STUDY OF GLUCOSE TOLERANCE AND
THE DYNAMIC PROPERTY OF INSULIN SECRETION.
ANALYSIS OF INTRAVENOUS GLUCOSE TOLERANCE TEST
WITH THE AID OF A CONTROL THEORY

By

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ABSTRACT

The dynamic property of insulin secretion in relation to glucose tolerance was investigated quantitatively during iv glucose tolerance tests in 237 cases. The following results were obtained; 1) Glucose clearance constant (k-value) was not constant but variable with time and should be expressed as a function of time, K(t). In normal glucose tolerance, K(t) became greater with time. 2) Glucose-induced insulin secretion was expressed as the function of a proportional plus derivative response to glucose concentration. A weighting function of derivative response, reflecting the insulin secretion per unit of rate of change in blood glucose concentration, was calculated from blood glucose concentration (input) and insulin concentration (output) by the deconvolution method. It was clearly shown that the gain in weighting function was small and the response was slow even in the individual whose glucose tolerance was slightly impaired. 3) The greater the weighting function, the larger the change in K(t).

Glucose homoeostasis is one of the most typical feedback control systems. The factor which controls glucose homoeostasis most powerfully seems to be the dynamic property of insulin secretion. The method for extracting parameters
which reflect glucose homoeostasis in relation to the dynamic property of insulin secretion theoretically has not been fully established.

Oral glucose tolerance tests (OGTT) have been widely applied to examine glucose tolerance and the insulin secretory ability, for the following reasons: 1) They are simple to perform. 2) The route of glucose administration is physiological. 3) Since many results have been accumulated, suitable criteria can be derived. On the contrary, intravenous glucose tolerance tests (IVGTT) are superior to OGTT as the means of investigating the quantitative relationship between glucose tolerance and the dynamic property of insulin secretion. This is because, for a quantitative evaluation, the intensity and time course of the glucose stimulus should be uniform whereas in OGTT, the mechanism of glucose absorption from the intestine and insulinogenic enterohormones confuse the interpretation of the results.

Among the methods for analyzing glucose assimilation during IVGTT quantitatively, the method of Hamilton & Stein (1942) and the method of Amatuilio et al. (1953) have been widely used, but in only a few studies was the validity of these models evaluated. Therefore, the present study was undertaken to determine the quantitative relationship between glucose tolerance and the dynamic property of insulin secretion by analyzing the data of IVGTT in 237 cases with the aid of a control theory.

MATERIALS AND METHODS

IVGTT were performed in 237 cases, of which 99 were normal, 56 were borderline and 82 were diabetics classified according to 50 g OGTT results by the criteria of the Japan Diabetic Society (Kuzuya et al. 1970); the glucose tolerance curve in which 60- and 120-min values were higher than 160 and 130 mg/100 ml, respectively, was defined as the diabetic type, and the glucose tolerance curve in which 60- and 120-min values were less than 140 and 100 mg/100 ml, respectively, was defined as the normal type. Intermediate curves were all defined as the borderline type.

Subjects whose obesity index was more than +20% determined from Metropolitan Life Insurance Table (Documenta Geigy Scientific Tables 1962) were not included in this study. Diabetic subjects whose fasting blood glucose concentrations were more than 150 mg/100 ml were excluded. All diabetic patients included in this study had not previously been treated with any medication.

After standard dietary preparation for at least 1 week, each individual was administered 0.3 g of glucose per kg body weight as a 40% solution in distilled water iv into an antecubital vein in exactly 3 min in the early morning after overnight-fasting. Samples were timed from the initiation of injection. Blood samples for glucose and insulin measurements were obtained at just before, 4, 7, 10 min, then every 5 min up to 60 min from the opposite antecubital vein. Plasma glucose concentrations were measured by the method of Hoffman (1937) using an Autoanalyzer. Immunoreactive insulin concentrations (IRI) in plasma were measured by the method of Hales & Randle (1963) in 106 cases, of which 33 were normal, 82 were borderline and 41 were diabetics, classified by means of the 50 g OGTT.

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DATA ANALYZING METHODS

1) Glucose clearance constant during IVGTT

In all 237 cases, glucose clearance constants (k-value) were determined by the method of Amaluzio et al. (1953), which is shown as the following equation;

$$\frac{dX(t)}{dt} = -k [X(t) - X(0)]$$

where X(0) and X(t) are the glucose concentrations pre-stimulated and at t min after the initiation of glucose injection, respectively. To determine k-value, ln [X(t) - X(0)] were plotted on the ordinate against time, t, on the abscissa, then the regression line was obtained by the least squares method by the computer (FACOM U-200, Fujitsu, Japan). The slope of the regression line obtained denoted k-value. In the original method, Amaluzio et al. (1953) for determining k-values, all the data from 10 to 60 min, or if [X(t) - X(0)] = 0 within 60 min, the data from 10 to the time when [X(t) - X(0)] = 1 were used.

In this study, K1, which is the slope of the line from 10 to 20 min, K2, which is the one from 20 to 30 min and K3, which is the one from 30 to 60 min, but if [X(t) - X(0)] = 0 within 60 min, the one from 30 to the time when [X(t) - X(0)] = 1 were determined, respectively in each subject. K1, K2 and K3 were expressed as per cent per min.

2) Quantification of insulin secretory response

Insulin secretory response is affected by the moment to moment plasma glucose concentration, so it is of no value to compare the plasma insulin concentration with different glucose responses during IVGTT for the purpose of estimating glucose-induced insulin secretory ability. To correct the insulin secretory response for plasma glucose concentration, in this study both the conventional insulinogenic index method and a weighting function method with the aid of a control theory were applied.

a) Insulinogenic index method. – The insulinogenic index was calculated in each subject by the method of Seltzer et al. (1967), by dividing the area circumscribed by the insulin curve (i.e. the increment above fasting level, $\Sigma$/insulin) by the corresponding area circumscribed by the glucose curve ($\Sigma$/glucose) from 0 to 10 min.

b) Weighting function method. – We have reported that the dynamic property of glucose-induced insulin secretion was simulated with the aid of a control theory, and the relationship between the stepwise input of glucose concentration and the biphasic response of insulin as an output was expressed successfully in the transfer function of proportional (glucose concentration per se) plus derivative (the rate of change in glucose concentration) response to glucose concentration (Kawamori et al. 1978).

By using this model, the following equation was obtained to express the relationship between plasma insulin concentration, y(t), and glucose concentration in plasma, x(t).

$$Y(s) = Gp(s) X(s) + Gd(s) sX(s)$$

where Y(s), X(s) and sX(s) are the Laplace transforms from y(t), x(t) and $\frac{dx(t)}{dt}$, respectively. Gp(s) and Gd(s) are the transfer functions of plasma glucose concentration

1) Laplace transformation is a useful means of analyzing linear systems. Firstly the function of time, t, is transferred to the function of complex number, s, and necessary calculations are performed. The results obtained are then inversely transferred to the function of time to determine the unknown function.
per se and the rate of change in plasma glucose concentration, respectively\(^2\). \(G_p(s)\) and \((G_d)\)s were calculated by the following processes from the data in each subject.

i) Plasma glucose concentration and insulin concentration in plasma were regarded as the stable state before glucose stimulation, so \(G_d(s) X(s) = 0\), then \(Y(s) = G_p(s) X(s)\) (3). By considering that \(Y(s)\) and \(X(s)\) were also constant before stimulation, \(Y(s)\) and \(X(s)\) could be expressed as \(Y(0)\) and \(X(0)\), respectively. So, \(G_p(s)\) was replaced as follows:

\[
G_p(s) = \frac{Y(s)}{X(s)} = \frac{Y(0)}{X(0)} = K_p
\] (4).

ii) From equation (2),

\[
G_d(s) = \frac{Y(s) - K_p X(s)}{s X(s)}
\] (5).

Weighting function, \(\omega\), is inversely Laplace transformed from \(G_d(s)\), would be calculated with the deconvolution method from \(G_p(s)\) and real values obtained during IVGTT with the aid of the following equation:

\[
y(r) = k_p x(r) + \sum \omega dx(i) \omega(r-i+1)
\] (6).

where \(x(i)\) (\(i = 0, 1, \ldots\)) was glucose concentration in plasma, \(y(i)\) (\(i = 0, 1, \ldots\)) was plasma insulin concentration, \(dx(i)\) was the rate of change in glucose concentration, \(dx(i)\) was determined by the equation,

\[
dx(i) = \frac{x(i+1) - x(i)}{T(i+1) - T(i)},
\]

where \(T(i)\) was the sampling time. If \(dx(i)\) was negative, \(dx(i)\) was set to be 0.

All the calculations mentioned above were done by using the computer (FACOM U-200, Fujitsu, Japan). Statistical analyses were carried out by paired \(t\)-tests.

**RESULTS**

1) **Glucose tolerance – analysis of the glucose assimilation curves**

The subjects were divided into 3 patterns according to the change in glucose clearance constant in the course of time. In pattern A \(K_2\) was larger than \(+120\%\) of \(K_1\), and \(K_3\) was larger than \(+120\%\) of \(K_2\). In pattern B \(K_3\) was larger than \(+120\%\) of \(K_2\), but difference between \(K_1\) and \(K_2\) was within \(120\%\). Remaining cases were designated as pattern C. Changes in mean glucose excess values in the course of time in 3 groups are shown in Fig. 1. Mean (\(\pm\) SEM) pre-stimulated glucose concentration in pattern A, B and C was \(81.6 \pm 1.8\), \(85.9 \pm 3.8\) and \(100.7 \pm 2.2\) mg/100 ml, respectively, and the differences among groups were not statistically significant. Mean (\(\pm\) SEM) values of \(K_1\), \(K_2\) and \(K_3\) in each group are shown in Table 1. In each of \(K_1\), \(K_2\) and \(K_3\), mean values

\(^2\) Weighting function is the output of the system in response to pulse input. Transfer function is Laplace transformed from weighting function. So weighting function is suitable for comparing systems because input to the systems is standardized.
**Fig. 1.**
Comparison of the glucose assimilation in three groups. Mean glucose excess values were plotted semilogarithmically against time on a linear axis.

**Table 1.**
Glucose clearance constant, $K_1$, $K_2$, $K_3$ value in each group.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>$K_1$</th>
<th>$K_2$</th>
<th>$K_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattern A (68)</td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.63</td>
<td>15.39</td>
<td>27.01</td>
</tr>
<tr>
<td></td>
<td>± SEM</td>
<td>0.52</td>
<td>2.44</td>
</tr>
<tr>
<td>Pattern B (43)</td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.86</td>
<td>3.58</td>
<td>9.64</td>
</tr>
<tr>
<td></td>
<td>± SEM</td>
<td>0.25</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>$P^*$</td>
<td>&lt; 0.025</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pattern C (126)</td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.41</td>
<td>2.32</td>
<td>2.60</td>
</tr>
<tr>
<td></td>
<td>± SEM</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>$P^{**}$</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Number of subjects in brackets.
* Pattern A versus pattern B.
** Pattern B versus pattern C.
were larger in pattern A than those of pattern B, and values in pattern B were larger that those of pattern C, and the differences were statistically significant. As compared with the results of OGTT, of 68 cases of pattern A, 62 (91.2%) were normal, 4 (5.9%) were borderline and only 2 (2.9%) showed diabetic curves. Of 43 cases of pattern B, 28 (65.1%) were normal, 12 (27.9%) were borderline and 3 (7.0%) were diabetic. Of 126 cases of pattern C, only 9 (7.1%) were diabetic.

2) Glucose-induced insulin secretory ability – Analysis of weighting function of derivative response to plasma glucose concentration

Mean (± SEM) values of insulin concentration in plasma and calculated weighting function, \( \omega \), which was the insulin secretion per unit of the rate of change in plasma glucose, in pattern A (24 cases), B (19 cases) and C (63 cases)
Comparison of cumulative area of weighting function in the first 10 min in three groups. The mean values ± SD are indicated for each group.

Fig. 3.

Relationship between cumulative area of weighting function and insulinogenic index in the first 10 min.

Fig. 4.
are shown in Fig. 2. In pattern A, a rapid initial peak and large gain in $\omega$ in 60 min were noted. As shown in Fig. 3, mean ($\pm$ so) cumulative area of $\omega$ in the first 10 min was $7.085 \pm 4.604 \times 10^2 \mu$U min/mg, $4.080 \pm 3.241$ and $0.609 \pm 1.138$, in pattern A, B and C, respectively. Statistically significant differences were recognized between pattern A and C and between B and C ($P < 0.001$), but no difference was found between pattern A and B. Among pattern C, in all 40 cases whose OGTT results were diabetic, $\omega$ in the first 4 min was nil.

3) Weighting function and insulinogenic index

The relationship between insulinogenic index in the first 10 min and the cumulative area of $\omega$ in the first 10 min was shown in Fig. 4. Significant correlation was observed ($r = 0.957$, $P < 0.001$).

**DISCUSSION**

1) Glucose tolerance

In the theory of control, as one of the methods of measuring the condition of the system, the analysis of the transient response resulting from giving error to the system has been used. As far as IVGTT is concerned, Hamilton & Stein (1942) proposed estimation of glucose tolerance by calculating glucose clearance constant, $k$, with the following equation:

$$\frac{dX(t)}{dt} = -k X(t) \quad (7)$$

This equation is unreasonable because if time, $t$, is infinite, blood glucose has to decrease to 0. Amatuizio et al. (1953) proposed that the rate of change in glucose concentration was proportional to the glucose excess value, [$X(t) - X(0)$] after intravenous glucose load. So glucose clearance constant has been calculated with the equation (1). In this model, the hypothesis is that glucose disposal rate is dependent on the increment in blood glucose concentration, and that the proportionality constant for negative feedback when blood glucose concentration goes up is constant regardless of time. If the glucose assimilation curve fits this model, it is possible to express its glucose tolerance quantitatively as glucose clearance constant, $k$. In other words, the glucose excess curve after iv glucose pulse loads is a straight line on semilogarithmic paper, and from the slope of the regression line, it is easy to determine $k$-value in each subject.

But as shown in this study, it was recognized that there were not only the cases whose glucose clearance constants were unchanged from 10 to 60 min (pattern C), but also the cases whose glucose clearance constant became larger with time (pattern A or B). When the results were compared with those of
OGTT, in the normal OGTT group 62.6% were categorized as pattern A, 28.3% were B and only 9.1% were C. In the diabetic group, 93.9% were pattern C, only 2.4% were A and 3.7% were B. So it was revealed that k-value became larger with time if the glucose tolerance was normal. From these results, we would like to suggest that the glucose clearance constant was not a proportionality constant, but variable with time and should be regarded as a function of time, and the following equation might be used to define glucose tolerance,

\[
\frac{dX(t)}{dt} = -k(t) [X(t) - X(0)] \tag{8}
\]

so, as parameters showing glucose tolerance, both intensity and the rate of change in glucose clearance constant should be evaluated in each subject.

2) The dynamic property of glucose-induced insulin secretion

To elucidate the reasons why the glucose clearance constant changes in the course of time, it is of great value to understand the dynamic property of insulin secretion in relation to glucose homoeostasis. One of the methods of measuring insulin secretory ability per unit of change in glucose concentration was investigated during IVGTT. Weighting functions were calculated from the blood glucose concentrations as input and plasma insulin concentrations as output during IVGTT. Plasma insulin concentration in peripheral veins is controlled by many factors, such as insulin secretion, degradation and distribution to the extracellular fluid. Factors except secretion of insulin are regarded as linear functions in this study because a significant positive correlation between peripheral and portal insulin concentrations was observed (Blackard & Nelson 1971), and because quantities of insulin degraded in the liver were rather stable even when insulin secretion increased over the physiological range (Kaplan & Madison 1959). In addition, it was shown that peripheral blood glucose concentrations reflected the portal blood glucose concentrations (Kanazawa et al. 1966). Hence, weighting function is considered to reflect the insulin secretion amount per unit of blood glucose stimulus.

To realize the insulin secretion pattern in response to glucose concentration, we focused attention on the biphasic response of insulin secretion in the perfused rat pancreas with glucose (Curry et al. 1968). The same phenomenon was also recognized in the human portal vein (Blackard & Nelson 1970). Then, this insulin secretion property was explained with the aid of a control theory as the proportional plus derivative response to blood glucose concentration (Kawamori et al. 1978). In practice, it is impossible to determine both proportional and derivative responses at the same time in each subject during IVGTT. Lerner & Porte (1972) reported that even in the diabetics whose acute insulin responses to glucose injection were diminished, the same steady-state insulin
levels as seen in normal subjects were recognized when blood glucose concentrations were increased with the constant infusion of 300 mg of glucose per min for 20 h. In other words, even in mild diabetics the proportional response to blood glucose concentration seems to be unaffected. Hence, in this study the proportional response in each subject was determined to remain intact. Then the dynamic property of insulin secretion per unit of rate of change in glucose concentration was expressed successively in each individual. Furthermore, the mechanism by which the dynamic property of insulin secretion acts upon the glucose tolerance was examined.

In the subjects whose glucose assimilation showed pattern A in which K(t) and the rate of change in K(t) was large, the rapid initial peak in $\omega$ was remarkable. In pattern B whose glucose tolerance seemed to be slightly impaired, the mean area of $\omega$ in the first 10 min was smaller than that of pattern A ($P < 0.05$), but on the contrary the mean area of $\omega$ in 60 min was not statistically different from that of pattern A. This means that in pattern B, insulin secretion was delayed in time, but was supplemented by the amount of secretion. In pattern C whose glucose tolerance was impaired, the initial secretory phase in $\omega$ was remarkably low or absent, and also the gain in 60 min was small. Furthermore, the time course of $\omega$ revealed that the larger the $\omega$, the larger the change in glucose clearance constant with time, showing that glucose tolerance was controlled by the insulin secreted based on the rate of change in glucose concentration in the plasma. To corroborate these findings, animal experiments to assess the role of derivative action-induced insulin in glucose regulation were performed by using our artificial beta cell system (Kawamori et al. 1978). In this system, the rate of insulin infusion was controlled by a derivative in addition to proportional response to blood glucose concentrations. IVGTT was performed in the normoglycaemic depancreatized dogs who were maintained on iv insulin infusion. The glucose assimilation curves and insulin infusion patterns were then examined. It was shown from these experiments that when the derivative response was added to the proportional response in the insulin infusion regulatory system, the insulin requirement was the smallest and glucose regulation was the best among the experimental groups. It was also shown that when insulin infusion was based only on the blood glucose concentration, it could not regulate the glucose assimilation curves following iv glucose challenges.

In 24 cases of pattern A, 3 cases showed the area of $\omega$ in the first 10 min being less than $2.40 \times 10^2 \mu U \ min/mg$ (Fig. 4). They were probably “low responders” proposed by Cerasi & Luft (1967). In addition to the cases included in this study, 11 potential diabetics whose OGTT results were normal but both whose parents were diabetic, were examined with this method. Glucose assimilation patterns were A in 1 case, B in 4, and C in 6 cases. In 10 cases out of 11, $\omega$ in the first 4 min was completely nil (data not shown).
3) Differences between insulinogenic index and weighting function

A significant positive correlation between insulinogenic index and the cumulative area of $\omega$ in first 10 min was observed, but there were many differences between them. This weighting function method is able to calculate the insulin secretion only based on the rate of change in plasma glucose concentration. It therefore might show useful for follow-up on subjects such as low responders, potential diabetics and those whose with glucose assimilation pattern B.

CONCLUSION

The following findings were obtained:

1) The glucose clearance constant (k-value) in normal glucose tolerance was not a constant with respect to time, but became larger with time.

2) The weighting function which reflects the insulin secretion per unit of rate of change in glucose concentration in plasma was calculated from the plasma glucose concentration as an input and plasma insulin concentration as an output by the deconvolution method. Then it was clearly shown that the gain in the weighting function was small and the response was slow even in the individual whose glucose tolerance was slightly abnormal.

3) The time course of the weighting function revealed that the larger the weighting function, the larger the change in glucose clearance constant with time, showing that glucose tolerance was controlled by the insulin secretion based on the change in glucose concentration in plasma.

This method which permits quantitation of insulin response against unit of change in plasma glucose concentration is considered to be effective for the diagnosis of the early stage of diabetes mellitus even before clinical manifestation and also useful for follow-up studies.

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