THE DEVELOPMENT OF A NONLINEAR MODEL TO DESCRIBE THE BLOOD GLUCOSE RESPONSE FOR THE DETERMINATION OF PRANDIAL INSULIN INFUSION

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We studied the relationship between the rate of intravenous glucose infusion and the blood glucose concentration at two different physiological levels of hyperinsulinemia in a depancreatized dog. The same degree of changes in the glucose infusion rate generated progressively larger increments in the blood glucose concentration. We then developed a Michaelis-Menten type kinetic model which could appropriately describe this nonlinear relationship of blood glucose response under the condition of hyperinsulinemia. In order to determine the prandial insulin infusion program, we used more simplified equations based on a nonlinear model. The simplified equations were demonstrated to be applicable for estimating the prandial subcutaneous insulin infusion program in the continuous subcutaneous insulin infusion system.

Keywords — insulin; pharmacodynamic model; blood glucose response; continuous subcutaneous insulin infusion; prandial insulin infusion; dog

INTRODUCTION

The postprandial serum insulin levels are known to rise to about 100 μ U/ml in healthy subjects and the postprandial blood glucose levels to about 400–500 mg/dl in diabetic patients.^{1,2)} The model for determining the postprandial serum insulin concentration *versus* time profile must therefore be able to describe the relationship between the blood glucose and insulin, even in these high concentrations. It is, however, known that glucose clearance is dependent on changes in blood glucose concentration and that increases in blood glucose concentration result in progressive decreases in glucose clearance.^{3–5)} From the viewpoint of application of mathematical models for the continuous subcutaneous insulin infusion (CSII) program, there has been no literature available concerning appropriate simplified models which can provide a concise description of the blood glucose-serum insulin relationship under the condition of physiological high insulin and glucose concen-

trations.

In the present study, the relationship between the blood glucose concentration and the rate of intravenous glucose infusion was studied in a depancreatized dog under the condition of hyperinsulinemia. We developed a nonlinear model to describe this relationship and the model was applied to determining the prandial subcutaneous insulin infusion program in the CSII system.

MATERIALS AND METHODS

Animal — One male beagle dog (body weight: 9 kg) was depancreatized under anesthesia with pentobarbital sodium (50 mg/kg, *i.v.*) after an overnight fast. The dog was allowed unrestricted access to water, and doses of 3.0 g of pancreatin (Beryzyum, Shionogi Pharmaceutical Co., Japan) and 150 g of dog-food (CD-5, Clea Co., Japan) were given twice a day. During a resting period from one experiment to another (1–4 weeks), single daily subcutaneous injec-

tions of equal amount (<8 U) of Actrapid MC® and Monotard MC® (Novo Industri., Copenhagen) were given to the depancreatized dog to control the high blood glucose. Dose adjustments were made based on the overnight fasting blood glucose levels.

Insulin Infusion Pump — A battery-powered preprogrammable continuous infusion pump ($126 \times 70 \times 26$ mm, 270 g, Terumo Co., Japan) was used. The infusion system was fitted with a finger pump and programmed at rates ranging from 0.02 to 2 ml/h by a programmable speed setting with 100 steps. The infusion rates were set at 15-min intervals.

Insulin and Glucose Continuous Intravenous Infusion — A continuous intravenous infusion of insulin at a rate of 9 mU/min in experiment A and 18 mU/min in experiment B with the insulin infusion pump was performed on the depancreatized dog with multistep rates of glucose infusion. The 40 U/ml insulin solution (Actrapid MC) was diluted with saline solution to concentrations of 0.6 U/ml (experiment A) and 1 U/ml (experiment B). Using another infusion pump (Terufusion-STC 521, Terumo Co., Japan), glucose were given intravenously to the same dog at rates of 60, 120, 180 and 240 mg/min in experiment A and at 120, 240 and 360 mg/min in experiment B. A 23 Gauge (G) $\times 5/8$ butterfly needle was inserted into the left forearm vein for the infusion of insulin and glucose. In experiment A, bolus injections of 0.4 units of insulin (Actrapid MC) were given into the left forearm vein, 4 and 24 min after the start of insulin infusion. In experiment B, a single bolus injection of 0.8 units of insulin (Actrapid MC) was given into the left forearm vein 32 min before the start of insulin infusion. During the insulin and glucose infusion, blood samples (1 ml each) for measurement of glucose concentration were withdrawn into a 1-ml tuberculin syringe *via* a 23 G $\times 1\ 1/2$ needle placed in the right forearm vein. At each glucose infusion rate, blood glucose levels were followed every 15 to 30 min with a reflectometer (Dextrostix and Glucometer, Miles Laboratory, Inc., U.S.A.) to follow steady-

state blood glucose levels. Blood glucose concentrations were determined by the glucose-oxidase method (Glucose-B-Test, Wako Chemical Co., Ltd., Japan). Blood samples (2 ml each) were withdrawn into 2-ml syringe *via* a 23 G $\times 1\ 1/2$ needle placed in the right forearm vein just prior to the test, 15 min (experiment A) or 30 min (experiment B) after beginning the infusion and every 1 h thereafter (experiments A and B) for measurement of serum insulin concentration.

Prandial Continuous Subcutaneous Insulin Infusion — The theoretically determined prandial insulin infusion program was applied to the depancreatized dog at 8:45 a.m. after maintaining the blood glucose level within the physiological range in the overnight fasted state by basal insulin infusion into the tissue of subcutaneous abdominal wall at a rate of 3 mU/min. One ml of 40 U/ml insulin solution (Actrapid MC) diluted with saline solution to a concentration of 6 U/ml was delivered *via* a 23 G $\times 5/8$ butterfly needle into the subcutaneous tissue of the anterior abdominal wall. The dog was given 150 g of dog-food at 9:00 a.m. Blood samples (2 ml each) were withdrawn into 2-ml syringe *via* a 23 G $\times 1\ 1/2$ needle placed in the forearm vein at 8:45, 9:00, 9:30, 10:00, 10:30, 11:00, 11:30, 12:00, 13:00, 14:00, 15:00, 16:00 and 17:00 for measurement of insulin and glucose concentrations.

Assay — The serum immunoreactive insulin concentration was measured by a double antibody method using Insulin RIA Kit® (Dinabot Radioisotope Laboratory, Japan).

RESULTS AND DISCUSSION

We examined the influence of blood glucose concentration on the rate of change of glucose in the body at two physiological levels of hyperinsulinemia in the depancreatized dog. As shown in Figs. 1 and 2, the average serum insulin levels were maintained at 41.7 ± 2.8 and 70.6 ± 6.9 μ U/ml during time periods of 120–420 min (experiment A) and of 120–345 min (experiment B). The average steady-state blood glucose levels were 113, 173 and 298 mg/dl at glucose in-

fusion rates of 120, 180 and 240 mg/min, respectively, in experiment A (Fig. 1). In experiment B, the average steady-state glucose levels were 74, 124 and 266 mg/dl at glucose infusion rates of 120, 240 and 360 mg/min, respectively (Fig. 2). The relationship between the glucose infusion rates and steady-state blood glucose levels at insulin levels of 41.7 and 70.6 $\mu\text{U}/\text{ml}$ are shown in Fig. 3. In an attempt to provide a basis for developing an appropriate subcutaneous insulin infusion program, the preceding article⁶⁾ has evaluated the Bolie model⁷⁾ to describe the relationship between the blood glucose and serum insulin concentrations. The model parameters α and β consider the disappearance of glucose from the blood as a linear function of blood glucose and insulin, respectively. Based on the parameter values for the first order rate con-

stants α and β obtained from the single bolus subcutaneous insulin injection experiments described in detail in the previous paper⁶⁾ ($\alpha = 0.0046 \text{ min}^{-1}$ and $\beta = 0.064 \text{ mg}/\mu\text{U}/\text{min}/10^2$), the Bolie model was applied to predict the glucose infusion rates at the three blood glucose levels as shown with broken-lines in Fig. 3. It was clearly shown that under the condition of physiological hyperinsulinemia the Bolie model could not predict the insulin-elicited blood glucose response.

Nonlinear Model

Previous studies^{8,9)} have shown that following the intravenous infusion of insulin and glucose, the splanchnic bed removes only 5% of the infused glucose, suggesting that peripheral tissues, probably muscle and adipose tissue, are primarily responsible for disposal of an intravenous glucose

FIG. 1. Time Course of the Blood Glucose and Serum Insulin Levels Resulting from Intravenous Insulin and Glucose Infusion in a Depancreatized Dog (Experiment A)

The short horizontal solid lines denote the steady-state blood glucose levels at each glucose infusion rate. The arrows at the bottom of panel indicate the bolus intravenous insulin injections. Key: (●), measured glucose concentration; (○), measured insulin concentration.

load. Moreover, the most important effect of insulin is its ability to increase the rate of glucose transport through the membranes of most cells with particular efficacy in skeletal muscle and adipose tissues. This effect has been considered to be due to the enhancement of the penetration of glucose by increasing the number of glucose transport sites.¹⁰⁻¹²⁾ However, the mechanism of insulin's stimulatory action on the entire glucose utilization process (glucose transport and metabolism) in muscle and adipose tissue is still unclear. Olefsky¹³⁾ reported that insulin could promote glucose oxidation independent of its ability to stimulate glucose transport. Chiasson *et al.*¹⁴⁾ showed that high physiological concentrations of insulin activated glycogen synthetase in voluntary skeletal muscle and that this effect was independent of changes in glucose uptake. It

is, therefore, not unreasonable to assume that insulin would increase the rate of the entire glucose utilization process.

In experiments A and B, an increase in glucose infusion rate resulted in an increase in blood glucose concentration at two insulin levels (Figs. 1 and 2), but the increase in glucose concentration was not proportional to the increase in glucose infusion rate (Fig. 3). To describe this nonlinear behavior of blood glucose, we developed the following Michaelis-Menten type kinetic model. The model assumes the presence of a glucose transport system with which glucose forms the transport site-glucose complex. Another assumption is that glucose utilization depends on the formation of the complex and is stimulated by the effect of insulin. Then, the overall process of glucose utilization can be visualized as,

FIG. 2. Time Course of the Blood Glucose and Serum Insulin Levels Resulting from Intravenous Insulin and Glucose Infusion in a Depancreatized Dog (Experiment B)

The short horizontal solid lines denote the steady-state blood glucose levels at each glucose infusion rate. The arrow at the bottom of panel indicates the bolus intravenous insulin injection. Key: (●), measured glucose concentration; (○), measure insulin concentration.

where k_{form} and k_{dis} are the association and dissociation rate constants between transport site and glucose, k_{util} is the rate constant of glucose utilization, and i is the serum insulin concentration. From these concepts, the rate of glucose utilization (G_u) can be described in terms of the steady state blood glucose (g) and the serum insulin concentrations as follows (see Appendix),

$$G_u = \frac{i \cdot V_m' \cdot g}{g + i \cdot K_m'} \quad \dots 1$$

where V_m' is the maximum rate of glucose utilization at unit insulin concentration, and K_m' is the half-saturation constant of the V_m' . Under the condition of the absence of exogenous glu-

cose, the rate of change of glucose in the body is described by the sum of the glucose utilization rate and the rate of endogenous glucose production. The rate of change of glucose in the body is then represented as follows,

$$\frac{dG}{dt} = - \frac{i \cdot V_m'}{g + i \cdot K_m'} \cdot g + G_p \quad \dots 2$$

where G and G_p are the amount of glucose in the body and the rate of endogenous glucose production, respectively.

When the serum insulin and blood glucose concentrations are at steady-state, the rate of exogenous glucose infusion is supposed to be equal to the rate of change of glucose in the body. Therefore, Eq. 2 can be rearranged to,

$$R_{g0} = \frac{i \cdot V_m'}{g + i \cdot K_m'} \cdot g - G_p \quad \dots 3$$

where R_{g0} is the rate of intravenous glucose infusion.

On the basis of the data obtained from experiments A and B, the parameter values for V_m' , K_m' , and G_p were estimated by using Eq. 3. The parameter values were calculated by a microcomputer (PC-8001, NEC Co., Japan) using the Marquard method.¹⁵⁾ This analysis assumed that the rate of endogenous glucose production was constant. The estimated parameter values for V_m' , K_m' , and G_p were 11.25 mg·ml/min/μU, 2.31 mg·ml/dl/μU, and 121 mg/min, respectively. The computer graphed theoretical curves shown in Fig. 4 which agreed with the measured experimental values. This means that the nonlinear model developed in this study can appropriately describe the nonlinear relationship between the blood glucose concentration and the rate of change of glucose in the body under the condition of hyperinsulinemia.

Endogenous glucose production is known to be completely suppressed at physiologically high insulin levels (60 μU/ml).^{5,16,17)} From this viewpoint the parameter values for V_m' and K_m' were reestimated by setting the values for G_p to 0 mg/min. The estimates for V_m' and K_m' were 12.2 mg·ml/min/μU and 4.9 mg·ml/μU, respectively. These parameter values, however,

FIG. 3. Relationship between the Glucose Infusion Rate and Steady State Blood Glucose Concentration at Insulin Levels of 41.7 (●) and 70.6 μU/ml (○)

Broken lines represent the estimated values using the Bolie model.

did not adequately describe the relationship between the blood glucose concentration and the rate of change of glucose. This implies that G_p has the characteristics of a lumped parameter accounting for not only the endogenous glucose production but the other unidentifiable variables.

Simplified Equations

Our primary objective in the present study was to develop an appropriate mathematical model to describe the relationship between the rate of change of glucose in the body and the blood glucose concentration and to apply the developed model to the determination of the prandial subcutaneous insulin infusion program in the CSII system. In the application of the nonlinear model described above (Eq. 2), three sets of measured values for steady-state blood glucose and serum insulin levels at three different glucose infusion rates must be obtained to estimate accurate parameter values for V_m' , K_m' , and G_p . Therefore, it was necessary to provide

more simplified equations accounting for the relationship between the rate of change of glucose and the blood glucose concentration from a standpoint of the practical clinical application.

If we approximate the relationship shown in Fig. 3 (solid lines) to be a straight-line with intercept zero, the rate of change of glucose, which is equal to the rate of glucose infusion under the experimental conditions employed, may be expressed as follows,

$$\frac{dG}{dt} = -A \cdot g \quad \dots 4$$

where G and g are the amount of glucose in the body and the blood glucose concentration at steady state, respectively, and A is the proportionality constant to relate the steady state glucose concentration to the rate of glucose disappearance from blood when the insulin concentration is maintained at a certain level. If we assume that the value for A changes proportionately to the serum insulin concentration ($A = a \cdot i$), Eq. 4 is rearranged to,

$$\frac{dG}{dt} = -a \cdot i \cdot g \quad \dots 5$$

where i is the serum insulin concentration, and a is the proportionality constant independent of i and g . The least squares regression analysis of the data shown in Fig. 3 yielded the values for A as 0.884 and 1.467 at the insulin levels of 41.7 and 70.6 $\mu\text{U/ml}$, respectively. The corresponding values for a were calculated to be 0.0212 and 0.0208 $\text{ml}^2 \cdot 10^2 / \text{min} / \mu\text{U}$, which were considered as the same. This suggested that Eq. 5 could describe the relationship between the rate of change of glucose and blood glucose concentration within the blood glucose levels ranging from 74 to 298 mg/dl .

Equations 4 and 5 assume linear conditions and the rate of change of glucose is dependent on the blood glucose concentration. However, the relationship between the rate of change of glucose and blood glucose concentration was shown to be described more appropriately by the saturation type kinetics (Eq. 2). That is, a plateau of the rate of change of glucose will be

FIG. 4. Simulation Curves for the Relationship between the Glucose Infusion Rate and Steady State Blood Glucose Level at Insulin Levels of 41.7 (●) and 70.6 $\mu\text{U/ml}$ (○)

The solid lines represent the estimated values using Eq. 3.

achieved when the blood glucose exceeds a certain concentration level. Therefore, there is the possibility that the rate of change of glucose estimated from Eq. 5 will become larger than the actual rate under the condition of hyperglycemia. Therefore, in our approach to providing simplified equations, we further assumed that the rate of change of glucose in the body was maintained at a plateau level above a certain glucose concentration (g_s). The rate of change of glucose at this plateau level g_s is then expressed as follows,

$$\frac{dG}{dt} = -a \cdot i \cdot g_s \quad \dots 6$$

An appropriate value for g_s was determined in the following manner. By using various values for g_s (200, 250, 300, 350 and 400 mg/dl), an interactive, direct-search computer program¹⁵⁾ estimated the optimal fitted value for a which would minimize the computed sum of the squares of difference between the rate of change of glucose estimated from Eq. 2 and that estimated from Eqs. 5 and 6. Parameter values used for K_m' , V_m' and G_p were 2.31 mg · ml/dl/μU, 11.25 mg · ml/min/μU and 121 mg/min, respectively, as determined previously. Starting with the initial estimate, the parameter value for a in the blood glucose concentration range of 0–600 mg/dl were adjusted until a minimum least

squares fit was obtained at the insulin levels of 30, 50, 70 and 90 μU/ml and the results are summarized in Table I. The most suitable value for g_s was 300 mg/dl and the parameter values for a were approximately equal to the values obtained from the least squares regression analysis of the actual measured data ($a = 0.0212$ and $0.0208 \text{ ml}^2 \cdot 10^2/\text{min}/\mu\text{U}$). In Fig. 5, the rate of change of glucose estimated from Eq. 5 (glucose concentration range: 0–300 mg/dl) and Eq. 6 (glucose concentration range: 300–600 mg/dl) using the optimal fitted value for a at each individual insulin level and the g_s value of 300 mg/dl (broken lines) is compared with that estimated from Eq. 2 (solid lines). It seemed that the broken-lines could approximate the solid-lines. This suggested that the nonlinear Eq. 2 could be simplified to Eqs. 5 and 6 to describe the relationship between the rate of change of glucose and the blood glucose concentration.

Prandial Continuous Subcutaneous Insulin Infusion in a Depancreatized Dog

Theoretically, it is possible that the postprandial blood glucose be held at a certain physiological level, if appropriate serum insulin levels are to be obtained with the inflow of exogenous glucose. Under these conditions, the inflow rate of exogenous glucose ($J(t)$) is added to the right hand side of Eq. 5 and the rate of change of glucose is set to zero. That is,

TABLE I. *The Optimal Fitted Values for a and the Sum of the Squares of the Difference*

Glucose levels for g_s	200 mg/dl		250 mg/dl		300 mg/dl		350 mg/dl		400 mg/dl	
	dif. ^{a)}	(a) ^{b)}	dif. ^{a)}	(a) ^{b)}	dif. ^{a)}	(a) ^{b)}	dif. ^{a)}	(a) ^{b)}	dif. ^{a)}	(a) ^{b)}
Insulin levels										
30 μU/ml	71562	(0.0267)	59006	(0.0222)	62279	(0.0192)	76788	(0.0171)	98713	(0.0157)
50 μU/ml	170099	(0.0301)	107320	(0.0251)	99176	(0.0217)	131743	(0.0194)	193056	(0.0178)
70 μU/ml	379010	(0.0297)	210168	(0.0248)	147389	(0.0215)	167030	(0.0193)	247851	(0.0176)
90 μU/ml	701361	(0.0285)	381752	(0.0238)	224383	(0.0207)	197563	(0.0185)	271181	(0.0170)
Total (Mean)	1322032	(0.0288)	758246	(0.0240)	533227	(0.0208)	573124	(0.0186)	810801	(0.0170)

a) The sum of the squares of the difference between the rate of change of glucose estimated from Eq. 2 and that estimated from Eqs. 5 and 6. *b)* The optimal fitted parameter values for a in Eqs. 5 and 6 ($\text{ml}^2 \cdot 10^2/\text{min}/\mu\text{U}$).

$$\frac{dG}{dt} = -a \cdot i(t) \cdot g' + J(t) = 0 \quad \dots 7$$

where $i(t)$ and $J(t)$ are the insulin concentration and the flow rate of exogenous glucose at time t , respectively, and g' is the desired glucose level after the ingestion of food. Rearranging this equation to solve for $i(t)$,

$$i(t) = \frac{J(t)}{a \cdot g'} \quad \dots 8$$

The postprandial insulin concentration *versus* time profile required to hold the blood glucose at a certain constant level can be calculated from this equation.

To maintain the blood glucose level within the normal range, the basal insulin infusion (3.33 mU/min) was started at 9:00 p.m. on the day previous to the start of the meal ingestion study. On the next day the dog was given 150 g of dog-food at 9:00 a.m. while basal insulin infusion was continued. Based on the obtained data on a time course of blood glucose concentrations presented in the preceding article,⁶⁾ the posthepatic glucose delivery rates ($J(t)$) were calculated by the deconvolution techniques^{18,19)} using Eqs. 5 and 6 (Fig. 6). Parameter values for a and g_s used in

FIG. 5. Simulation Curves for the Relationship between the Rate of Change of Glucose in the Body and Glucose Level at Insulin Levels of 30, 50, 70 and 90 $\mu\text{U/ml}$

The solid lines represent the estimated values using Eq. 2. The broken lines represent the estimated values using Eqs. 5 and 6.

this calculation were 0.021 $\text{ml}^2 \cdot 10^2 / \text{min} / \mu\text{U}$ and 300 mg/dl, respectively. Distribution volume of glucose used was 34 dl, which was estimated from the back extrapolation of the glucose decay curve for semilogarithmic plots of the measured glucose concentration *versus* time data obtained from the previous experiment of intravenous bolus injection of 5 g of glucose.⁶⁾

FIG. 6. Time Course of Blood Glucose Concentration Profile Resulting from the Ingestion of 150 g of Dog-food During Basal Subcutaneous Insulin Infusion (Upper Panel) and the Calculated Posthepatic Glucose Delivery Rates (Lower Panel)

FIG. 7. Time Profile of Serum Insulin Concentration Required to Hold the Postprandial Blood Glucose Concentration at 120 mg/dl

The total amount of glucose delivered to the periphery was then calculated to be about 56 g.

For the depancreatized dog used in this study, the time profile of the postprandial serum insulin levels after the ingestion of dog-food was calculated as shown in Fig. 7. In this calculation, the g' value was set to 120 mg/dl and the value for a was that obtained previously ($a=0.021 \text{ ml}^2 \cdot 10^2/\text{min}/\mu\text{U}$). The subcutaneous insulin infusion program required to reconstruct this insulin pattern was then determined by our method.²⁰⁾ A total amount of insulin required for the ingestion of 150 g of dog-food was calculated to be about 7.6 units.

As shown in Fig. 8, when the total amount of 7.6 units of insulin was infused over a period of 8 h at variable infusion rates to reconstruct the desired serum insulin pattern, the measured serum insulin concentrations did not differ greatly from the predetermined levels. The measured blood glucose was very close to the desired level of 120 mg/dl until 14:00 in spite of the ingestion of 150 g of dog-food at 9:00. The blood glucose levels were lowered to about 50 mg/dl toward the end of the insulin infusion period (later than 15:00). For practical purposes, however, the duration of prandial insulin infusion on

each meal will be about 4.5 h at the most.^{21,22)} From these results, our approach to determining the postprandial subcutaneous insulin infusion program was demonstrated to be successful in its ability to control the blood glucose concentration after the ingestion of dog-food in the depancreatized dog.

In conclusion, the nonlinear model (Eq. 2) developed in this study describe the relationship between the rate of change of glucose in the body and the blood glucose concentration under the condition of hyperinsulinemia. The simplified equations (Eqs. 5 and 6) may be used to estimate the prandial subcutaneous insulin infusion program in the CSII system.

APPENDIX

If the total concentration of transport site (Tr) is Tr_0 and after time t the concentration of transport site-glucose complex is ($Tr-g$), then the concentration of free transport site available for further reaction is ($Tr_0 - Tr-g$). The rate of formation of the complex is proportional to this concentration and glucose concentration (g),

$$\frac{d Tr-g}{dt} = k_{\text{form}} \cdot (Tr_0 - Tr-g) \cdot g \quad \dots 9$$

FIG. 8. Time Course of Blood Glucose and Serum Insulin Levels by Prandial Insulin Subcutaneous Infusion in a Depancreatized Dog

Key: (Δ), predetermined pattern of insulin concentration; ($\bullet$), measured blood glucose concentration; ($\circ$), measured serum insulin concentration. Bars denote subcutaneous insulin infusion rate. The horizontal broken line denotes a desired glucose level (120 mg/dl).

The rate of disappearance of the complex, by the reverse of this first order reaction, is,

$$\frac{dTr-g}{dt} = k_{dis} \cdot Tr-g \quad \dots 10$$

But the complex also disappears by utilization of glucose,

$$\frac{dTr-g}{dt} = i \cdot k_{util} \cdot Tr-g \quad \dots 11$$

When the reaction is proceeding at steady-state, the sum of the disappearance rates equals the rate of formation,

$$k_{form} \cdot (Tr_0 - Tr-g) \cdot g = k_{dis} \cdot Tr-g + i \cdot k_{util} \cdot Tr-g \quad \dots 12$$

Rearranging Eq. 12 to solve for $Tr-g$,

$$Tr-g = \frac{Tr_0 \cdot g}{g + (k_{dis} + i \cdot k_{util})/k_{form}} \quad \dots 13$$

If it is assumed that $i \cdot k_{util} \gg k_{dis}$, $(k_{dis} + i \cdot k_{util})/k_{form}$ may be approximated to $i \cdot k_{util}/k_{form}$. Replacing the term involving the two constants (k_{util} and k_{form}) by a single constant K_m' gives,

$$Tr-g = \frac{Tr_0 \cdot g}{g + i \cdot K_m'} \quad \dots 14$$

If, on the other hand, it is assumed that $i \cdot k_{util} \ll k_{dis}$, $(k_{dis} + i \cdot k_{util})/k_{form}$ may then be approximated to k_{dis}/k_{form} . Replacing the term involving the two constants by a single constant K_m gives,

$$Tr-g = \frac{Tr_0 \cdot g}{g + K_m} \quad \dots 15$$

The rate of glucose utilization (G_u) is equal to $i \cdot k_{util} \cdot Tr-g$, and Eq. 14 is rearranged to,

$$G_u = \frac{i \cdot k_{util} \cdot Tr_0 \cdot g}{g + i \cdot K_m'} \quad \dots 16$$

Then Eq. 15 is rearranged to,

$$G_u = \frac{i \cdot k_{util} \cdot Tr_0 \cdot g}{g + K_m} \quad \dots 17$$

At high glucose concentrations, the glucose utilization occurs at its maximum rate, V_{max} , and in this situation it is reasonable to assume that

$Tr-g = Tr_0$. Thus, $V_{max} = i \cdot k_{util} \cdot Tr_0$. Replacing the term involving the two constants (K_{util} and Tr_0) in Eq. 16 by a single constant V_m' gives,

$$G_u = \frac{i \cdot V_m' \cdot g}{g + i \cdot K_m'} \quad \dots 18$$

And Eq. 17 becomes,

$$G_u = \frac{i \cdot V_m' \cdot g}{g + K_m} \quad \dots 19$$

where V_m' is the maximum rate of glucose utilization at unit insulin concentration and K_m' is the half-saturation constant of the V_m' . Validity of the derived equations (Eqs. 18 and 19) was assessed from the experimental data reported by Rizza *et al.*¹⁷⁾ who studied the rate of glucose utilization in healthy human subjects. Rearranging Eqs. 18 and 19 to solve for i/G_u gives,,

$$\frac{i}{G_u} = \frac{K_m'}{V_m' \cdot g} \cdot i + \frac{1}{V_m} \quad \dots 20$$

$$\frac{i}{G_u} = \frac{K_m + g}{V_m' \cdot g} \quad \dots 21$$

FIG. 9. Correlation between Serum Insulin/Glucose Utilization (i/G_u) and Serum Insulin Level (i)

Experimental data from Rizza *et al.*, ref. 17).
 $y = 0.0929x + 5.95, r = 0.998$.

Equation 20 indicates that if g is constant, a plot of i/G_u versus i should yield a straight-line with intercept $1/V_m'$ and slope $K_m'/(V_m' \cdot g)$. Equation 21 indicates that if g is constant, i/G_u should also be constant.

A plot of i/G_u vs. i shown in Fig. 9 yielded a straight line with a slope and we concluded that Eq. 18 was valid to describe the relationship between glucose utilization rate and glucose concentration at high insulin levels.

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