Evaluating pancreas function by meal tolerance test (MTT) in diabetes

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Abstract

Background: Diabetic nutritional treatment involves the discussion of Low Carbohydrate Diet (LCD) and Calorie Restriction (CR). Authors have initiated and developed LCD in Japan and continued clinical research. In this study, we investigated glucose variability in patients with type 2 diabetes mellitus (T2DM). Subjects and Methods: Subjects were 60 T2DM patients of 62.7 years in average with its fasting immunoreactive insulin (IRI) less than 5μU/mL. Methods include basal blood test, daily profile of blood glucose and insulinogenic index (IGI) for 70g of carbohydrate (0-30min) in CR breakfast. Correlation among these and comparison in 4 groups categorized by Morbus value were analyzed. Results: Basal data revealed HbA1c 7.9%, daily glucose 222 mg/dL in average, and Triglyceride 83 mg/dL, Morbus value 150, HOMA-R 1.1, HOMA-β 11.0 in median. Delta Ratio of IGI and AUC ratio of IGI showed significant correlations with M value and HbA1c (p<0.01). Discussion and Conclusion: Meal Tolerance Test (MTT) has been recently used for convenient methods and meaningful results. AUC ratio suggests a little superior than Delta ratio for its higher correlation coefficient. These results would become the basal data in this field, and further development of related research is expected in the future.

Keywords: area under the curves (AUC), insulinogenic index (IGI), type 2 diabetes mellitus (T2DM), morbus value (M value), delta ratio of IGI, AUC ratio of IGI

Introduction

As to diabetes mellitus, the prevalence of diabetes has been increasing worldwide, and it would become not only medical problem, but also social, economic and ecological problems [1]. Diabetes has a variety of complications with micro-angiopathy and macro-angiopathy. The former includes neuropathy, retinopathy and neuropathy, and furthermore, the latter includes large vessels impairment and dysfunction of head, heart and lower leg [2].

For diabetic prevention and treatment, several diabetic societies have presented their guidelines until now. There was recently the proposal of changes in the guideline about the goal of treatment for diabetes. American Diabetes Association (ADA) has given the comments in 2017 [3], which was followed by the joint algorithm of European Diabetes Society (EASD) 2012 [4]. In succession, American College of Physicians (ACP) has
proposed the change in standard value concerning the goal of HbA1c value [5], where the management goal for HbA1c in most type 2 diabetic patients would be 7% or more and less than 8%. This seemed to be a large impact for several diabetic societies. Against the concept of ACP, ADA made an objection comment immediately [6]. Thus, diabetic management has been in discussion among several guidelines from medical societies, leading to better clinical practice with accumulated evidences.

For years, the problem about carbohydrate intake has been continued. Diabetic nutritional treatment can be generally classified into 2 representative groups. One is Calorie Restriction (CR) diet, and another is Low Carbohydrate Diet (LCD) [7,8]. The former means mainly the restriction fat and calorie restriction, while the latter means reduced amount of carbohydrate. LCD has been known for clinical effects such as weight reduction and several beneficial aspects.

Originally, LCD was started by Atkins and others in North American region and European countries [9]. After that, authors and colleagues started to introduce LCD projects in Japan and developed LCD through lots of books, seminars, presentation in medical conferences and papers [10,11]. We also developed social movement through Japan Low Carbohydrate Diet Promotion Association [10]. We have continued clinical practice for diabetes with three useful LCD formula meals, which are petit LCD, standard LCD, super LCD) [11]. Furthermore, we already presented various research reports concerning LCD, M value, ketone bodies and related investigation [12-14].

As we have continued diabetic research using LCD and CR, we have reported the proposal for clinically new index which is simple and useful method. It has been similar method and calculation of insulinogenic index (IGI) against 75g oral glucose tolerance test (75gOGTT). Subjects have breakfast with 70g of carbohydrate, fat and protein in it, which is one of the meal tolerance test (MTT). Furthermore, the response of blood glucose and immunoreactive insulin (IRI) would be measured [15]. It is called insulinogenic index (IGI)-Carbohydrate70g (IGI-Carbo70), and seems to play a role of simple and useful clinical diabetic practice. We develop this evaluation method, and continue further investigation concerning IGI and average glucose, M value and measurement of Delta (increment) ratio and Area Under the Curves (AUC) ratio of IGI in this study.

**Subjects and Methods**

Subjects enrolled in this study were 60 patients with type 2 diabetes mellitus (T2DM). For evaluation and treatment for T2DM, they were admitted to the hospital. We have performed the standard diabetic examination protocol for CR and LCD program. Regarding the necessary elements and condition of the patients, the following items were included. i) medical diagnosis was T2DM, ii) type 1 diabetes mellitus (T1DM) and special type of DM were excluded, iii) patients who had already have insulin therapy were excluded, iv) patients whose body mass index (BMI) was 35 and more than 35 were excluded, v) patients whose IRI level was 5 and more than 5 μU/mL were excluded.

Methods for the study were according to our examination protocol for diabetes with the meal of CR and LCD. In this study, the following procedures were used.

(i) In regard to research protocol, patients are to take the standard meal of CR on day 1 and 2, and LCD after day 3, with 1400 kcal/day each. In the case of current study, we used the data of meal tolerance test (MTT) in the morning of day 2, and the data of daily glucose profile 7 times a day on day 2.

(ii) In the morning of day 2 after overnight fasting, fundamental biomarkers related to diabetes were measured. They included glucose, HbA1c, IRI, complete blood count, liver and kidney function, lipids and so on.

(iii) On day 2 just after drawing blood samples for basal items, patients were to take breakfast of standard formula. It included 70g of carbohydrate, protein and fat. As to this breakfast of CR, PFC ratio was 15% of protein, 25% of fat and 60% of carbohydrate. The content of this standard meal was due to the standard guideline of diabetes meal that was proposed by Japan Diabetes Society (JDS) [16].

(iv) The content of the breakfast was calculated as follows: The meal has 1400 kcal/day and the ratio of the carbohydrate is 60%, then 840 kcal was from the carbohydrate per day. One third of 840 kcal is 280kcal, and 280kcal of carbohydrate equals to 70g of carbohydrate as a breakfast.

(v) MTT was performed in the following: Pre and post 30 min of breakfast, blood sample was drawn for blood glucose and IRI. After breakfast for 30 minutes, the subjects were indicated to keep still on the chair on sitting position.

(vi) The examination of daily profile of blood glucose was done during Day 2. Blood samples were drawn 7 times a day. They were 0800, 1000, 1200, 1400 1700, 1900, 2200h. From these results, average blood glucose value and also Morbus (M) value were obtained using the standard formula calculation for M value.

**Morbus value**

As one of the biomarker for indicating average blood glucose level and also the mean amplitude of glycemic excursions (MAGE), M value has been introduced [17,18]. Consequently, M value suggests the degree of
Consequently, M value suggests the degree of hyperglycemia and also the degree of high fluctuation or swinging of blood glucose in a day. The data of M value has been calculated by the way of logarithmic transformation. It can suppose the deviation of glucose level and swinging level from ideal glucose level [17-19]. The level of M value is calculated by the method of logarithmic transformation, which means the deviation of glucose from ideal glucose value [17-19].

There is the formula to calculate the M value in the following way. At first, the basic equation is that \( M = MBS + MW \), and M value is the total of MBS and MW. Secondly, MW is \( \frac{\text{maximum blood glucose} - \text{minimum glucose}}{20} \). Moreover, MBS is the mean of MBSBS. When these equations are summarized, MBSBS is the individual M-value for each blood glucose, calculated as \( (\text{absolute value of } [10 \times \text{log (blood glucose level/120)})^3 \) [17-20]. The result of M value has been clinically evaluated as follows: normal range is less than 180, borderline is from 180 to 320, abnormal levels are from 180 to 320.

**Insulinogenic index for MTT**

According to the results of glucose and IRI on 0 and 30 min in the MTT, two kinds of IGI were calculated. In the case of 75gOGTT, IGI has been indicated to speculate pancreas function by the ability of secretion of insulin. The formula is that the increment (delta) of insulin (30min – 0 min) / increment (delta) of blood glucose (30min – 0min). In this article, it equals to the ‘Delta Ratio of IGI for Carbo70’.

We tried another evaluating method, taking the advantage of the Area Under the Curves (AUC) describing the responses of glucose and insulin. By comparing the area size, we call the ratio between IRI and glucose as the ‘AUC Ratio of IGI for Carbo70’. To summarize the both calculation methods, two biomarkers were as follows: the Delta Ratio of IGI for Carbo70 is defined as \( (\text{IRI at 30min} – \text{IRI at 0min} \times 1000) / (\text{Glucose at 30min} – \text{Glucose at 0min}) \) (μU/mL x h)/ (mg/dL). On contrast, the AUC Ratio of IGI for Carbo70 is defined as \( (\text{AUC of IRI for 0-30min} \times 1000) / (\text{AUC of glucose for 0-30min}) \) (μU/mL x h)/ (mg/dL x h).

**Glucose variability of a day**

Regarding to the daily profile of blood glucose, 7 times of blood samples were drawn on day 2. From the obtained data, average blood glucose on day 2, and M value were investigated. According to the previous study, there were almost the similar data of comparison between 7-times sampling and 20 times sampling [19,20].

**Statistical analysis**

In current study, data were revealed by mean and standard deviation, and also by the median and quartile of 25% / 75% in several biomarkers. The latter are described as median [25%–75%] inserted numerical value in the parenthesis. With regard to the statistical calculation, the correlation coefficients were used for the study, in which Spearman test has been utilized on analytical evaluation [21].

**Ethical Standard**

This research was conducted in compliance with the ethical principles based upon the Declaration of Helsinki. In addition, additional commentary was done in 2004 General Assembly Tokyo, Japan. These were conducted with Personal Information Protection Law and in reference to “Standards for the Implementation of Clinical Trials (GCP), an ordinance of the Ministry of Health, Labour and Welfare No. 28 of March 27, 1997. Furthermore, there was the “Ethical Guidelines for Epidemiology Research” presented by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare.

Authors and colleagues had an ethical committee consisting of professionalisms, such as physician, nurse, pharmacist and other experts in the legal specialty. We have discussed enough and confirmed that current study is valid and agreed with all members. We have obtained informed consents and written paper agreements from the subjects. Current study was registered by National University Hospital Council of Japan (ID: #R000031211).

**Results**

**Basal data**

Several data of the subjects in the morning on Day 2 were shown in Table 1. There were average data about 62.7 years in age, 7.9% in HbA1c, 222 mg/dL in glucose, respectively. There were median data about 150 in M value, 1.1 in HOMA-R, 82.5 mg/dL in Triglyceride, respectively.

Responses of Glucose and insulin for Carbo70 were shown in Table 2. From the data of 0 min and 30 min, Delta Ratio of IGI for Carbo70 and AUC ratio of IGI for Carbo70 were 0.12 [0.07–0.21] and 3.1 [2.3-4.6], respectively.

**Correlation between IGI and M value**

IGI was calculated by 2 methods, one is Delta Ratio of IGI for Carbo70, and another is AUC Ratio of IGI for Carbo70. There was significant correlation between Delta Ratio of IGI for carbo70 and M value (p<0.01) (Figure 1a). Similarly, there was significant correlation between AUC Ratio of IGI for carbo70 and M value (p<0.01) (Figure1b). Compared the both, the latter showed higher correlation coefficient, with the results of \( R^2 = 0.16 \) vs 0.45, respectively.
**Correlation between IGI and HbA1c**

There was significant correlation between Delta Ratio of IGI for carbo70 and HbA1c (p<0.01) (Figure 2a). Similarly, there was significant correlation between AUC Ratio of IGI for carbo70 and HbA1c (p<0.01) (Figure 2b). Compared to the both, the latter showed higher correlation coefficient, with the results of $R^2$ = 0.16 vs 0.30, respectively.

**Comparison of M value and HbA1c in 4 groups**

Subjects (N=60) were classified into 4 groups according to the data of M value (n=15, each). M value in median in the 4 group was 15, 77, 227, 625, respectively (Figure 3a). HbA1c value was shown in Figure 3b.

**Table 1. Subjects and basal data.**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Number (M/F)</th>
<th>Mean±SD</th>
<th>Median [25% - 75%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>age (years old)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 (35/25)</td>
<td>62.7±10.6</td>
<td>60 (35/25)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Glucose profile</th>
<th>HbA1c (%)</th>
<th>fasting glucose (mg/dL)</th>
<th>average glucose (mg/dL)</th>
<th>Morbus value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.9±1.7</td>
<td>168±54.5</td>
<td>222±82.1</td>
<td>264±296</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>150[40-410]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insulin resistance</th>
<th>fasting glucose (mg/dL)</th>
<th>Fasting IRI</th>
<th>HOMA-R</th>
<th>HOMA-β</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>166±53.9</td>
<td>2.9±1.1</td>
<td>1.2±0.6</td>
<td>13.3±9.1</td>
</tr>
<tr>
<td></td>
<td>156[117-208]</td>
<td>3.0[2.3-3.9]</td>
<td>1.1[0.8-1.6]</td>
<td>11.0[7.3-16.6]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lipid profile</th>
<th>Triglyceride (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>LDL-C (mg/dL)</th>
<th>LDL/HDL ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>115±87.0</td>
<td>71.1±19.8</td>
<td>133±38.2</td>
<td>2.0±0.7</td>
</tr>
<tr>
<td></td>
<td>82.5[60.7-143]</td>
<td>66.5[568-82.8]</td>
<td>136[107-157]</td>
<td>1.8[1.3-2.5]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Renal function</th>
<th>Creatinine (mg/dL)</th>
<th>Uric Acid (mg/dL)</th>
<th>Ccr (ml/min)</th>
<th>Ccr (L/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.72±0.15</td>
<td>4.9±1.2</td>
<td>94.3±25.2</td>
<td>135±35.9</td>
</tr>
<tr>
<td></td>
<td>0.72[0.62-0.79]</td>
<td>4.8[4.0-5.6]</td>
<td>94.0[78.3-109]</td>
<td>134[112-153]</td>
</tr>
</tbody>
</table>

**Table 2. Responses of Glucose and insulin for Carbo70**

<table>
<thead>
<tr>
<th>Response of glucose</th>
<th>Mean±SD</th>
<th>Median [25% - 75%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>before (0 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after (30 min)</td>
<td>168±54.5</td>
<td>156[117-209]</td>
</tr>
<tr>
<td>delta (0-30 min)</td>
<td>218±61.8</td>
<td>210[165-271]</td>
</tr>
<tr>
<td></td>
<td>51.9±27.8</td>
<td>46[32-64]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Response of Insulin</th>
<th>Mean±SD</th>
<th>Median [25% - 75%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>before (0 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after (30 min)</td>
<td>2.9±1.1</td>
<td>3.0[2.3-3.9]</td>
</tr>
<tr>
<td>delta (0-30 min)</td>
<td>10.2±7.7</td>
<td>8.7[6.3-11.9]</td>
</tr>
<tr>
<td></td>
<td>7.4±7.5</td>
<td>5.4[3.6-8.8]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Delta Ratio of IRI/Glu</th>
<th>Mean±SD</th>
<th>Median [25% - 75%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta of glucose</td>
<td>51.9±27.8</td>
<td>46[32-64]</td>
</tr>
<tr>
<td>Delta of insulin</td>
<td>7.4±7.5</td>
<td>5.4[3.6-8.8]</td>
</tr>
<tr>
<td>Delta of Ratio</td>
<td>0.17±0.19</td>
<td>0.12[0.07-0.21]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AUC Ratio of IRI/Glu</th>
<th>Mean±SD</th>
<th>Median [25% - 75%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC of glucose</td>
<td>277±83.2</td>
<td>263[201-349]</td>
</tr>
</tbody>
</table>
Figure 1. Correlation between Delta/AUC Ratio and M value. 1a: Correlation between Delta Ratio of IGI and M value. 1b: Correlation between AUC Ratio of IGI and M value.

Figure 2. Correlation between Delta/AUC Ratio and HbA1c. 2a: Correlation between Delta Ratio of IGI and HbA1c. 2b: Correlation between AUC Ratio of IGI and HbA1c.
Figure 3. Comparison of M value and HbA1c in 4 groups. 3a: Comparison of M value in 4 groups. 3b: Comparison of HbA1c in 4 groups.

Figure 4. Comparison of Delta Ratio and AUC Ratio in 4 groups. 4a: Comparison of Delta Ratio of IGI in 4 groups. 4b: Comparison of AUC Ratio of IGI in 4 groups.
Discussion

Discussion on CR and LCD has been continued for years. In this perspective, authors have reported clinical research in two axes. As the first axis, standard meals of CR and LCD were provided and related biomarkers were measured and compared. Among these research, M value has been calculated which indicates average blood glucose and the degree of glucose fluctuation [15,22]. As the second axis, breakfast with 70g of carbohydrate from the standard CR meal has been tried for the response of glucose and insulin. This is one of the MTT similar to 75gOGTT [15,23,24].

Recently, MTT has been used more for the evaluation of pancreas function. Breakfast has been frequently applied, in which mixed macronutrients are included [25,26]. As an example, there are carbohydrate 50%, fat 35%, protein 15%, 450 kcal, including 56g of carbohydrate [25,26].

From two axes mentioned above, this study is the combination of the both. Daily profile of blood glucose and M value represent glucose variability in a day [22,27,28]. Furthermore, Delta or AUC ratio of IGI suppose the insulin secretion for 30 min. Consequently, this study would be related with the pathophysiological background of T2DM. The distribution of the data in M value was larger than that of HbA1c, suggesting that M value may be more useful with less overlap area than HbA1c, and that M value has benefit of indicating of both average glucose and mean amplitude of glycemic excursions (MAGE) into one numerical value [22,27,28].

In 60 cases of this study, median fasting blood glucose was 156 mg/dL, and the median average blood glucose was 210 mg/dL. The 7-times sampling method has reported to be the same result of 20-times sampling and continuous glucose monitoring (CGM). Probably, CGM will certainly be frequently used to the future, but at present this method is simple and useful for grasping blood glucose variability.

In this study, the median increase of glucose and IRI increase for Carbo70 were 46 mg/dL and 5.4 μU/mL, respectively. The Delta Ratio of IGI for Carbo 70 values were 0.17 on average and 0.12 in median. Regarding this numerical value, there is a previously related reports.

There was similar MTT report by Cozma et al. in which formula breakfast has 500 kcal in calorie and 55% of carbohydrate [29]. It contains 69g of carbohydrate, which is similar to our protocol with 70g of carbohydrate. Due to their protocol, they excluded the cases whose fasting glucose was more than 180 mg/dL, because of little insulin responses. Calculated from the data of Cozma et al. [29], supposed data of Delta ratio of IGI is 0.39, and AUC ratio of IGI is 12.3, which was similar to our results of those in group 1 and 2. Both data are similar in the carbohydrate loading 69g vs 70g, and in the insulinogenic index. The correlation with M value was compared between Delta ratio and AUC ratio. Significant correlation was observed in both cases, but higher correlation was found in the latter as $R^2 = 0.45$. The reason is speculated to the large variance in the Delta calculation method.

Similarly for the correlation with HbA1c, both Delta ratio and AUC ratio were compared. The latter showed a higher correlation as $R^2 = 0.30$. This would be probably due to wider distribution. Furthermore, compared with M value and HbA1c, the correlation of HbA1c with Delta and AUC ratio is lower in the latter. M value is calculated from the average blood glucose of the day examined, while HbA1c is assumed to be the average over the past month. These situation would be involved in the difference between M value and HbA1c.

As for 4 groups, HbA1c data tended to overlap each other, whereas the M value showed little overlap. This is probably from the fact that M value indicates average and fluctuation of glucose, which would be considered to show larger difference in the numerical value.

In the study of 4 groups, Delta ratio and AUC ratio were compared. In the former, the median value overlapped, but in the latter, the median value decreased in the group from 1 to 4. Accordingly, in the 4th groups with high average blood glucose, the decrease in insulin secretion ability is obviously recognized. It seems to be clearer in AUC ratio rather than delta ratio. Consequently, AUC ratio seems to be a little superior to Delta ratio as an analysis method of IGI.

In the clinical setting from now on, meal tolerance test (MTT) using Carbo 70g can be applied instead of 75gOGTT. Moreover, calculation method includes both delta ratio and AUC ratio. From current results, AUC ratio may be useful for clinical diabetic research, associated with assessment way for grouping.

There are several methods to suppose insulin response to carbohydrate loading in order to examine pancreatic function [30]. Conventionally, Intravenous Glucose Tolerance Test (IVGTT) and OGTT have been prevalent [31]. In recent years, MTT has been introduced and adopted in clinical practice and research.

There are some reports of MMT. One formula meal is high-protein Boost-HP (237ml, Vevey, Switzerland) consisting of carbohydrate 33g, protein 15g, fat 6g [32]. Its PFC ratio is 25:20:55, and speculated Delta IGI for Carbo 33g would be 1.6 in average. Another formula for MMT is a breakfast with 450 kcal and PFC = 15:35:50 [33]. In this case, carbohydrate dose seemed to be 56g in the breakfast.

Recent study showed 2 types of formula breakfast. One is carbo-breakfast with PFC = 15:20:65%, and another is protein-breakfast with PFC = 35: 20: 45% [34]. When same lunch were provided to carbo-group and protein-group, the latter showed higher insulin response and lower glucose increase. This is called ‘second-meal
phenomenon, keeping the glucose variability controlled. By the ingestion of mixed meal loading, GLP-1-induced insulin secretion has been observed [35]. For preload of mixed nutrient, glucose tolerance was decreased according to the severity level of T2DM [36]. From these, to study the responses to nutrient ingestion would clarify the pathophysiological function of T2DM, leading to improvement of glucose variability.

There are limitation of this study. Various research due to MTT have been found. Because the content has complex macronutrients, they may have unstable speed and degree of digestion and absorption, various kinds of mixture ratio or unexpected response of insulin secretion. However, our current research would become a fundamental data for future research.

Conclusion

In summary, we investigated 60 T2DM patients for the daily profile of blood glucose, average glucose and Morbus value. Furthermore, we studied IGI of insulin/glucose (0-30min) for Carbo70 and calculated the Delta Ratio of IGI for Carbo70 and the AUC Ratio of IGI for Carbo70. Obtained data were compared and correlations among those were investigated. AUC Ratio of IGI seemed to be a little superior for clinical research than Delta Ratio of IGI. These results would be the basal data in this field, and further development of related research is expected in the future.

Acknowledgement

As for this paper, some part of them was presented in the 90th Annual Congress of Japan Diabetes Society (JDS), Tokyo, 2018. The authors appreciate all staffs and patients for their understanding and cooperation.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Abbreviation

AUC: Area Under the Curve; IGI: Insulinogenic Index; T2DM: Type 2 diabetes mellitus; M value: Morbus value; IRI: immunoreactive insulin

References


