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First phase release coefficient of insulin in subjects with normal glucose tolerance on glucose infusion analyzed by computer simulation

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Abstract

We report here a mathematical model using computer simulation to solve the phase fractionation coefficient (f) of instantaneous insulin release on glucose infusion. By extensive model testing with the cited parameters obtained from the literature, the values of the factor f were shown to lie in range of 0.93 ± 0.02 (mean $\pm$ 2S.D., $n = 15$), indicating that the high pulsatile bolus of glucose by i.v. infusion may trigger acute insulin release (AIR) corresponding to a fraction of more than 90% of the stored insulin release in the first phase from the secretory granules of pancreatic β cells. In addition, the value of the factor f was shown to be independent of both the glucose infusion method and the non-insulin-dependent uptake of glucose.

Keywords: Phase fractionation coefficient; Insulin release; Glucose infusion

1. Introduction

Early in 1969, Porte and Pupo had speculated a two-pool system concept to describe the insulin response to glucose in man (Porte and Pupo, 1969). However, the response pattern is highly nonlinear. In response to intravenous (i.v.) glucose, insulin is released in a biphasic pattern (Porte and Pupo, 1969; Nesher and Cerasi, 2002). The first phase (acute) insulin response to glucose begins within 1 min after an i.v. glucose bolus, peaks between 3 and 5 min, and lasts for up to 10 min. The second phase insulin response to glucose begins just after the glucose bolus but is not evident until 10 min later and lasts as long as the hyperglycemia persists (Cerasi, 1992; Elrick et al., 1964; Darren et al., 2006).

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Both oral and intravenous glucose tolerance tests are widely used to evaluate glucose metabolism in man. Intravenous methods permit the calculation of a specific rate constant for glucose utilization from a relationship of blood glucose and the time course of tracing the experimentation, whereas oral glucose tolerance testing (OGTT) is not a precise or reproducible method, because it could be handicapped by the variable of individual intestinal absorption. Elrick et al. (1964) had reported that glucose administered orally evokes a significantly greater insulin response than does glucose administered intravenously.

The acute insulin response (AIR) following intravenous glucose stimulation, which has been defined as the mean of the increments in serum immuno-reactive insulin (IRI) above baseline at 3–5 min, appears to be an important determinant of carbohydrate tolerance (Lerner and Porte, 1971). Thus, while the OGTT is a good measure of overall glucose tolerance, the intravenous glucose tolerance test (IVGTT) is preferable for evaluating glucose-regulated insulin secretion and β cell function (Elrick et al., 1964).

Bergman and colleagues (Bergman et al., 1985; Pacini et al., 1982) developed the minimal model approach, based on analysis of a frequently sampled i.v. glucose tolerance test (FSIGT). In vivo glucose tolerance is determined by both insulin-dependent and non-insulin-dependent processes. Two important metabolic parameters related to these two processes are estimated by the minimal model-insulin sensitivity (S_I), which characterizes insulin action on glucose kinetics, and glucose effectiveness (S_G), which characterizes non-insulin-dependent glucose kinetics at basal insulin (Ni et al., 1997). However existing approaches to FSIGT analysis are based on data of endogenous insulin secretion so that it could be applicable to a wide variety of clinical problems. While its method of resolution has long been handicapped by lacking a relevantly proper experimental approach, to our knowledge, we are the first to present such a mathematical model using computer simulation to solve for the phase fractionation factor of the stored insulin release.

2. The Model Derivation

The model describing the phase fractionation of the insulin release initiated either by a single glucose bolus or a hyperglycemic clamp test is exhibited in Appendix A from which some major equations that were used in the computer simulation are summarized in Table 1.

3. Materials and Methods

3.1. Subjects

Twelve volunteers without any family history of diabetes were involved in experimentation after given informed consent.

Table 1
A summary of the major equations relevant to the glucose–insulin interaction

Use of equation	Equation	Remark
Glucose utilization	$\gamma = R_{max}(C_{inf} - C_{pl})/K_s + (C_{inf} - C_{pl})$	
Phase 1: net insulin release	$\left(\frac{dN}{dt}\right)_{net,(1)} = \left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big\vert^{t_{inf}}\right] - \frac{(dN_c/dC)R_{max}(C_{inf}-C_{pl})}{K_s+(C_{inf}-C_{pl})}$	Only the stored insulin is concerned, normally this takes 10 min
Phase 2: net insulin release	$\left(\frac{dN}{dt}\right)_{net,(2)} = \left[\frac{1-f}{f}\right]\left\{\left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big\vert^{t_{inf}}\right]\right\} + \left(\frac{dN_s}{dC}\right)\left\{\frac{R_{max}(C_{inf,10}-C_{pl})}{K_s+(C_{inf,10}-C_{pl})}\right\} - \left(\frac{dN_c}{dC}\right)\left\{\frac{R_{max}(C_{inf,10}-C_{pl})}{K_s+(C_{inf,10}-C_{pl})}\right\}$	Involving both the insulin from the residual stored and the de novo synthesis. For normal subjects, phase 2 lasts for 60 min, however it only becomes significant at 10 min post a single glucose bolus.
Overall: net insulin release	$\left(\frac{dN}{dt}\right)_{net,overall} = \left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big\vert^{t_{inf}}\right] - \frac{(dN_c/dC)R_{max}(C_{inf}-C_{pl})}{K_s+(C_{inf}-C_{pl})]} + \left[\frac{1-f}{f}\right]\left\{\left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big\vert^{t_{inf}}\right]\right\} + \left(\frac{dN_s}{dC}\right)\left\{\frac{R_{max}(C_{inf,10}-C_{pl})}{K_s+(C_{inf,10}-C_{pl})]}\right\} - \left(\frac{dN_c}{dC}\right)\left\{\frac{R_{max}(C_{inf,10}-C_{pl})}{K_s+(C_{inf,10}-C_{pl})]}\right\}$	By a single glucose bolus; named herein; the model of insulin response to glucose bolus (MIRGLuB)
Overall: net insulin release in hyperglycemic clamp	$\left(\frac{dN}{dt}\right)' = \left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big\vert^{t_{inf}}\right] - \left(\frac{dN_c}{dC}\right)\left\{\frac{R_{max}(C_{inf}-C_{pl})}{K_s+(C_{inf}-C_{pl})}\right\} + \left[\frac{1-f}{f}\right]\left\{\left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big\vert^{t_{inf}}\right]\right\} + \left(\frac{dN_s}{dC}\right)\left\{C_{inf} - \left\{\frac{R_{max}(C_{inf}-C_{pl})}{K_s+(C_{inf}-C_{pl})}\right\}\right\} - \left(\frac{dN_c}{dC}\right)\left\{\frac{R_{max}(C_{inf}-C_{pl})}{K_s+(C_{inf}-C_{pl})}\right\}$	By hyperglycemic clamp test at a certain glucose concentration C_{inf}; the model of insulin response to hyperglycemic clamp test (MIRHyCT)

Table 2
Model testing using the cited values obtained from the single glucose bolus experimentations

Test run	C_{inf} (mg/dL)	C_{pl} (mg/dL)	R_{max} (mg/dL min)	K_s (mg/dL)	dN_r/dC (mU/L)/(mg/dL)	$Y_{Nr/G}$ (mU/L)/(mg/dL)	dN_c/dC (mU/L)/(mg/dL)	$Y_{Nc/G}$ (mU/L)/(mg/dL)	f	Fig. no.	Relevant reference
1	300	90	20.0	213	1.40	0.650	1.30	1.30	0.914	1	This paper
2	175	90	17.0	138	1.00	0.233	1.30	1.30	0.934	2	Lerner and Porte (1971)
3	300	90	18.0	230	0.94	0.233	1.80	1.80	0.938	3	Bergman et al. (1987) and Galvin et al. (1992)
4	300	90	18.0	230	0.88	0.233	1.80	1.80	0.937	NS	Lerner and Porte (1971) and Bergman et al. (1987)
5	300	90	18.0	230	0.78	0.233	1.80	1.80	0.936	NS	Bergman et al. (1987) and Osei and Schuster (1995)
6	300	90	18.0	230	0.78	0.233	0.94	0.94	0.928	NS	Bergman et al. (1987) and Osei and Schuster (1995)
7	235	90	17.0	178	0.60	0.140	1.00	1.00	0.936	NS	Lerner and Porte (1971)
8	250	75	18.0	188	0.50	0.233	0.70	1.80	0.920	NS	Henriksen et al. (1994)
9	350	92	21.0	254	0.50	0.150	1.10	1.10	0.938	4	Pacini and Bergman (1986)
10	396	90	21.0	285	0.49	0.180	0.80	0.80	0.929	NS	McCulloch et al. (1993)
11	235	90	17.0	178	0.49	0.100	1.00	1.00	0.944	NS	Lerner and Porte (1971)
12	376	90	20.0	272	0.30	0.100	0.80	0.80	0.937	NS	Ezenwaka et al. (1993)

NS: figure not shown.

Table 3
Model testing using the cited values obtained from the hyperglycemic clamp tests

Test run	C_{inf} (mg/dL)	R_{max} (mg/dL min)	K_s (mg/dL)	dN_r/dC (mU/L)/(mg/dL)	$Y_{Nr/G}$ (mU/L)/(mg/dL)	dN_c/dC(mU/L)/(mg/dL)	$Y_{Nc/G}$ (mU/L)/(mg/dL)	f	Fig. no.	Relevant reference
13	300	18.0	230	0.40	0.330	0.80	0.80	0.930	5	Polonsky et al. (1988) and Tillil et al. (1988)
14	225	18.0	171	0.30	0.260	0.30	0.30	0.930	NS	Tillil et al. (1988)
15	185	17.0	144	0.42	0.280	0.20	0.20	0.930	NS	Gulli et al. (1992)

NS: not shown.

They were non-obese, ambulant, healthy, aged 20–30 years, and were normal glucose tolerant as having been confirmed by the 75-g OGTT.

3.2. Infusion Protocol

After a 12-h overnight fast, intravenous catheters were placed in antecubital veins of both arms. One of the catheters was used for intravenous 50% glucose solution infusion, while the other, for blood sampling. Two basal blood samples (−20, 0 min) were obtained, followed immediately by an injection of 50% glucose solution (0.3 g/kg) within 2 min beginning at time 0. Following the glucose injection, blood samples (3 mL) were collected at time 2–6, 8, 10, 15, 19, 22, 25, 30, 40, 60, 80, 100, 120, 140, 160, and 180 min for determination of plasma glucose and insulin concentrations.

3.3. Glucose Assay

Glucose concentrations were measured with an Automatic Analyzer (AU600, Olympus, Japan).

3.4. Insulin Assay

Serum insulin levels were measured with a commercial coated tube radioimmunoassay procedure (INS-IRMA, BioSource, Europe S.A.)

3.5. Estimation of the Phase Fractionation Coefficient

3.5.1. Computer Simulation

A self-established software written with "Borland C++ Builder 6.0" (copyright reserved by Dr. Wen-Chih Chou, the second co-author) was used for computer simulation, from which the fractionation factor, f, was obtained. The software is too huge to be attached herein but could be available on request.

Model testing was performed by using the data cited in Tables 2 and 3, and Figs. 1–5. Two experimental categories were tested:

(A) by a single glucose bolus (data cited in Table 2 were used),
(B) by a hyperglycemic clamp study (data cited in Table 3 were used).

3.5.2. Statistical Treatment

The values of the phase fractionation coefficient f obtained by simulation as shown in Tables 2 and 3 were treated statistically to yield a value at a CI = 0.95.

4. Results and Discussion

4.1. Model Testing to Solve for the Partition Coefficient f

Model testing using the cited values (6, 10–19; Tables 2 and 3) gave a fractionation coefficient of

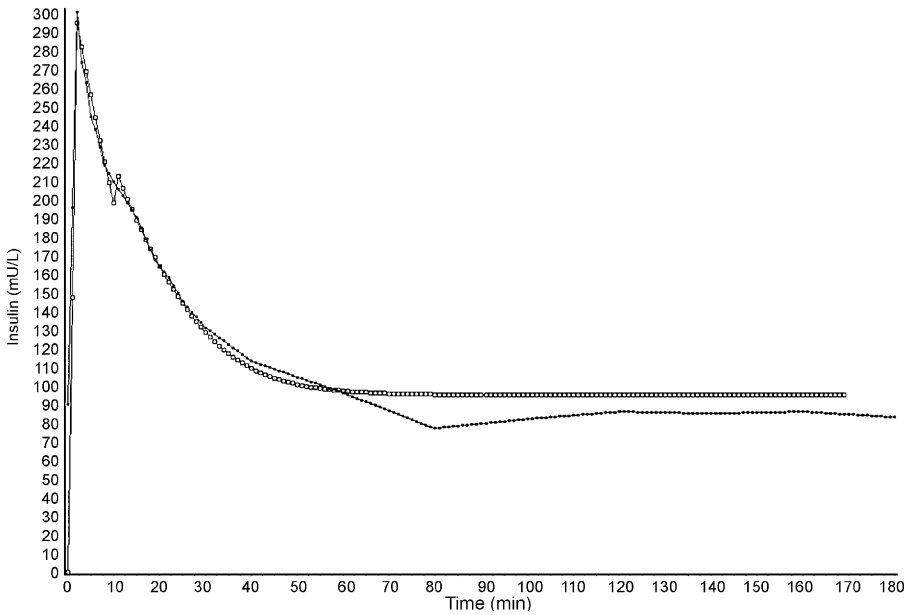

Fig. 1. The time course of insulin release post a FSIGT test (solid line, experimental; dotted line, simulated). The parameters: $C_{inf} = 300$ mg/dL, $C_{pl} = 90$ mg/dL, $K_s = 213$ mg/dL, $R_{max} = 20.0$ mg/(dL min), $dN_r/dC = 1.40$ (mU/L)/(mg/dL), $Y_{Nr/G} = 0.65$ (mU/L)/(mg/dL), $dN_c/dC = 1.30$ (mU/L)/(mg/dL), $Y_{Nc/G} = 1.30$ (mU/L)/(mg/dL); $f = 0.914$. A self-established software written with "Borland C++ Builder 6.0" (copy right reserved by Dr. Wen-Chih Chou, the second co-author) was used for computer simulation, from which the fractionation factor, f, was obtained.

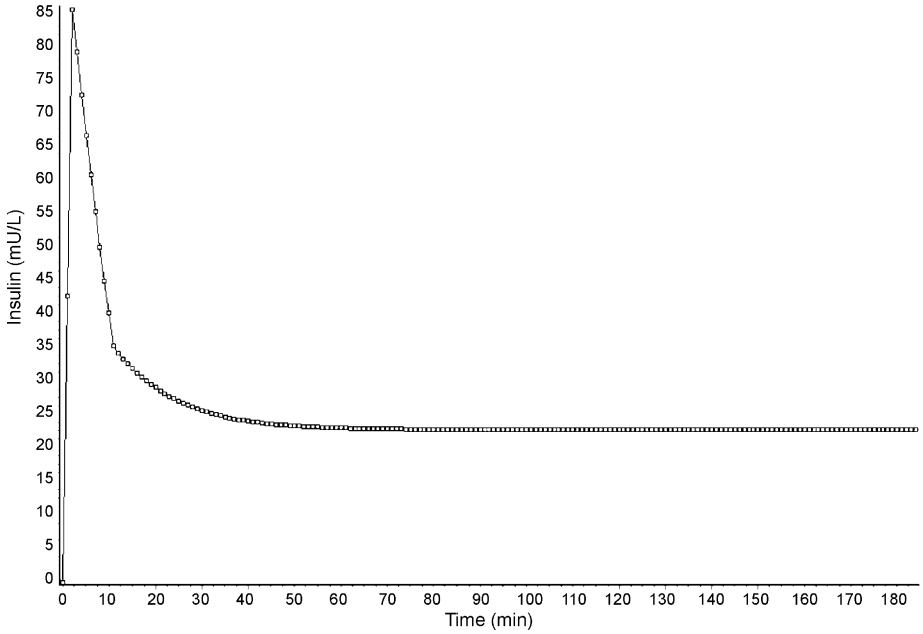

Fig. 2. Model test run 2. The parameters: $C_{inf} = 175$ mg/dL, $C_{pl} = 90$ mg/dL, $K_s = 138$ mg/dL, $R_{max} = 17.0$ mg/(dL min), $dN_r/dC = 1.00$ (mU/L)/(mg/dL), $Y_{Nr/G} = 0.233$ (mU/L)/(mg/dL), $dN_c/dC = 1.30$ (mU/L)/(mg/dL), $Y_{Nc/G} = 1.30$ (mU/L)/(mg/dL); $f = 0.934$.

0.93 ± 0.02 ($n = 15$), a phenomenon which is quite consistent with the acute insulin release (AIR) from the secretory storage compartment in pancreatic β cells. Table 4 shows the parameters that are best fittings in the model testing.

High pulsatile bolus of glucose had been found to trigger a large amount of AIR. In order to diminish the glucose concentration to restore to the normal plasma level in such an emergency, a majority of the stored insulin (>90%), acting as the first aid weapons,

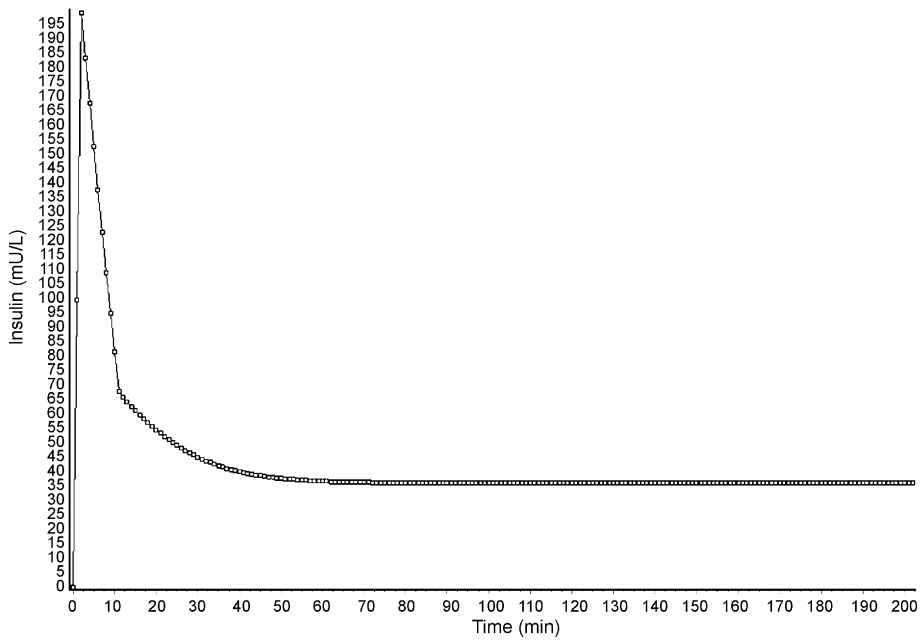

Fig. 3. Model test run 3. The parameters: $C_{inf} = 300$ mg/dL, $C_{pl} = 90$ mg/dL, $K_s = 230$ mg/dL, $R_{max} = 18.0$ mg/(dL min), $dN_r/dC = 0.94$ (mU/L)/(mg/dL), $Y_{Nr/G} = 0.233$ (mU/L)/(mg/dL), $dN_c/dC = 1.80$ (mU/L)/(mg/dL), $Y_{Nc/G} = 1.80$ (mU/L)/(mg/dL); $f = 0.938$.

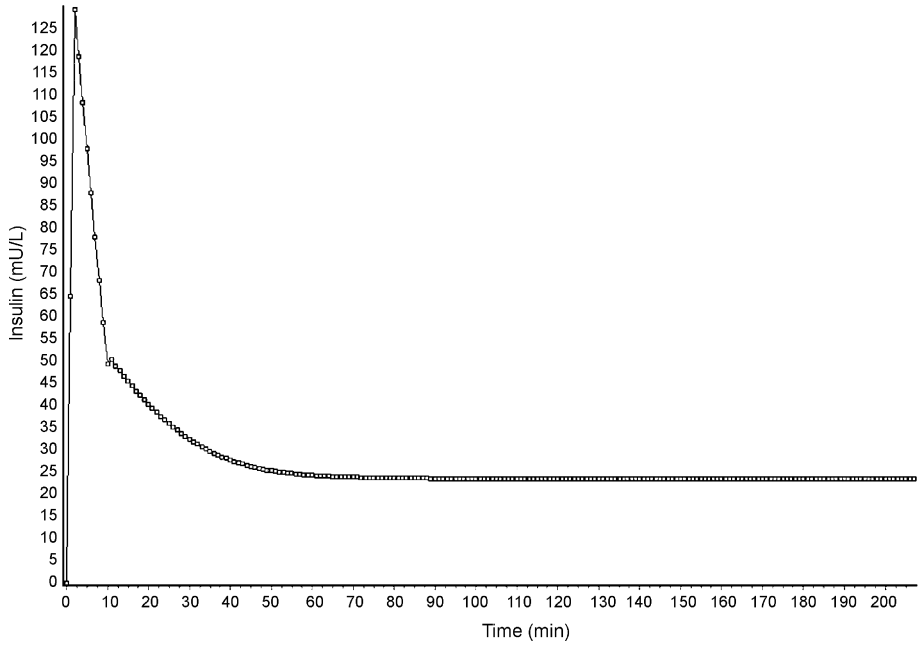

Fig. 4. Model test run 9. The parameters: $C_{inf} = 350$ mg/dL, $C_{pl} = 92$ mg/dL, $K_s = 254$ mg/dL, $R_{max} = 21.0$ mg/(dL min), $dN_r/dC = 0.50$ (mU/L)/(mg/dL), $Y_{Nr/G} = 0.150$ (mU/L)/(mg/dL), $dN_c/dC = 1.10$ (mU/L)/(mg/dL), $Y_{Nc/G} = 1.10$ (mU/L)/(mg/dL); $f = 0.938$.

is reasonably expected to be released instantaneously in a large amount in the first phase (the first 10 min post the glucose infusion), leaving a rather sufficient long time to initiate the second phase insulin release, which virtually involves the release of the portion of stored insulin (<10%) remained post first phase and the subsequent insulin de novo synthesis, the latter is concomitantly initiated up in reflecting the plasma glucose concentration and the glucose utilization.

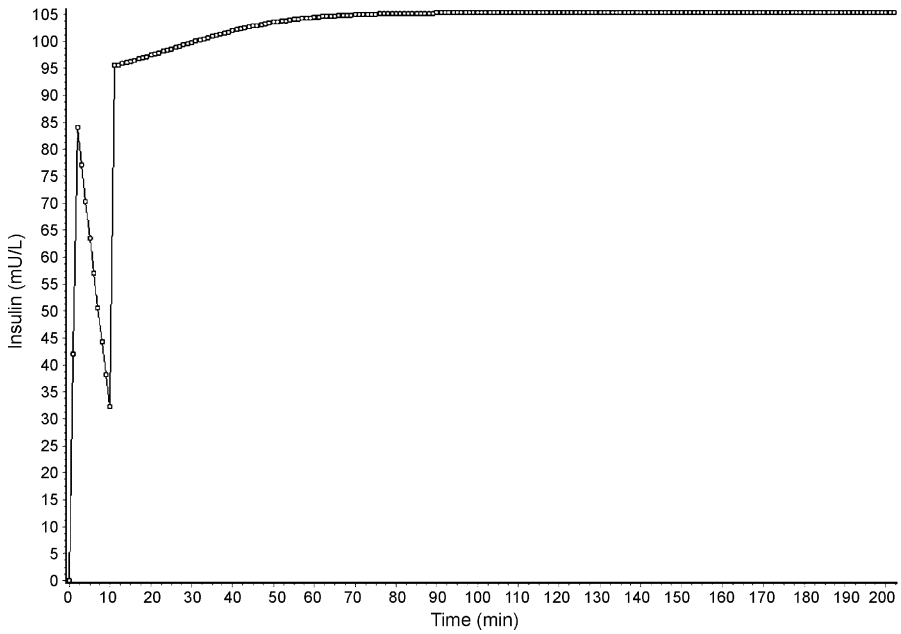

Fig. 5. Model test run 13 for hyperglycemic clamp. The parameters: $C_{inf} = 300$ mg/dL, $C_{pl} = 90$ mg/dL, $K_s = 230$ mg/dL, $R_{max} = 18.0$ mg/(dL min), $dN_r/dC = 0.40$ (mU/L)/(mg/dL), $Y_{Nr/G} = 0.33$ (mU/L)/(mg/dL), $dN_c/dC = 0.80$ (mU/L)/(mg/dL), $Y_{Nc/G} = 0.80$ (mU/L)/(mg/dL); $f = 0.930$.

Table 4
A summary of the best fitting parameters for computer simulation by this model

Notations	Best fitting parameters dimension and units
C_{inf}	175–396 mg/dL
C_{pl}	75–92 mg/dL
K_s	138–285 mg/dL
R_{max}	17–21 mg/(dL min)
dN_c/dC	0.70–1.80 (μU/mL)/(mg/dL)
dN_r/dC	0.30–1.40 (μU/mL)/(mg/dL)
dN_s/dC	0.100–0.625 (μU/mL)/(mg/dL)

Literature cited: Darren et al. (2006), Lerner and Porte (1971), Bergman et al. (1987), Galvin et al. (1992), Osei and Schuster (1995), Henriksen et al. (1994), Pacini and Bergman (1986), McCulloch et al. (1993), Ezenwaka et al. (1993), Polonsky et al. (1988), Tillil et al. (1988), Gulli et al. (1992) and Soad et al. (1994).

5. Conclusion

In conclusion, to our knowledge, this is the first study to discover the phase fractionation factor f to be 0.93 ± 0.02, which defines the differentiating release of stored insulin from the secretory granules in pancreas between the first (with factor f) and the second phases (with factor $1-f$). Our model testing in addition revealed a fact that the fractionation factor f was always remained nearly at a constant value of 0.93, a fact further implying the independence of factor f on the non-insulin-dependent glucose utilization.

Appendix A. The Model

All the notations used in the manuscript are listed in Table A1.

A.1. Model Derivation

A diagrammatic model for normal steady state regulation of plasma glucose is presented in Fig. A1.

A.1.1. The Mass Balance for Glucose

The mass balance for glucose in the model for normal steady state regulation of plasma glucose as shown in Fig. A1 is expressed as

$$\left(\frac{1}{V}\right)\{[(G_{inf}+G_{lv}+G_{fd}+G_{neo})] - [(G_{ms}+G_{ft})+(G_{bn}+G_{nilv}+G_{nipp})]\} = \left(\frac{1}{V}\right)G_{pl} \quad (A1)$$

where the collective term, $(G_{inf}+G_{lv}+G_{fd}+G_{neo})$, is the inflow of glucose to plasma; whereas the one,

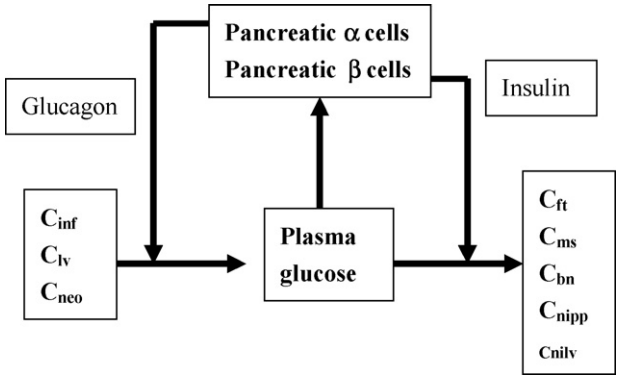

Fig. A1. The diagrammatic model for normal steady state regulation of plasma glucose.

$[(G_{ms}+G_{ft})+(G_{bn}+G_{nilv}+G_{nipp})]$ is the consumption term, in which the term, $(G_{ms}+G_{ft})$, is the insulin-dependent, while the other one, $(G_{bn}+G_{nilv}+G_{nipp})$, non-insulin-dependent activities; V, the individual blood volume in dL, which is a function of body weight, body surface area, body height, and probably sex deviation.

Alternatively, Eq. (A1) is simplified and more commonly expressed as

$$(C_{inf}+C_{lv}+C_{fd}+C_{neo})-(C_{ms}+C_{ft}) - (C_{bn}+C_{nilv}+C_{nipp}) = C_{pl} \quad (A2)$$

which means that normal subjects are at a pseudo-steady state at which the plasma glucose G_{pl} is always held nearly constant. Hence we have from Eq. (A2), the differential form:

$$(dC_{inf}+dC_{lv}+dC_{fd}+dC_{neo}) - (dC_{ms}+dC_{ft}+dC_{bn}+dC_{nilv}+dC_{nipp}) = 0 \quad (A3)$$

which states that the increment of glucose inflow must be balanced by the decrement of glucose utilization in plasma as well as in other tissues. It is reasonably considered that within a limited short period after glucose infusion such as in FSIGT, only the excess amount in normal subjects has to be degraded in order to restore the original dynamic steady state (Eq. (A3)).

In case of FSIGT, the terms C_{lv} and C_{neo} on the left-hand side of Eq. (A2) are instantaneously and temporarily suppressed because of feed back inhibition, thus all the terms C_{lv}, C_{fd}, and C_{neo} vanish, leading to

$$C_{inf}-(C_{ms}+C_{ft})-(C_{bn}+C_{nilv}+C_{nipp}) = C_{pl} \quad (A4)$$

Moreover, assume that the fractionation of the stored insulin release from the secretory compartment of pan-

Table A1
Notations used in this thesis

Notation (*)	Unit	Definition
G_{bn} (or C_{bn})	mg (or mg/dL)	Number of mg (or mg/dL) of glucose uptake by brain
G_{fd} (or C_{fd})	mg (or mg/dL)	Number of mg (or mg/dL) of glucose from feeding
G_{ft} (or C_{ft})	mg (or mg/dL)	Number of mg (or mg/dL) of glucose uptake by adipose or fatty tissue
G_{inf} (or C_{inf})	mg (or mg/dL)	Number of mg (or mg/dL) of glucose by infusion
G_{lv} (or C_{lv})	mg (or mg/dL)	The number of mg (or mg/dL) of glucose produced by liver glycolysis
G_{ms} (or C_{ms})	mg (or mg/dL)	Number of mg (or mg/dL) of glucose uptake by muscle
G_{neo} (or C_{neo})	mg (or mg/dL)	Number of mg (or mg/dL) of glucose from gluconeogenesis;
G_{nilv} (or C_{nilv})	mg (or mg/dL)	The non-insulin-dependent flow of glucose (in mg or mg/dL) into liver from plasma
G_{nipp} (or C_{nipp})	mg (or mg/dL)	The non-insulin-dependent consumption of glucose (in mg or mg/dL) by peripheral tissue
G_{pl} (or C_{pl})	mg (or mg/dL)	Number of mg (or mg/dL) of glucose present in plasma
K_s	mg/dL	The half saturation constant
R_{max}	mg/dL min	The maximum specific glucose utilization rate achievable when $(C_{inf}-C_{pl}) > K_s$
V	dL	Individual blood volume
$(dC/dt)_{inf}\|^{t\,inf}$	(mg/dL)/t_{inf} min	The glucose infusion rate within an infusion time interval of t_{inf} min
$(dN/dt)_{consum,(1)}$	mU min/L	The consumption rate of insulin in phase 1
$(dN/dt)_{consum,(2)}$	mU min/L	The consumption rate of insulin in phase 2
$(dN/dt)_{net,overall}$	mU min/L	The overall net gain of insulin release rate
$(dN/dt)'$	mU min/L	the overall net gain of insulin release rate in a hyperglycemic clamp experimentation
$(dN/dt)_{net,(1)}$	mU min/L	The net gain rate of insulin in phase 1
$(dN/dt)_{net,(2)}$	mU min/L	The net gain rate of insulin in phase 2
$(dN/dt)_{total,consum}$	mU min/L	Total insulin consumption rate
$(dN/dt)_{total,rel}$	mU min/L	Total insulin release rate
$(dN/dt)_{total,store}$	mU min/L	Release rate of the total amount of insulin previously stored in the secretory gland
$(dN/dt)_{total,syn}$	mU min/L	Total amount of insulin produced by de novo synthesis in the pancreatic β cells
$(dN/dt)_{store,(1)}$	mU min/L	The insulin release rate from the stored insulin in phase 1
$(dN/dt)_{store,(2)}$	mU min/L	The insulin release rate from the stored insulin in phase 2
$(dN/dt)_{syn,(1)}$	mU min/L	The phase one insulin de novo synthetic rate
$(dN/dt)_{syn,(2)}$	mU min/L	The phase two insulin de novo synthetic rate
(dN_c/dC)	(μU/mL)/(mg/dL)	Insulin consumption rate per mg of glucose consumed
(dN_r/dC)	(μU/mL)/(mg/dL)	Insulin equivalent units release rate per mg of glucose infused
(dN_s/dC)	(μU/mL)/(mg/dL)	The insulin yield factor; the insulin production rate per mg of glucose consumed by de novo synthesis
f		The partition factor of pre-stored insulin release between phase 1 and phase 2
γ	mg/dL min	The specific glucose utilization rate

(*) $C = G/V$.

creas is independent of the non-insulin-dependent terms, and that Monod Equation (James and Ollis, 1986) is applicable in describing the glucose degradation with its relevant insulin consumption and/or the corresponding insulin biosynthesis, thus for glucose utilization, we have

$$\gamma = \frac{R_{max}(C_{inf} - C_{pl})}{K_s + (C_{inf} - C_{pl})} \tag{A5}$$

where γ represents the specific glucose utilization rate, R_{max} the maximum specific glucose utilization rate achievable when $(C_{inf} - C_{pl}) > K_s$, K_s the half saturation constant, and the amount $(C_{inf} - C_{pl})$ is the amount of excess substrate glucose that has to be degraded in order to restore the original dynamic steady state.

A.1.2. The Mass Balance for Insulin

As mentioned above, insulin release involves two phases, the net gain of insulin releasing rate must be balanced by the difference of the total insulin release rate and the total insulin consumption rate as shown in the following equation:

$$\left(\frac{dN}{dt}\right)_{net,overall} = \left(\frac{dN}{dt}\right)_{total,rel} - \left(\frac{dN}{dt}\right)_{total,consum} \tag{A6}$$

where

$$\left(\frac{dN}{dt}\right)_{total,rel} = \left(\frac{dN}{dt}\right)_{total,store} + \left(\frac{dN}{dt}\right)_{total,syn} \tag{A7}$$

To consider the individual contribution of each phase, Eq. (A7) is further split into

$$\left(\frac{dN}{dt}\right)_{total,rel} = \left(\frac{dN}{dt}\right)_{store,(1)} + \left(\frac{dN}{dt}\right)_{store,(2)} + \left(\frac{dN}{dt}\right)_{syn,(1)} + \left(\frac{dN}{dt}\right)_{syn,(2)} \quad (A8)$$

According to the literature (Porte and Pupo, 1969; Nesher and Cerasi, 2002; Cerasi, 1992; Elrick et al., 1964; Darren et al., 2006), the amount of insulin from the de novo synthesis in phase 1 is negligible, hence Eq. (A8) is simplified as

$$\left(\frac{dN}{dt}\right)_{total,rel} = \left(\frac{dN}{dt}\right)_{store,(1)} + \left(\frac{dN}{dt}\right)_{store,(2)} + \left(\frac{dN}{dt}\right)_{syn,(2)} \quad (A9)$$

In essence, phase 1 merely releases a fraction of the pre-stored insulin. While phase 2 is releasing the residual part of it, the majority of the second phase release, in fact, is from the de novo synthesis.

Literature (Cerasi, 1992; Darren et al., 2006) cited that in the first phase the acute insulin release (AIR) is instantaneous. The problem rises, "How much is the fraction to be released as the acute insulin release (AIR) in phase 1 from the stored insulin in secretory granules of β cells in response to the FSIGT?". In kinetic form, the insulin balance in the phase 1 is

$$\left(\frac{dN}{dt}\right)_{net,(1)} = \left(\frac{dN}{dt}\right)_{store,(1)} - \left(\frac{dN}{dt}\right)_{consum,(1)} \quad (A10)$$

where

$$\left(\frac{dN}{dt}\right)_{store,(1)} = \left(\frac{dN}{dt}\right)_{total,store} \quad (A11)$$

$$= \left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big|^{t_{inf}}\right] \quad (A12)$$

Here the symbol f is the fraction of the total stored insulin to be released in the first phase (Eq. (A11)), the parameter $[(dC/dt)_{inf}|^{t_{inf}}]$ the glucose infusion rate within a time interval of t_{inf} min which is always ranging from 1 to 3 min; the term, (dN_r/dC) is the insulin equivalent units released per mg of glucose infused into plasma. Moreover, phase 1 persists about 10 min (Cerasi, 1992; Darren et al., 2006), within which time the released insulin in phase 1 would be consumed up. The overall consequence is, in reality, quantitatively depending upon two factors, i.e. the rate $[(dC/dt)_{inf}|^{t_{inf}}]$, and the amount ($C$) of glucose uptake (Chen and Porte, 1976) (Eq. (A12)) and the degree of insulin sensitivity, with larger responses occurring in insulin resistant individuals (Kahn et al., 1993).

Obviously, the second term on the right-hand side of Eq. (A10) is controlled by the glucose utilization rate (Eq. (A5)), thus on transformation of which yields

$$\left(\frac{dN}{dt}\right)_{consum,(1)} = -\frac{(dN_c/dC)R_{max}(C_{inf} - C_{pl})}{K_s + (C_{inf} - C_{pl})} \quad (A13)$$

Here the parameter (dN_c/dC) is the insulin consumption equivalent units per mg of glucose utilized. Substitution of Eqs. (A12) and (A13) into Eq. (A10) gives

$$\left(\frac{dN}{dt}\right)_{net,(1)} = \left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big|^{t_{inf}}\right] - \frac{(dN_c/dC)R_{max}(C_{inf} - C_{pl})}{K_s + (C_{inf} - C_{pl})} \quad (A14)$$

or

$$(dN)_{net,(1)} = \left\{\left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big|^{t_{inf}}\right] - \frac{(dN_c/dC)R_{max}(C_{inf} - C_{pl})}{K_s + (C_{inf} - C_{pl})}\right\} dt|^{t=10} \quad (A15)$$

In contrast, the net insulin response in the second phase depends primarily not only on insulin stores, but is also regulated by de novo protein synthesis within the β cells of pancreas (Song et al., 2000), hence on balancing with the consumption rate gives

$$\left(\frac{dN}{dt}\right)_{net,(2)} = \left(\frac{dN}{dt}\right)_{store,(2)} + \left(\frac{dN}{dt}\right)_{syn,(2)} - \left(\frac{dN}{dt}\right)_{consum,(2)} \quad (A16)$$

where

$$\left(\frac{dN}{dt}\right)_{store,(2)} = (1 - f)\left(\frac{dN}{dt}\right)_{total,store} \quad (A17)$$

$$\left(\frac{dN}{dt}\right)_{syn,(2)} = \left(\frac{dN_s}{dC}\right)\left\{\frac{R_{max}(C_{inf,10} - C_{pl})}{K_s + (C_{inf,10} - C_{pl})}\right\} \quad (A18)$$

and

$$\left(\frac{dN}{dt}\right)_{consum,(2)} = \left(\frac{dN_c}{dC}\right)\left\{\frac{R_{max}(C_{inf,10} - C_{pl})}{K_s + (C_{inf,10} - C_{pl})}\right\} \quad (A19)$$

Table A2
A collection of the cited parameters

$(dC/dt)_{inf}\|^{t_{inf}}$	C_{inf} (mg/dL)	C_{pl} (mg/dL)	R_{max} (mg/dL min)	K_s (mg/dL)	dN_r/dC (μU/mL/mg/dL)	$(dN/dt)_{store,(1)}$	Reference
(300–90 mg/dL)/2 min	300	90	20	213		889.4 ± 478.9 mU/L	This paper
	288	92	18	230			Henriksen et al. (1994)
	315	90	18	230			Soad et al. (1994)
	265	88	12.4	215			Cutfield et al. (1990)
(350–92 mg/dL)/2 min, 0.3 g/kg	350	92	32	269			Pacini and Bergman (1986)
	246	84	18	211			Ni et al. (1997)
(387–90 mg/dL)/0.5 min, 0.3 g/kg; (315–90 mg/dL)/3 min, (0.5 g/kg)	387	90				4015 ± 419 pM (=559.2 ± 58 mU/L), 4274 ± 545 pM (=595.3 ± 75.9 mU/L)	McCulloch et al. (1993)
	315	90					
(297–90 mg/dL)/1 min, 0.3 g/kg; (290–90 mg/dL)/4 min, 0.5 g/kg	297	90					Colman et al. (1992)
	290	90					
(376–68 mg/dL)/1 min; 0.3 mg/(L/min)	376	68			0–10 min: 4.1 ± 0.04 mU/(L/mM) (=0.23 ± 0.0 (mU/L)/(mg/dL), 10–182 min: 3.2 ± 0.25 mU/L/mM (=0.18 ± 0.02 (mU/L)/(mg/dL)	435.6 ± 5.6 mU min/L	Ezenwaka et al. (1993)

Table A3
A collection of the cited parameters

dN_r/dC (μU/mL/mg/dL)	dN_s/dC (μU/mL/mg/dL)	dN_c/dC (μU/mL/mg/dL)	$(dC/dt)_{inf}\|^{t\,inf}$ (mg/dL)/t_{inf} min	$(dN/dt)_{store,(1)}$ mU min/L or μU min/mL	Reference
			(300–90)mg/dL/2 min	889.4 ± 478.9 mU/L	This paper
				840 ± 13 mU min/L	Bergman et al. (1987)
			(387–90)mg/dL/0.5 min; (0.3 g/kg); (315–90)mg/dL)/3 min; (0.5 g/kg)	4015 ± 419 pM (=559.2 ± 58 mU/L) 4274 ± 545 pM (595.3 ± 75.9 mU/L)	McCulloch et al. (1993)
			5 g/m^2/0.5 min; 0.5 g/kg/3 min	454 mU min/L	McNair et al. (1995)
16.90 ± 2.28 (mU/L)/(min/mM) (=0.94 ± 0.13/(mU/L)/(min)/(mg/dL)					Galvin et al. (1992)
0–10 min: 4.1 ± 0.04 mU/(L/mM) (=0.23 ± 0.0 (mU/L)/(mg/dL)	10–182 min: 3.2 ± 0.25 (= 0.18 ± 0.02 mU/L/mg/dL)		(376–68 mg)/dL)/1 min; (0.3 mg/1 min)	435.6 ± 5.6 mU min/L	Ezenwaka et al. (1993)
1.82 ± 0.2 (mU/L)/(min)/(mg/dL)	9.9 ± 1.0 (mU/L)/(min)/(mg/dL)				Ward et al. (1990)
154.81/11.8–4.33 = 1.15 (mU/L)/(mg/dL) (Ameican); 127.2/12.6–4.37 = 0.86 (mU/L)/(mg/dL) (African)					Osei and Schuster (1995)
68.3/139 = 0.49 (mU/L)/(mg/dL) (3–5 min)					Lerner and Porte (1971)

respectively, where the notation $(1-f)$ in Eq. (A17) indicates the fraction of stored insulin remains post first phase release and is subsequently released in the second phase, while the term $(dN/dt)_{syn,(2)}$ in Eq. (A18) denotes the rate of new protein synthesis within the β cells and (dN_s/dC) is the insulin yield factor. $(dN/dt)_{consum,(2)}$ in Eq. (A19) is the insulin consumption rate in phase 2, and (dN_c/dC) is the insulin equivalent units per mg glucose consumed, respectively. Since phase 2 normally starts from the 10th minute post glucose infusion, hence the starting concentration of glucose in phase 2 is denoted as $C_{inf,10}$.

Alternately, the total stored insulin release rate is modified from Eq. (A11) to give

$$\left(\frac{dN}{dt}\right)_{store,total} = \left(\frac{1}{f}\right)\left\{\left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big|^{t_{inf}}\right]\right\} \tag{A20}$$

while the proportion of phase 2 is

$$(1-f)\left(\frac{dN}{dt}\right)_{store,total} = \left[\frac{1-f}{f}\right]\left\{\left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big|^{t_{inf}}\right]\right\} \tag{A21}$$

Substitution of Eqs. (A18), (A19) and (A21) into Eq. (A16), we have the net insulin gain in phase 2 as

$$\begin{aligned}\left(\frac{dN}{dt}\right)_{net,(2)} = {} & \left[\frac{(1-f)}{f}\right]\left\{\left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big|^{t_{inf}}\right]\right\} \\ & + \left(\frac{dN_s}{dC}\right)\left\{\frac{R_{max}(C_{inf,10}-C_{pl})}{K_s+(C_{inf,10}-C_{pl})}\right\} \\ & - \left(\frac{dN_c}{dC}\right)\left\{\frac{R_{max}(C_{inf,10}-C_{pl})}{K_s+(C_{inf,10}-C_{pl})]}\right\}\end{aligned} \tag{A22}$$

Combination of Eqs. (A14) and (A22) yields the overall net response of insulin to glucose bolus involving phases 1 and 2 by FSIGT.

$$\begin{aligned}\left(\frac{dN}{dt}\right)_{net,overall} = {} & \left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big|^{t_{inf}}\right] - \frac{(dN_c/dC)R_{max}(C_{inf}-C_{pl})}{K_s+(C_{inf}-C_{pl})} + \left[\frac{1-f}{f}\right]\left\{\left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big|^{t_{inf}}\right]\right\} \\ & + \left(\frac{dN_s}{dC}\right)\left\{\frac{R_{max}(C_{inf,10}-C_{pl})}{K_s+(C_{inf,10}-C_{pl})}\right\} - \left(\frac{dN_c}{dC}\right)\left\{\frac{R_{max}(C_{inf,10}-C_{pl})}{K_s+(C_{inf,10}-C_{pl})}\right\}\end{aligned} \tag{A23}$$

A.2. The Model of Insulin Response to Glucose Bolus (MIRGLuB)

Eq. (A23) is named herein; the model of insulin response to glucose bolus (MIRGLuB). Normally the time relapse required to restore the original dynamic steady state in Eq. (A23) takes about 60 min in normal subjects.

A.3. The Model of Insulin Response to Hyperglycemic Clamp Test (MIRHyCT)

Alternatively, the overall insulin response in a hyperglycemic clamp experimentation is obtained by modification of Eq. (A23) to give

$$\begin{aligned}\left(\frac{dN}{dt}\right)' = {} & \left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big|^{t_{inf}}\right] \\ & - \left(\frac{dN_c}{dC}\right)\left\{\frac{R_{max}(C_{inf}-C_{pl})}{K_s+(C_{inf}-C_{pl})}\right\} \\ & + \left[\frac{1-f}{f}\right]\left\{\left(\frac{dN_r}{dC}\right)\left[\left(\frac{dC}{dt}\right)_{inf}\Big|^{t_{inf}}\right]\right\} \\ & + \left(\frac{dN_s}{dC}\right)\left\{C_{inf}-\left\{\frac{R_{max}(C_{inf}-C_{pl})}{K_s+(C_{inf}-C_{pl})}\right\}\right\} \\ & - \left(\frac{dN_c}{dC}\right)\left\{\frac{R_{max}(C_{inf}-C_{pl})}{K_s+(C_{inf}-C_{pl})}\right\}\end{aligned} \tag{A24}$$

As shown in Eq. (A24), in a hyperglycemic clamp test, insulin production is evoked and persists at a steady constant value through the course as long as the concentration of glucose is maintained at C_{inf}.

Some major useful equations discussed in the above that are relevant to glucose–insulin interaction are summarized in Table 1.

A.3.1. Parameter Collection

A collection of the parameters obtained by a single glucose bolus is presented in Tables A2 and A3, some additional data by the hyperglycemic clamp test were reported by Polonsky et al. (1988), Tillil et al. (1988) and Gulli et al. (1992).

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