Insulin Value-added after Taking Glucose *vs* Blood Glucose Value-added after Sixty minutes Taking Glucose as a Good Index for Estimating Beta Cell Function by Non-specific Insulin Assay

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ABSTRACT

Objective: *To determine the clinical significance of measuring serum true insulin (TI) in overweight and the non-obese with varying degrees of glucose tolerance, we estimated βeta cell (β-cell) function by calculating indices.*

Methods: *Serum true insulin, immunoreactive insulin (IRI), and glucose level in fasting and during an oral glucose tolerance test (OGTT) were measured in 32 individuals with normal glu-cose tolerance (NGT), 42 individuals with impaired glucose tolerance (IGT), 27 individuals with Type 1 diabetes mellitus (DM1), two-hour post-prandial glucose (2hPBG) ≤ 15 mmoL/L, 28 individuals with Type 2 diabetes mellitus (DM2), 2hPBG ≤ 20 mmoL/L, 29 individuals with Type 3 diabetes mellitus (DM3), 2hPBG ≤ 20 mmoL/L.*

Results: *The differences in βeta cell function among NGT, IGT, DM1, DM2, DM3 were apparent when, the ratio of the increasing serum insulin and plasma glucose levels after 60 minutes glucose loading (ΔI60/ΔG60) and the homeostasis model assessment-β-cell (HOMA-β) were calculated by TI and ΔI60/ΔG60 which was calculated by IRI still decreased appropriately in NGT, IGT, DM1, DM2, DM3. However, the function of βeta cells was estimated in the overweight group higher than in the control group when evaluated by HOMA-β and modified β-cell function index (MβCI), but not by ΔI60/ΔG60. We thought that ΔI60/ΔG60 was a good choice when evaluating β-cell's secretory function, especially when TI could not be measured.*

Conclusion: *The increasing serum insulin and plasma glucose levels after 60 minutes glucose loading was a widely used index which applied not only to diabetes but also to overweight.*

Keywords: βeta cell function, glucose tolerance test, true insulin

Valor Agregado de Insulina Tras la Ingestión de Glucosa vs Valor Agregado de Glucosa en Sangre Luego de la Ingestión de Glucosa Después de Sesenta Minutos como un Buen Indice para Evaluar la Función de las Células Beta Mediante el uso de Ensayo de Insulina no Específico

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RESUMEN

Objetivo: *Determinar la significación clínica de la medición de la insulina verdadera (TI) en suero en personas con sobrepeso y no obesas con diversos grados de tolerancia a la glucosa, y evaluar la función de las células betas mediante el cálculo de índices.*

Métodos: *La insulina verdadera en suero, la insulina inmunorreactiva (IIR), y el nivel de glucosa en ayunas y durante una prueba de tolerancia a la glucosa oral (PTGO), fueron medidos*

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en 32 individuos con tolerancia normal a la glucosa normal (TNG), 42 individuos con alteración de la tolerancia a la glucosa (ATG), 27 individuos con diabetes mellitus tipo 1 (DM1), glucosa postprandial de dos horas (2hPBG) ≤ 15 mmoL/L, 28 individuos con diabetes mellitus tipo 2 (DM2), 2hPBG ≤ 20 mmoL/L, 29 individuos con diabetes mellitus tipo 3 (DM3), 2hPBG ≤ 20 mmoL/L.

Resultados: *Las diferencias en función de las células βeta entre TNG, ATG, DM1, DM2, DM3 fueron evidentes cuando el ratio de la insulina sérica en aumento y los niveles de plasma después de 60 minutos de carga de glucosa (ΔI60/ΔG60) y la evaluación del modelo homeostático para las células βeta (HOMA-β) fueron calculados mediante TI y ΔI60/ΔG60 que se calculó por IIR disminuyó todavía apropiadamente en TNG, ATG, DM1, DM2, DM3. Sin embargo, se estimó que la función de las células βeta en el grupo con sobrepeso era mayor que en el grupo control cuando se evaluó por HOMA-β y el índice de función de las células βeta modificado (MβCI), pero no por ΔI60/ΔG60. Pensamos que ΔI60/ΔG60 fue una buena elección a la hora de evaluar la función secretora de las células βetas, especialmente cuando no se podía medir TI.*

Conclusión: *Aumentar los niveles insulina sérica y glucosa en plasma después de 60 minutos de carga de glucosa, fue un índice ampliamente utilizado que se aplicó no sólo a la diabetes, sino también al sobrepeso.*

Palabras claves: Función de las células βeta, prueba de tolerancia a la glucosa, insulina verdadera

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INTRODUCTION

Insulin resistance and βeta cell's (β-cells) dysfunction are two different aspects in different stages of glucose tolerance, but it is still controversial which is the main reason for Type 2 diabetes (1, 2).

The prevalence of insulin resistance in obese subjects as revealed by previous studies makes it very difficult to accurately investigate the function of β-cell's in these individuals (3, 4).

The effect of insulin resistance causes us, to not know the exact β-cell function or insulin sensitivity from insulin level or glucose level, so we generally use the index to evaluate β-cell function in order to minimize the impact caused by insulin resistance (5). The index include: homeostasis model assessment-β-cell (HOMA-β), modified β-cell function index (MβCI), the ratio of the increasing serum insulin and plasma glucose levels after glucose loading (ΔI/ΔG), all based on the value of insulin (6–8). Homeostasis model assessment-β-cell estimates insulin secretion (9) and is calculated by [20* FINS)/(FPG-3.5], (fasting insulin level (FINS) and fasting glucose level [FPG]). It is simple and used widely in the research of β-cell function. ΔI/ΔG (6–8) is the ratio of insulin and blood glucose value. The ratio of 30 minutes or 60 minutes after glucose was taken is used most commonly. ΔI/ΔG is the recognized index to estimate β-cell function and was used for several years. Modified β-cell function index (10) was proposed by Li, a Chinese scholar and calculated by (FINS * FPG)/(PG2h + PG1h–2FPG). It is still unknown if MβCI is a better index for β-cell function.

The above indices all need to use insulin level, although the method for obtaining them is completely different. Therefore, to determinate specific serum true insulin level properly becomes the key factor for evaluation of the β-cell function and insulin sensitivity. Insulin which is measured by traditional radioimmunoassay is called immunoreactive insulin [IRI] (11), including insulin and proinsulin and other insulin analogues (12, 13). So most researchers have accepted IRI over evaluated β-cell function (14), but how much the IRI affects the index of β-cell function and which index is affected still mostly is unknown.

We determined serum true insulin (TI), IRI and calculated an index of β-cell function, respectively including: HOMA-β, MβCI, ΔI60 /ΔG60 in overweight and non-obese persons with varying degrees of glucose tolerance to study any difference in the clinical significance of measuring serum TI and IRI.

SUBJECTS AND METHODS

One hundred and fifty-eight patients with varying degrees of glucose tolerance were chosen randomly from outpatients and routine physical examination performed.

Informed consent was obtained from patients before enrolment. Serum true insulin, IRI were measured during an oral glucose tolerance test (OGTT) in 32 individuals with normal glucose tolerance (NGT), 42 individuals with impaired glucose tolerance (IGT), 27 individuals with Type 2 diabetes mellitus (DM) group 1, 2hPBG ≤ 15 mmoL/L), 28 individuals with Type 2 diabetes mellitus (DM) group 2, 2hPBG ≤ 20 mmoL/L), 29 individuals with Type 2 diabetes mellitus (DM) group 3, 2hPBG > 20 mmoL/L). The study population was further subdivided into a non-obese body mass index ((BMI) < 25) and an overweight (BMI ≥ 25) group.

Blood lipids, blood pressure, height, weight, waist circumference and hip circumference were determined at the same time. ΔI60/ΔG60, HOMA-β, MβCI (10) were calculated, respectively by TI and IRI in order to analyse the diffferent changes of β-cell function with varying degrees of glucose tolerance.

Serum true insulin kit was the products of US Linco (catalog number, HI-14K), TI concentrations were analysed using a double-antibody radioimmunoassay (RIA) technique with guinea pig antihuman insulin antibodies, human insulin standard and mono-[125 I-Tyr] human insulin. Cross-reaction between TI and intact proinsulin, des-31, 32-proinsulin less than 0.2%, and cross-reaction between TI and des-64, 65-proinsulin was 76%, but des-64, 65-proinsulin only accounted for 5–10% of the total three insulin analogues. The intra- and interassay co-efficients of variation of the insulin assay were less than 3%.

Immunoreactive insulin kit was the product of the Atomic Energy Institute in China and the cross-reaction was 100% with proinsulin and insulin analogues.

All statistical data were handled with the SPSS software package, *t*-test and variance of non-normal distribution data were analysed after taking the natural logarithm.

Ethics statement

The study protocol was approved by the Clinical Research Ethics Committee of the Harbin Medical University (IRB No.050426). Informed consent was confirmed by the Institutional Review Board.

RESULTS

Age, gender, waist hip ratio, systolic blood pressure, diastolic blood pressure, cholesterol, triglycerides were not significantly different among all groups and BMI has no significant difference in obese (OB) subgroups or in non-obese (NOB) subgroups (Table 1).

Table 1: General data of group with varying degrees of glucose tolerance (± S)

GROUP	N(M/F)	age (Y)	BMI (kg/m^2)	Waist-hip ratio (mmHg)	Systolic blood pressure (mmHg)	Diastolic blood pressure	TG (mmol/L)	cholesterol (mmol/L)	OGTT				HbA_{1c} %
									0`	60`	120`	180`	
NGT-OB	16 (9/7)	40.3 ± 15.8	30.20 ± 4.92	0.88 ± 0.07	124.1 ± 27.1	85.0 ± 14.5	1.80 ± 0.94	4.05 ± 1.54	4.24 ± 0.38	5.85 ± 0.83	5.74 ± 0.98	4.31 ± 0.98	5.57 ± 0.52
NGT-NOB	16 (8/8)	39.1 ± 10.1	21.77 ± 2.23	0.82 ± 0.05	119.5 ± 22.9	79.1 ± 16.4	1.12 ± 0.22	4.61 ± 0.89	4.77 ± 0.46	6.31 ±1.1	5.65 ± 1.35	4.72 ± 0.86	5.53 ± 0.61
IGT- OB	25 (14/11)	42.1 ± 11.5	29.56 ± 3.51	0.87 ± 0.05	127.6 ± 13.08	88.4 ± 10.08	1.97 ± 1.19	4.83 ± 1.03	4.98 ± 0.70	9.93±1.86	8.28 ± 1.62	4.71 ± 1.42	6.31 ± 0.69
IGT- NOB	17 (8/9)	42.5 ± 8.8	22.57 ± 1.68	0.84 ± 0.06	116.54 ± 16.51	75.38 ± 6.91	2.01 ± 1.64	4.87 ± 2.00	5.32 ± 0.68	9.72±1.52	8.19 ± 1.59	5.35 ± 1.29	6.49 ± 0.35
DM1- OB	12 (6/6)	42.3 ± 6.3	28.35 ± 2.35	0.88 ± 0.06	126.22 ± 25.87	86.60 ± 14.30	2.37 ± 2.02	5.85 ± 1.40	6.38 ± 0.79	14.91±1.92	12.38 ± 1.54	7.35 ± 2.28	7.88 ± 0.86
DM1-NOB	15 (8/7)	47.6 ± 6.2	23.82 ± 1.02	0.86 ± 0.07	119.28 ± 13.26	78.66 ± 9.63	2.69 ± 1.59	5.66 ± 1.69	6.36 ± 0.72	13.37±2.62	12.63 ± 1.88	6.39 ± 0.12	7.95 ± 0.91
DM2- OB	11 (6/5)	44.6 ± 9.7	27.56 ± 3.65	0.86 ± 0.07	132.35 ± 22.45	89.36 ± 16.23	2.20 ± 1.56	5.56 ± 1.36	8.42 ± 1.31	18.08±2.93	17.97 ± 1.88	14.26 ± 3.50	8.96 ± 0.81
DM2-NOB	17 (8/9)	52.31 ± 5.6	24.32 ± 0.89	0.85 ± 0.06	126.65 ± 12.63	80.96 ± 11.56	2.89 ± 1.60	5.78 ± 1.69	10.12 ± 2.71	16.99±1.06	17.90 ± 1.17	12.92 ± 2.1	8.69 ± 0.35
DM3- OB	12 (6/6)	49.4 ± 12.3	29.35 ± 4.86	0.90 ± 0.06	139.36 ± 20.16	89.45 ± 18.96	2.16 ± 1.62	5.20 ± 1.55	12.49 ± 2.54	23.63±3.04	23.13 ± 2.04	18.71 ± 2.36	11.25 ± 0.93
DM3-NOB	17 (10/7)	53.21 ± 4.3	22.89 ± 0.98	0.85 ± 0.07	130.56 ± 16.53	92.56 ± 11.23	2.63 ± 1.56	5.42 ± 1.23	12.61 ± 4.21	24.3±4.96	26.33 ± 3.80	20.33 ± 3.18	10.93 ± 1.32

Table: BMI: Body mass index; TG: triglyceride; OGTT: oral glucose tolerance test; HbA_{1c}: glycated haemoglobin; NGT-OB: normal glucose tolerance – obese subgroup; NGT-NOB: normal glucose tolerance – non-obese subgroup; IGT-OB: impaired glucose tolerance – obese subgroup; IGT-NOB: impaired glucose tolerance – non-obese subgroup; DM1: diabetes mellitus type 1; DM2: diabetes mellitus type 2; DM3: diabetes mellitus type 3 (OB: obese subgroup; NOB: non-obese subgroup).

Figure 1 showed the results of TI, IRI and CP during OGTT in each group. True insulin, IRI and CP were measured during an oral glucose tolerance test (OGTT). Serum true insulin, IRI, CP were higher in the IGT group than in others, and TI, IRI, CP decreased gradually with blood sugar rising.

Fig. 1A: Overweight group Fig. 1B: Non-obese group

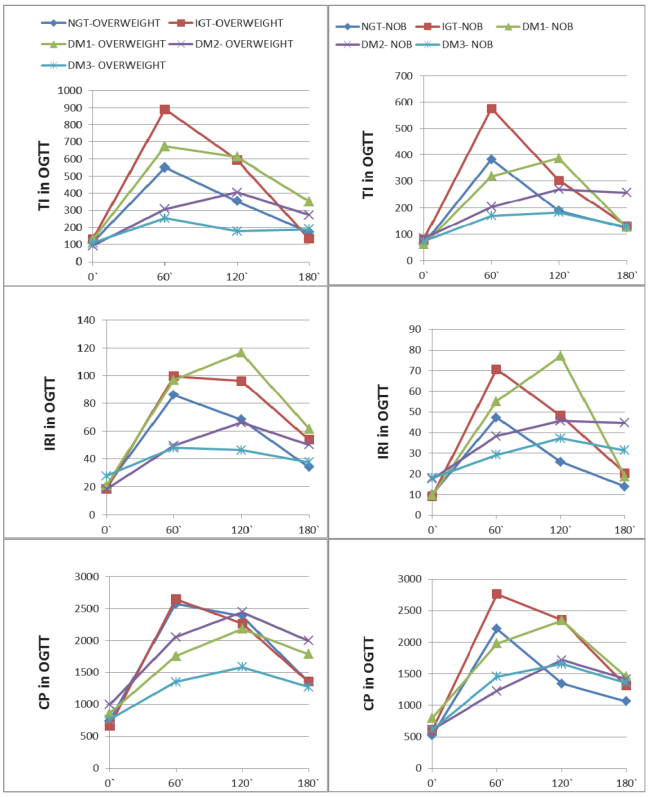

Fig. 1: TI, IRI and CP in OGTT in the overweight group (Fig. 1A) and in the non-obese group (Fig. 1B).

NGT: normal glucose tolerance; IGT: impaired glucose tolerance; DM1: 2hPBG ≤ 15 mmoL/L; DM2: 2hPBG ≤ 20 mmoL/L; DM3: 2hPBG > 20 mmol/L; TI: true insulin; IRI: immunoreactive insulin; CP: C-peptide

Figure 2 shows that HOMA-β, MβCI, ΔI60/ΔG60 decreased in proper order in NGT, IGT, DM groups and significant difference was found between adjacent groups ($p < 0.05$), irrespective of overweight or non-obesity. The Beta cell's secretory function was misjudged in overweight by HOMA-β and MβCI. The figure shows that the β-cell secretory function was better in overweight than in non-obesity with normal glucose tolerance. This conclusion was obviously inconsistent with the actual situation.

Fig. 2A: Overweight group Fig. 2B: Non-obese group

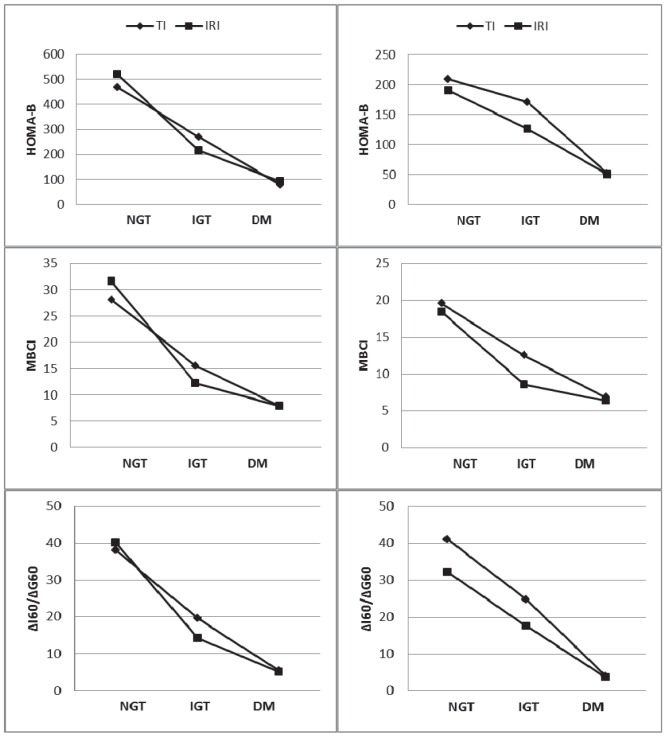

Fig. 2: Comparisons among NGT *vs* IGT *vs* DM in the overweight group (Fig. 2A) and in the non-obese group (Fig. 2B).

NGT: normal glucose tolerance; IGT: impaired glucose tolerance; DM: diabetes mellitus; HOMA-β: homeostasis assessment model estimates insulin sensitivity, is calculated by (20 * FINS) / (FPG-3.5); MβCI: (FINS * FPG) / (PG2h + PG1h-2FPG).

ΔI60: insulin value-added after 60 mins of taking glucose; ΔG60: blood glucose value-added after 60 mins taking glucose; FINS: fasting insulin level; FPG: fasting glucose level.

The index HOMA-β calculated by TI was 125.18 ± 58.02, 68.11 ± 44.90 and 42.12 ± 29.75 in the overweight subgroup ($p < 0.05$), and 69.33 ± 25.63, 47.32 ± 33.69 and 37.22 ± 31.03 in the non-obesity subgroup of DM1, DM2, DM3 ($p < 0.05$).

ΔI60/ΔG60 were 11.97 ± 7.15, 3.10 ± 2.65 and 1.33 ± 1.29 in the overweight subgroup (pairwise comparison, $p < 0.01$); 6.22 ± 2.20, 3.60 ± 2.22 and 2.13 ± 2.15 in non-obesity subgroup in DM1, DM2 and DM3 groups in turn (pairwise comparison, $p < 0.01$ and $p < 0.05$) by using TI. The results by using IRI were 10.45 ± 6.42, 3.15 ± 2.21 and 1.13 ± 0.91 in the overweight subgroup (pairwise comparison, $p < 0.01$); 7.33 ± 5.90, 2.56 ± 2.00 and 1.20 ± 1.51 in the non-obese subgroup in DM1, DM2 and DM3 groups in turn (pairwise comparison, $p < 0.01$

and $p < 0.05$). ΔI60/ΔG60 calculated by IRI were 40.12 ± 23.04 *vs* 32.21 ± 39.22 ($p > 0.05$), by TI were 38.09 ± 26.70 *vs* 40.97 ± 34.42 ($p > 0.05$) in the overweight and the non-obese subgroups with normal glucose tolerance, and had no significant difference. The HOMA-β were 467.71 ± 209.65 *vs* 208.93 ± 117.26 ($p < 0.01$), and the MβCI were 28.06 ± 24.41 *vs* 19.53 ± 14.60 ($p < 0.05$) calculated by TI.

Homeostasis model assessment-β-cell (TI, IRI), MβCI (TI, IRI), ΔI60/ΔG60 (TI, IRI) decreased in proper order in NGT, IGT, DM groups and significant difference was found between adjacent groups ($p < 0.05$). However, only HOMA-β (TI), ΔI60/ΔG60 (TI, IRI) decreased in proper in DM1, DM2, DM3 group and significant difference was found between adjacent groups ($p < 0.05$).

Fig. 3A: Overweight group Fig. 3B: Non-obese group

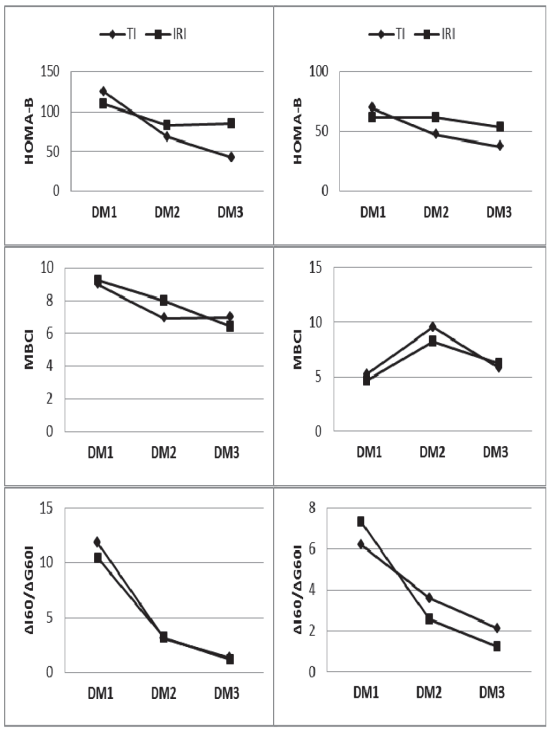

Fig. 3: DM1 *vs* DM2 *vs* DM3 in the overweight group (Fig. 3A) and in the non-obese group (Fig. 3B).

Only HOMA-β (TI), ΔI60/ΔG60 (TI, IRI) decreased in proper in DM1, DM2, DM3 group and significant difference was found between adjacent groups ($p < 0.05$).

DM: diabetes mellitus; DM1:2hPBG ≤ 15 mmol/L; DM2: 2hPBG ≤ 20 mmol/L; DM3: 2hPBG > 20 mmol/L; HOMA-β: homeostasis assessment model estimates insulin sensitivity, is calculated by (20 *FINS) / (FPG-3.5).

TI: serum true insulin; IRI: immunoreactive insulin; PBG: postprandial blood glucose; ΔI60: Insulin value-added after 60 mins taking glucose; ΔG60: Blood glucose value-added after 60 mins of taking glucose; FINS: fasting insulin level; FPG: fasting glucose level.

Correlation analyses between TI values and IRI values
We calculated the relationship between TI and IRI values and r = 0.9645, $p < 0.01$ according to the groups: NGT, IGT and DM; r = 0. 9363, $p < 0.01$ according to the groups: DM1, DM2 and DM3. So TI values and IRI values were positively correlated.

DISCUSSION

Hyperglycaemic lamp technique is the most classical technique for evaluating β-cell function, but it is difficult to use widely due to expense and is cumbersome and complex. In recent years, scientists have created a number of simple and economical methods for evaluating β-cell function. This study attempts to find a more practical index to evaluate the function of β-cell. So we calculated widely used ΔI/ΔG, HOMA-β and MβCI index to compare the value of different indices for β-cell function. Serum true insulin and IRI was calculated separately in order to compare the difference between them in patients with varying degrees of glucose tolerance.

This study showed that all indices can evaluate the β-cell secretory function well when the subject is in NGT, IGT or of the DM group, and no matter TI or IRI. The results in each adjacent group showed significant differences ($p < 0.05$, Fig. 2). However, the distinguishing ability of the studied index for β-cell secretory function was totally different when the subject was in DM1, DM2, DM3 group with different blood glucose levels.

The index HOMA-β which was calculated by TI, but not by IRI, could distinguish the secretory function of β-cell clearly, which gradually decreased in the DM1, DM2, DM3 groups ($p < 0.05$, Fig. 2).

Homeostasis model assessment-β-cell calculated by IRI had no significant difference among DM1, DM2, DM3 group regardless of overweight or non-obesity. So TI should be determined when HOMA-β is used for evaluating β-cell secretion in diabetes.

ΔI60/ΔG60 has a strong ability to distinguish secretory function of β-cell in those with different levels of blood glucose calculated by TI or IRI (15); the best results can be seen even if IRI was determined.

The reason for this may be that the index calculated by using the different (margin) of insulin level between different time in the same exactly person. This means that the error of evaluating IRI was reduced because the ratio of proinsulin and other insulin analogue in IRI was sim-ilar in the same person even if the level of IRI was inap-propriately over-estimated by proinsulin and insulin analogue in diabetes during OGTT. This paper showed that ΔI60/ΔG60 can be used to analyses β-cell secretory

function by determining IRI if TI cannot be determined.

ΔI60/ΔG60 can also be used to evaluate β-cell secretory function in overweight non-obesity no matter if calculated by TI or IRI. But the HOMA-β and MβCI indices do not have this ability. When used HOMA-β and MβCI, the β-cell secretory function was better in overweight than in non-obesity with normal glucose tolerance. This conclusion was obviously inconsistent with the actual situation. The β-cell secretory function was misjudged in obesity by HOMA-β and MβCI because the two indies were calculated by fasting insulin level, which is better in overweight than non-obesity because of insulin resistance and ΔI60/ΔG60 evaluated β-cell secretory function of overweight correctly once again, avoiding the impact of elevated fasting insulin by using the difference (margin) of insulin level among different times in the OGTT.

ΔI60/ΔG60, HOMA-β and MβCI can all evaluate β-cell secretory function well in subjects with NGT or IGT regardless of TI or IRI. Homeostasis model assessment-β-cell and ΔI60/ΔG60 can evaluate β-cell secretory function well in subjects with diabetes if TI was determined. If only IRI but not TI can be determined, ΔI60/ΔG60 needs to be used in diabetes. In summary, the results indicate that ΔI60/ΔG60 was a good choice when evaluating β-cell secretory function, especially if TI could not be measured. ΔI60/ΔG60 was a widely used index which applied to not only diabetes but also obesity.

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