cohort was relatively small, we were unable to stratify these participants into 3 groups. Further well-designed study with larger population will be valuable to support our study results. Secondly, we did not repeat the protocol in the same individual twice, so the reproducibility cannot be demonstrated in our study. However, even with these limitations, we still believe that our finding could be reliable and informative.

In conclusion, although this is simple study with limited n number, the result is important. We have demonstrated that SPIS-O is highly correlated with the gold standard, i.e., the SPIS-L. Since it is easier to be performed, future researches focusing on SPIS by using OGTT might be expedited.

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Abbreviations:

T2DM, type 2 diabetes; SI, insulin sensitivity; IR, insulin resistance; FPIS, first-phase insulin secretion; SPIS, second-phase insulin secretion; LDGGI, low-dose graded glucose infusion test; ISR, insulin secretion rate; FPG, fasting plasma glucose; NGT, normal glucose tolerance; PreDM, Prediabetes; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPI, fasting plasma insulin; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-, homeostasis model assessment of beta-cell function; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; SPIS-L: second phase insulin secretion from low-dose

graded glucose infusion test; FPIS-O, first phase insulin secretion from oral glucose tolerance test, SPIS-O: second-phase insulin secretion from oral glucose tolerance test; TIS, total insulin secretion from oral glucose tolerance test.

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