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ORIGINAL

Evaluation of second phase insulin secretion with simple surrogates derived from the mixed meal tolerance test in patients with type 2 diabetes

Wei-Cheng Lian[1)], Jiunn-Diann Lin[2)], Jin-Biou Chang[3), 4)], Chun-Hsien Hsu[5)], Chun Pei[6)], Chung-Ze Wu[2)], Chang-Hsun Hsieh[7)], Dee Pei[8)] and Yen-Lin Chen[9)]

[1)] *Division of Endocrinology and Metabolism, Department of Internal Medicine, Buddhist Dalin Tzu Chi General Hospital and College of Medicine, Tzu Chi University, Taiwan, R.O.C.*
[2)] *Division of Endocrinology, Department of Internal Medicine, Shuang Ho Hospital, School of Medicine, School of College, Taipei Medical University, Taipei, Taiwan, R.O.C.*
[3)] *Department of Pathology, National Defense Medical Center, Division of Clinical Pathology, Tri-Service General Hospital, Taipei, Taiwan, R.O.C.*
[4)] *School of Medical Laboratory Science and Biotechnology, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan, R.O.C.*
[5)] *Department of Family Medicine, Cardinal Tien Hospital, School of Medicine, Fu-Jen Catholic University, New Taipei City, Taiwan, R.O.C.*
[6)] *Graduate School of Gerontic Technology and Service Management, Nan Kai University of Technology, Nan Tou County, Taiwan, R.O.C.*
[7)] *Division of Endocrinology and Metabolism, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, R.O.C.*
[8)] *Division of Endocrinology and Metabolism, Department of Internal Medicine, Cardinal Tien Hospital, Medical School, Fu Jen Catholic University, New Taipei City, Taiwan, R.O.C.*
[9)] *Department of Pathology, Cardinal Tien Hospital, Medical School, Fu Jen Catholic University, New Taipei City, Taiwan, R.O.C.*

Abstract. The major contributors to the pathogenesis of type 2 diabetes are impaired insulin action and insulin secretion, including second phase insulin secretion (2nd ISEC). This study aimed to compare surrogates derived from the mixed meal tolerance test (MTT) with 2nd ISEC derived from modified low-dose graded glucose infusion (M-LDGGI) in patients with type 2 diabetes. We were subsequently able to decide which surrogate would be performed easily and accurately. Twenty type 2 diabetes patients were enrolled. They received both MTT and M-LDGGI. The standardized MTT meals were provided at 8:00 A.M. and 12:00 P.M. The M-LDGGI was a simplified version of the Polonsky method; only two 80-min stages of glucose infusion (2 and 6 mg/kg/min) were given. The slopes of the insulin to glucose curve during the test were regarded as the 2nd ISEC. First, we used the area under the insulin curve (AUC_{IN}) during MTT to quantify the 2nd ISEC. The best correlated AUC_{IN} was from 60-240 min. Second, the slopes between any two time points of the plasma insulin to glucose level ($SLOPE_{I/G}$) were also assessed. The time period best correlated with 2nd ISEC was from 0-120 min ($SLOPE_{0\text{-}120}$). Finally, the insulin-to-glucose ratio (IG_r) of each time point was used to estimate the 2nd ISEC, and the best correlation was observed at 180 min. In conclusion, estimating 2nd ISEC surrogates derived from MTT proved to be possible. The most accurate surrogate is the $SLOPE_{0\text{-}120}$, while IG_{r180} is another less precise but more convenient method.

Key words: Type 2 diabetes, Second phase insulin secretion, Mixed meal tolerance test, Modified low dose graded glucose infusion test

IT IS WELL KNOWN that the underlying pathophysiologies of type 2 diabetes are insulin resistance and impaired insulin secretion [1]. Of these 2 defects, both Caucasian and Japanese data showed that decreased insulin secretion played an important role in triggering the occurrence of diabetes [2-4]. However, very

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Correspondence to: Yen-Lin Chen, Department of Pathology, Cardinal Tien Hospital, No 362, Chung Cheng Road, Xindian, Taipei County 23137, Taiwan, R.O.C. E-mail: jdlin1971@yahoo.com.tw

Abbreviations: 1st ISEC, first phase insulin secretion; 2nd ISEC, second phase insulin secretion; HOMA-β, homeostasis model assessment-β cell; FSIGT, frequent sampling intravenous glucose tolerance test; M-LDGGI, modified low-dose graded glucose infusion; MTT, mixed meal tolerance test; OGTT, oral glucose tolerance test; AUC_{IN}, area under curve of plasma insulin levels between 2 time points; $SLOPE_{I/G}$, slope of the plasma insulin to glucose ratio between 2 time points; IG_r, insulin/glucose ratio

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few studies have focused on the role of insulin secretion compared to insulin resistance. Some studies have shown that first phase insulin secretion (1st ISEC) nearly disappears even before type 2 diabetes is diagnosed [5-8], although second phase insulin secretion (2nd ISEC) remains partially functional after the diagnosis [9]. This is important for glucose homeostasis when patients are still being treated with oral hypoglycemic agents. The role of 2nd ISEC while important has been underestimated and less discussed in the literature.

There are several methods of measuring ISEC, such as homeostasis model assessment-β cell (HOMA-β) [10], the oral glucose tolerance test (OGTT) [11], and the frequent sampling intravenous glucose tolerance test (FSIGT) [12]. However, most of these tests measure 1st ISEC only. At the same time, although low-dose graded glucose infusion (LDGGI) is considered to be more accurate and is focused on 2nd ISEC [13], it takes 4 hours and requires a mathematical method known as 'deconvolution' for calculation. This method requires special computer programs and thus cannot be done even in many research facilities. These two conditions limit the wide application of LDGGI. Our group proposed a modified LDGGI (M-LDGGI) and described its use in our previous study [14]. In brief, compared with the LDGGI, the M-LDGGI is shorter and contains fewer time points for blood drawing. At the same time, no deconvolution is needed.

Recently, due to the development of glucagon-like peptide-1 receptor agonists (GLP-1RAs) and dipeptidyl peptidase IV (DPP-4) inhibitors, the concept of insulin being stimulated by nutrients, other than plasma glucose only, entering the gastrointestinal tract has been more greatly appreciated [15-18]. Thus, a mixed meal tolerance test (MTT) that contains all nutrients might be a better challenge. Indeed, some studies have investigated the use of MTT for estimating ISEC. However, most of these previously published MTT methods still require deconvolution in the calculation [19]. Again, this prohibits the widespread use of MTT.

The purpose of the present study was to find a relatively simple and yet accurate method for quantifying 2nd ISEC. To accomplish this goal, both MTT and M-LDGGI were used with 20 subjects with type 2 diabetes. M-LDGGI was taken as the standard for quantifying 2nd ISEC. Different surrogates derived from MTT were calculated and compared with M-LDGGI by simple correlation. Thus, we could ascertain which MTT surrogate could be used to estimate 2nd ISEC more accurately in type 2 diabetes patients in daily practice.

Subjects and Methods

Subjects

We enrolled 20 subjects with type 2 diabetes (10M/10F), aged between 40-65 years, who had received only oral hypoglycemic agents (OHA) from our outpatient clinic in Cardinal Tien Hospital. All these subjects were diagnosed as type 2 diabetes by 75g OGTT before they received OHA. The mean duration of diabetes was about 8.0 ± 3.4 years (0.5 years to 9.5 years) (Table 1). Other than diabetes, the subjects had no significant medical diseases or history of diabetic ketoacidosis, nor had they taken any medications known to affect insulin sensitivity and/or β-cell function during the study period. After explaining the research details, written informed consent was obtained from each individual. The study was reviewed and approved by the Institutional Review Board (CTH-101-2-5-027).

Each participant undertook both the MTT and M-LDGGI randomly with an interval of at least three days between the two tests. The tests were performed at 8:00 A.M. with subjects in the sitting position after a 10-hour overnight fast. An intravenous catheter was placed in each forearm, one for blood sampling and one for glucose infusion (only during M-LDGGI). The sampling catheters were kept patent by slow infusion

Table 1 Demographic and clinical data of the study subjects

	Total
n	20
Age (years)	51.0 ± 8.3
Duration of type 2 diabetes (years)	8.0 ± 3.4
Body mass index (kg/m^2)	25.1 ± 3.2
Hemoglobin A1c (%)	8.3 ± 1.9
Systolic blood pressure (mmHg)	123.5 ± 15.0
Diastolic blood pressure (mmHg)	79.3 ± 11.5
Fasting plasma glucose (mmol/L)	7.2 ± 2.0
Fasting plasma insulin (pmol/L)	61.4 ± 65.6
Total cholesterol (mmol/L)	4.8 ± 1.2
HDL-cholesterol (mmol/L)	1.4 ± 0.5
Triglyceride (mmol/L)	1.4 ± 0.5
2nd ISEC (pmol/mmol)	0.12 ± 0.12
HOMA-β	93.3 ± 160.1

Data are shown as mean ± standard deviation.
HDL-cholesterol, high-density lipoprotein cholesterol; 2nd ISEC, second phase insulin secretion derived from modified low-dose graded glucose infusion; HOMA-β, homeostatic model assessment of β-cell function.

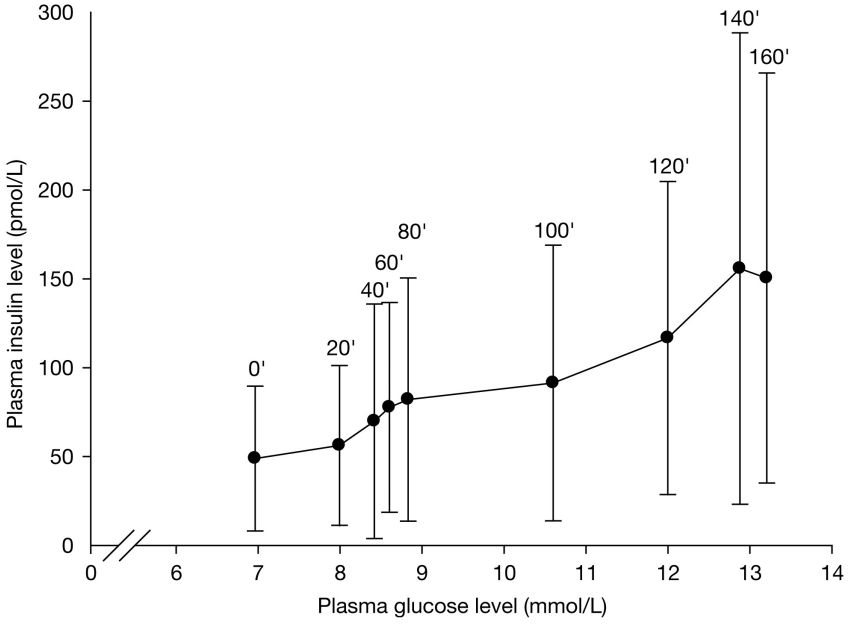


Fig. 1 Plasma glucose and insulin concentration at each time point (min) during the modified low-dose graded glucose infusion test (Data are shown as means ± standard deviation)

of 0.9% saline.

After the catheters were inserted, the fasting samples were taken. All the other blood samplings were taken hourly until 4:00 P.M. to determine the plasma glucose and insulin concentration. The standardized MTT meals were isocaloric (30 kcal/kg), and each meal contained (as a percentage of the total energy) 15% protein, 33% fat, and 52% carbohydrates. The meals were provided at 8:00 A.M. and 12:00 P.M. and had 20% and 40% of the daily total caloric intake, respectively.

M-LDGGI: The M-LDGGI is a simplified version of the original stepped intravenous infusion. Compared to the six stages proposed by Byrne *et al*. [13], we administered two stages of glucose infusion (20% dextrose) only at the rate of 2 mg/kg/min, followed by 6 mg/kg/min. Each infusion was maintained for 80 min and blood samples were obtained at 20-min intervals for the measurement of plasma insulin and glucose levels. A regression line was drawn based on the plasma insulin levels (*y*-axis) against the plasma glucose levels (*x*-axis, Fig. 1) at each time point. The slope of this line was considered to be the standard for the 2nd ISEC in this study.

The blood samples were centrifuged immediately and stored at –30C$^{\circ}$ until time of analysis. Plasma insulin was measured with a commercial solid phase radioimmunoassay kit (Coat-A-Count Insulin Kit, Diagnostic Products Corp., Los Angeles, CA, USA). The cross-reactivity of the insulin assay with proinsulin at midcurve was about 40%. Intra- and inter-assay coefficients of variance for insulin were 3.3 and 2.5%, respectively. Plasma glucose was measured using a glucose oxidase method (YSI 203 Glucose Analyzer, Scientific Division, Yellow Springs Instrument Co. Inc., Yellow Springs, OH, USA). Serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were measured using the dry, multilayer analytical slide method in the Fuji DR-Chem 3000 analyzer (Fuji Photo Film Corp., Minato-ku, Tokyo, Japan). The level of HbA1C was evaluated by the ion-exchange high pressure liquid chromatography (HPLC) method (Bio-Rad Variant II, Hercules, CA, USA).

Surrogates for 2nd ISEC

We used 3 different methods to calculate 2nd ISEC from the MTT.

1. Area under curve of plasma insulin levels between 2 time points (AUC_{IN}, Table 2). AUC was calculated using the trapezoidal rule.
2. Slope of the plasma insulin-to-glucose ratio between 2 time points ($SLOPE_{I/G}$): This method further takes the effect of time of insulin secretion into account and is supposed to be more accurate (Table 3).
3. The insulin/glucose ratio (IG_r): Since insulin secre-

Table 2 The area under the curve of insulin level against time (AUC_{IN}) during the mixed meal tolerance test and its correlation with second phase insulin secretion derived from the modified low-dose graded glucose infusion

Time (min)	$AUC_{time\text{-}insulin}$ (pmol/L ×min)	r	*p*
0 - 60	9098.6 ± 10004.1	0.543	0.016
0 - 120	21694.5 ± 21865.9	0.585	0.009
0 - 180	29642.6 ± 26934.8	0.597	0.007
0 - 240	33832.5 ± 29757.7	0.611	0.005
60 - 120	12595.9 ± 12070.4	0.575	0.010
60 - 180	20544.0 ± 17499.3	0.593	0.007
60 - 240	24733.8 ± 20410.8	0.621	0.005
120 - 180	7948.1 ± 6153.5	0.570	0.121
120 - 240	12138.0 ± 9360.8	0.588	0.008
180 - 240	4189.8 ± 3518.3	0.527	0.021

Data are shown as means ± standard deviation.

Table 3 The slopes of two time points for plasma insulin to glucose levels ($SLOPE_{I/G}$) during the mixed meal tolerance test and their correlation with second phase insulin secretion derived from the modified low-dose graded glucose infusion

Time (min)	Slope	r	*p*
0 - 60	0.60 ± 0.76	0.672	0.003
0 - 120	0.19 ± 1.54	0.807	0.000
60 - 180	0.40 ± 0.56	0.680	0.004
60 - 240	0.48 ± 0.60	0.737	0.000
120 - 180	0.38 ± 0.55	0.606	0.013
120 - 240	0.51 ± 0.71	0.771	0.000
180 - 240	0.47 ± 1.06	0.511	0.052

Data are shown as means ± standard deviation.

Table 4 The plasma insulin/glucose ratio (IG_r) at each time point during the mixed meal tolerance test and its correlation with second phase insulin secretion derived from the modified low-dose graded glucose infusion

Time (min)	IGr (pmol/mmol)	r	*p*
0	9.73 ± 11.4	0.223	0.358
60	26.1 ± 31.9	0.608	0.007
120	23.5 ± 21.5	0.454	0.051
180	14.6 ± 13.8	0.613	0.005
240	9.89 ± 9.19	0.511	0.025

Data are shown as means ± standard deviation.

tion is related to the plasma glucose level, this method could take the plasma glucose level into consideration (Table 4).

To compare our new indices from MTT with other available indices, the homeostatic model assessment of β-cell function (HOMA-β) was also calculated [10].

Statistical analysis

Statistical analysis was performed with SPSS Version 10.0 for Windows (SPSS, Chicago, IL). Data are shown as mean ± standard deviation (S.D.). As 2nd ISEC, HOMA-β, AUC_{IN}, $SLOPE_{I/G}$, and IG_r showed a right-skew distribution, log transformation was done before further analysis. Correlations were evaluated by Pearson's correlation. The Bland-Altman plot was used to evaluate the agreement between the new method and the gold standard. Using r-to z transformation, z statistics were calculated and were used to determine the difference between any two r values. All statistical tests were two-sided and $p \leq 0.05$ was considered to be significant.

Results

The demographic data and indices of β-cell function of the study subjects, including the 2nd ISEC derived from M-LDGGI, and HOMA-β are found in Table 1. The standard 2nd ISEC used in this study, which is the slope of the regression line, is also presented (Fig. 1).

The first surrogate we used to estimate 2nd ISEC was AUC_{IN}. All the AUC_{IN} were significantly correlated with the 2nd ISEC (Table 2). Among them, the AUC of 60-240 min was found to have the best correlation (AUC_{60-240}) (r = 0.621, p = 0.005).

With regard to the $SLOPE_{I/G}$ between any time point and its relationship with the 2nd ISEC, the slope with the highest r value was between 0-120 min ($SLOPE_{0-120}$, r = 0.807, p = 0.000) (Table 3). Finally, the relationships between IG_r and 2nd ISEC were calculated (Table 4). The single most significant time point for the IG_r was at 180 min (IG_{r180})(r = 0.613, p = 0.005). There was no difference among these r values for different AUC_{INS}, SLOPE or IGr, respectively. At the same time, there was no difference between the r values derived from AUC_{60-240}, $SLOPE_{0-120}$ and IGr_{180}. The correlations and Bland-Altman plots between AUC_{60-240} (Panels A, B), $SLOPE_{0-120}$ (Panels C, D), and insulin/glucose$_{180}$ (Panels E, F), and the 2nd ISEC are shown in Fig. 2. From these Bland-Altman plots, we found good agreement between the new indices and the 2nd ISEC.

Pearson's correlation was used to compare the most widely used surrogate, HOMA-β. However, there was no significant correlation between the 2nd ISEC and HOMA-β (r = 0.303, p = 0.207).

Discussion

The aim of this study was to evaluate which surrogates are best correlated during MTT with the 2nd ISEC in patients with type 2 diabetes. There were 3 different analyses of MTT, as we have shown in the Materials and Methods section. The first method we used was AUC_{IN}. The drawback of this method is the necessity of two time points to do the calculation. Of all the time periods, the best correlated AUC_{IN} was from 60-240 min (r = 0.621). This finding is not completely novel since a similar study was also done with children with normal glucose tolerance tests. Bacha *et al.* showed that, compared to either the 30 min or 60 min insulinogenic index (IGI), AUC_{IN} during both OGTT and MTT were better correlated with the 2nd ISEC calculated by the hyperglycemia clamp [20]. One important finding that is worth noting is that the range of the r values of AUC_{IN} was relatively narrow (0.527 to 0.621). This implies that AUC_{IN} might be a more reliable method than other surrogates discussed in this study.

The second surrogate we used was the $SLOPE_{I/G}$ between any two points during MTT. To our knowledge, this is a totally novel method to quantify the 2nd ISEC. The beauty of this method is that the effect of time and glucose effects on insulin secretion are taken into consideration. However, the method requires two time points to obtain the slope, which makes it less convenient. From our data, the best correlated time period was $SLOPE_{0-120}$. Our data are not surprising, since Tura *et al.* showed that that IGI in the first 30 min of the oral glucose tolerance test (IGI: Δ Insulin0-30/Δ Glucose0-30) was highly related to the 1st ISEC [21]. In that study, FSIGT to quantify the 1st ISEC was used. The researchers further postulated that a similar ratio of glucose and insulin at 90-min time points might be related to 'late secretion'. Although the best correlated time period for our first surrogate was from 0-120 min (r = 0.807), it still covered the 90-min time point and thus was compatible with their suggestion. It should be noted that the correlations between SLOPE and 2nd ISEC after 90 min still existed in the current study. This could be partially explained by the fact that during MTT, slow digestion due to multiple nutrients might play a role in this extension of the significant time period.

The last surrogate we used to estimate the 2nd ISEC was the IG_r. Among all the time points, the IG_r at 180 min had the highest r value (0.613) (Table 4). This finding is compatible with our two previous methods, since the 2nd ISEC was expected sometime after 60 min. Despite these significant results, we still consider the IG_r to be less accurate than the $SLOPE_{I/G}$, because of its lower r value. However, this method does have some important implications. First, in the aforementioned study, Tura *et al.* [21] used a clamp to quantify the 2nd ISEC, which is a well-recognized method. By being consistent with their results, our data are further validated. Second, although less accurate, this method only needs one time point to do the estimation. This makes the IG_r more widely applicable in regular health facilities.

It would be of interest to compare our new indices with the traditional surrogates, the HOMA-β. Our three indices derived from MTT showed better correlation with the 2nd ISEC than the traditional index. The

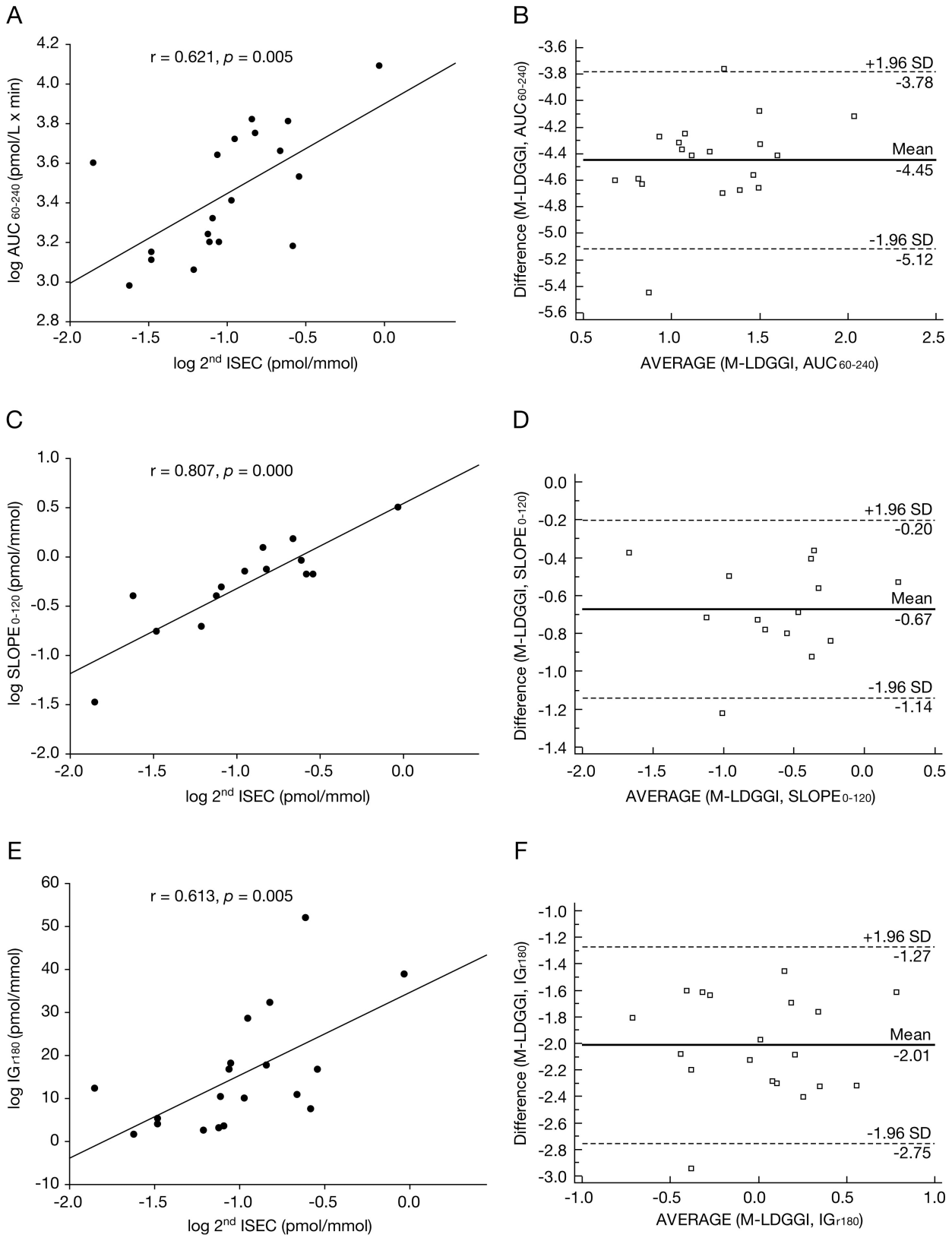


Fig. 2 The correlations and Bland-Altman plots between insulin of area under curve $_{60\text{-}240}$ (Panel A, B), SLOPE$_{0\text{-}120}$ (Panel C, D), and insulin/glucose$_{180}$ (Panel E, F) and second phase insulin secretion

concept behind HOMA-β is how much insulin can be driven per unit of glucose. It represents a static insulin secretion during the fasting status. On the other hand, both MTT and M-LDGGI are relatively more physiological, since they assess the dynamic response after glucose load. These results might contribute to the fact that HOMA-β did not show a better association with the 2nd ISEC than indices derived from MTT.

There are some limitations in our study. First, we chose a less well-documented method, the M-LDGGI, as the "gold standard" in our study. In theory, either the hyperglycemia clamp or LDGGI might be a better method. However, the M-LDGGI is less time- and effort-consuming. This method has been described by our group elsewhere [14]. Second, it is generally agreed that measuring C-peptide during MTT might be more accurate than insulin. However, we did not have the data for C-peptide. It would be interesting to compare the calculated insulin secretion between C-peptide and insulin. This would be an important issue in future studies. Third, we did not repeat MTT in the same individual twice, so the reproducibility of the new indices cannot be demonstrated in our study. Finally, although all subjects received OGTT before they received OHA, only fasting plasma glucose and post-challenge 120 min glucose levels were measured. Many interesting indices derived from OGTT such as insulinogenic index 120 min, Stumvoll index and sigma insulin 0-120/sigma glucose 0-120, which required insulin levels, were unable to be calculated. It would be our future plan to do the comparison of the differences between indices from MTT and those from OGTT in type 2 diabetes.

In conclusion, we have demonstrated that it is possible to estimate 2nd ISEC surrogates derived from MTT easily in daily practice. The most accurate surrogate is the $SLOPE_{0\text{-}120}$. At the same time, IG_{r180} is another less precise but more convenient method that can be be used.

Conflict of Interest

No conflict of interest.

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