RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2008.34.077

ScienceAsia 34 (2008): 077-086

Modelling Glucose-Insulin Feedback Signal Interchanges Involving β-Cells with Delays

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Received 4 Jun 2007
Accepted 19 Oct 2007

ABSTRACT: In this paper, we studied a mathematical model system of delay-differential equations which describe the dynamic behavior of the glucose-insulin feedback system which involved β-cells, incorporating two time delays, one being the glucose triggered insulin production delay τ_g, and the other being the hepatic glucose delay τ_i. This secretory mechanism was governed by nonlinear and time-delayed feedforward and feedback signal interchanges. Moreover, delayed responses to the changes in β-cell density were incorporated. The model system was analyzed to investigate the effect of delays on the dynamic behavior of the system and the possibility of periodic solution which simulate sustained oscillations frequently observed in the experiments.

KEYWORDS: delay-differential equation, insulin secretion, Hopf bifurcation theory, mathematical model.

INTRODUCTION

The primary control of insulin secretion is a directed negative feedback system between the pancreatic β-cells and the concentration of glucose in the blood flowing to them as shown in Figure 1. An elevated blood glucose level, such as during the intake of a meal, directly stimulates the β-cells to synthesize and release insulin. The increased insulin level, in turns, reduces the level of plasma glucose to normal, promoting the use and storage of nutrients. Conversely, a fall in blood glucose below normal, such as during fasting, directly inhibits insulin secretion. Lowering of the rate of insulin secretion shifts the metabolism from the absorptive to the postabsorptive phase. Thus, this simple negative-feedback system can maintain a relatively constant supply of glucose to the tissues essentially without requiring the participation of nerves or other hormones[1].

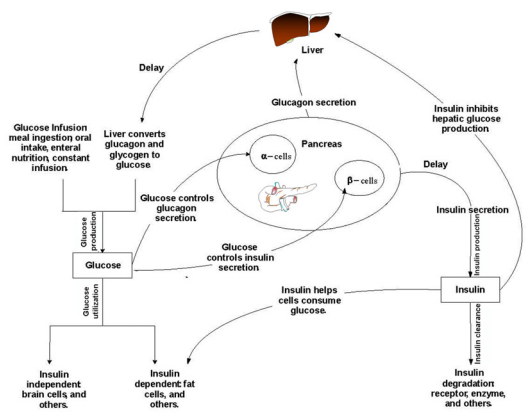

Fig 1. Two time delays in glucose-insulin regulatory system (adapted from the work of Li *et al.*[16]).

In 1960, Yalow and Berson[2] reported on the plasma insulin concentrations observed during a standard 100 grams oral glucose tolerance test. They found that the plasma insulin concentrations in early maturity-onset diabetic patients who had never been treated with insulin and in nondiabetic patients did not differ markedly. In addition, they found that nondiabetic patients usually showed peak insulin concentrations at 0.5 hour or 1 hour and a decline by 2 hours. In contrast, insulin concentrations in diabetic patients showed a lesser increase at 0.5 hour but continued to rise to a peak at 2 hours. In this study, it was reported that the integrated averaged insulin concentrations during 2 hours glucose tolerance test was higher in diabetics than in nondiabetics.

In a later work by Ackerman *et al.*[3], a mathematical model was proposed for the glucose-tolerance test, consisting of the following two autonomous first order differential equations:

$$\frac{dx}{dt} = a_1 y(t) - a_2 x(t) + E_1^{'}, \tag{1}$$

$$\frac{dy}{dt} = -a_3 y(t) - a_4 x(t) + E_2^{'} + I(t) \tag{2}$$

where $x(t)$ and $y(t)$ represent the insulin and glucose concentrations, which are the concentration differences from their fasting levels, respectively. The term $a_1y(t)$ represents the net rate of increase of insulin due to glucose, $a_2x(t)$ is the rate of insulin removal independent of glucose, $a_3y(t)$ is the rate of glucose removal independent of insulin, and $a_4x(t)$ is the rate of glucose reduction due to insulin. E'_1 and E'_2 are constant rates of increase independent from the other components. $I(t)$ is the rate of increase of blood glucose due to absorption from the intestines.

Later, the effect of insulin on diabetic and normal patients was investigated in the work of Reaven and Miller[4]. In this study, they pathologically divided the patients into 3 groups; normoglycemic, mild diabetic, and severe diabetic patients. They found that insulin concentrations of normal patients and those of mild diabetic patients were not significantly different during any time interval. Insulin concentrations of normal patients were significantly higher than those with severe diabetics only during the first hour interval. They also studied the relationship between the patterns of glucose and insulin responses in these groups. In normoglycemic patients,they observed that the relationship was characterized by a rapid fall in both glucose and insulin concentrations within the time between one and two hours. It was observed that the slower rate of fall in glucose concentration between one and two hours was associated with a constant insulin level in mild diabetics. In severe diabetics, the glucose concentration fell at an even slower rate that was associated with a continuous rise in insulin concentration.

More recently, Molnar *et al*.[5] studied the plasma immunoreactive insulin patterns in twenty-four patients composed of normal, stable diabetic, and unstable diabetic subjects. They found that the immunoreactive insulin and blood glucose levels were positively associated. High immunoreactive insulin was observed in normal patients, while the association between insulin and glucose levels in unstable diabetics was negative. In this study, crystalline zinc insulin was used for controlling the blood sugar elevations. The immunoreactive insulin-blood glucose was less negative, during the treatment of diabetic coma with short-acting insulin, than with intermediate-acting insulin. The chaotic behavior during 48-hour observation period of the plasma immunoreactive insulin and blood glucose patterns in diabetics was also reported in this study. Moreover, they modified the model Equations (1)-(2) as follows.

If there is a decrease in insulin secretion due to a reduction to $\frac{1}{N}$ of the number n of β-cells, then the plasma glucose increases until nearly normal insulin levels are obtained. Thus, the plasma glucose level is a function of the β-cells capacity $\frac{N}{n}$ and this assumption leads to the replacement of the term -$a_3y(t)$ in Equation (2). The term $I(t)$, which is the rate of increase of blood glucose due to intestinal absorption, is replaced by $b_2W(t)y(t)$, for some positive constant b_2, where $W(t)$ is the specific rate of gastrointestinal absorption. Therefore, Equation (2) was modified in Molnar *et al*.[5] as

$$\frac{dy}{dt} = \frac{b_1 N}{z(t)} - a_4 x(t) + E_2^{'} + b_2 W(t) y(t) \tag{3}$$

where b_1 is the rate constant and $z(t)$ is the density of β-cells in the proliferative phase.

In 1982, Swenne[6] studied the kinetics of β-cells proliferation in cultured fetal pancreatic cells of rats. The results indicated that an increase in glucose concentration increased the number of β-cells entering the proliferative phase.

Then, in 1987, Bajaj and Rao [7] proposed a model system of three nonlinear differential equations which incorporated β-cells kinetics in the glucose-insulin feedback system as follows.

$$\frac{dx}{dt} = R_1 y - R_2 x + C_1 \tag{4}$$

$$\frac{dy}{dt} = \frac{R_3 N}{z} - R_4 x + C_2, \tag{5}$$

$$\frac{dz}{dt} = R_5 y(T - z) + R_6 z(T - z) - R_7 z, \tag{6}$$

where $R_1, R_2, \ldots, R_7, C_1$ and C_2 are rate constants. x and y represent the plasma insulin and glucose concentrations above the basal levels, respectively. The number of β-cells in the proliferative phase is denoted by z, and N is the normal number of β-cells. T is the total number of dividing and non-dividing β-cells that is assumed to be constant. In Equation (6), the first term accounted for the increase in z due to the interaction between the plasma glucose above the fasting level and the non-dividing β-cells, while the second term represented the increase in the dividingβ-cells from the interaction between the dividing and non-dividing β-cells. Finally, the last term was the rate of reduction in the β-cells density, proportional to its current level with a rate constant R_7. This system was solved by the Runge Kutta Merson integration scheme. From the numerical simulations, the proposed model closely approximated glucose-insulin relationship to the extent that it gave a good qualitative agreement with the experimental observations.

Since β-cells function in a negative feedback loop has been established to take a predominant role in regulating both the basal plasma and insulin concentrations[7,8,9], it is reasonable to assume that the rate constants R_1, R_2, C_1, should depend on the density of β-cells in the proliferative phase z.

Thus, in 2001, Lenbury *et al.*[9] studied a mathematical model which had been adapted from that of Bajaj and Roa[7] in order to take into account the role of the number of β-cells in the regulation of plasma insulin level, following the suggestion made by Turner *et al.*[10] that the β-cells function in the negative feedback loop played a predominant role in the insulin-glucose regulation system. The insulin rate of change $\frac{dx}{dt}$ in Equation (4) should therefore be replaced by the rate $\frac{1}{z}\frac{dx}{dt}$ specific to the activated β-cells. Hence, we were led to the following model system.

$$\frac{dx}{dt} = r_1 zy - r_2 zx + c_1 z = z(r_1 y - r_2 x + c_1), \quad (7)$$

$$\frac{dy}{dt} = \frac{R_3 N}{z} - R_4 x + C_2 + w, \quad (8)$$

$$\frac{dz}{dt} = R_5(y - \hat{y})(T - z) + R_6 z(T - z) - R_7 z, \quad (9)$$

$$\frac{dw}{dt} = -\alpha w + \alpha p w^2, \quad (10)$$

where $x(t)$ and $y(t)$ now represent the insulin and glucose concentrations above their basal levels, respectively, $w(t)$ is the gastrointestinal absorption term, $\hat{y}$ is the glucose fasting level above the basal level while α and p determine the rapidity of glucose absorption, and $r_1, r_2, c_1, R_3, R_4, C_2, R_5, R_6, R_7$ are positive rate constants. More details of the model's derivation may be found in the work of Lenbury *et al.*[9].

Recently, several works on glucose-insulin regulatory system incorporated time delays in the insulin response to the glucose stimulation. There appeared to be two significant time delays in the system. One was glucose triggered insulin production delay, and the other one was hepatic glucose response delay. Based on the clinical data reported by Palumbo *et al.*[11], the time delay τ_g of insulin production in response to elevated glucose was observed to be 23.5 min. According to Prager *et al.*[12], the time delay τ_i in hepatic glucose level adjustment in response to insulin variation was generally smaller than τ_g. It is reasonable, therefore, to assume that τ_i lies in the range 0-23.5 min.

In the work of Engelborghs *et al.*[13], two models were proposed in the form of delay differential equations. The first model was for ultradian insulin oscillation, in which, however, the glucose triggered insulin production delay was missing. With the other one, they tried to model the exogenous insulin infusion under the assumption that the exogenous insulin infusion took the same form as the internal insulin production.

Some analytical work can be seen in the work of Bennett and Gourley[14], in which the authors proposed an alternative model which incorporated a time delay in a model of the glucose-insulin interaction. The model system consisted of three delay differential equations. Analytical result was obtained on some sufficient conditions for global stability.

In the same year, Mukhopadhyay *et al.*[15] studied a single-distributed-delay differential model that described the rate of glucose concentration variation due to both insulin-dependent and insulin-independent net glucose tissue uptake, as well as to constant liver glucose production. They assumed that pancreatic insulin secretion at time t depended on the earlier effects of glucose concentrations, up to distributed delay time τ. They found that the interactions among different steps of a complex biochemical and cellular transport chain within the pancreatic cell made the hypothesis of a single discrete delay less plausible than that of a distributed delay, where the concentrations of glucose at different times in the past had different relevance to the present insulin secretion rate.

In 2006, Li *et al.*[16] proposed a mathematical model for glucose-insulin regulatory system, applying two explicit time delays in the insulin response to the glucose stimulation for better understanding of this regulatory system and the ultradian insulin secretory oscillations for the cases of continuous enteral and constant glucose infusion as in Figure 1. Their simulation results indicated that the time delay τ_g played a key role in the occurrence of sustained glucose-insulin regulatory oscillations and insulin secretory oscillations. Moreover, the time delay τ_i was found to also play a key role in sustaining the oscillatory patterns in this feedback control system. As a result, they suspected that one of the possibly many causes of ultradian insulin secretion oscillations was the time delay of the insulin secretion stimulated by the elevated glucose concentration.

Recently, Palumbo *et al.*[11] introduced a family of delay-differential equation models of the glucose-insulin system to simulate the Intra-Venous Glucose Tolerance Test and allied experimental procedures of diabetological interest. Local stability was investigated in a pair of interesting member models: one, a discrete-delay differential system; the other, a distributed-delay system reducing to an ordinary differential system. A study of the global stability properties was performed, while from simulations it could be conjectured that the models considered were not satisfied for the physiological parameters values. However, their models did not consider the role of β-cells and there are few published reports on mathematical modelling and analysis of β-cells mediated glucose-insulin regulatory system with time delays.

In this paper, we studied a mathematical model system of delay-differential equations which describes the dynamic behavior of β-cells kinetics in a glucose-insulin feedback system derived from the model studied by Lenbury *et al.*[9], incorporating two time delays, one

being the glucose triggered insulin production delay τ_g, and the other being the hepatic glucose delay τ_i. Formulation of the model system provided a qualitative framework for a better understanding of the delayed responsive mechanisms in which secreted insulin requires time in transport before its increase in the bloodstream induces the expected effect that may be felt at the target site to give rise to a reduction in the glucose level. Moreover, delayed responses to the changes in β-cell density were incorporated. The model system was analyzed to investigate the effect of delays on the dynamic behavior of the system and the possibility of periodic solution which simulates sustained oscillations frequently observed in the experiments.[5,16,18-22]

Mathematical Model

We now modify the nonlinear model for glucose-insulin feedback control via β-cells proposed in the study by Lenbury *et al*.[9] in order to incorporate time-delays, τ_g in the insulin secretion in response to elevated level of glucose following the clinical evidence reported by Palumbo *et al*.[11] as mentioned above, and τ_i in the glucose drop in response to increased insulin level, following the clinical evidence reported by Prager *et al*.[12] previously mentioned as well. Since there has been no report on the delay in β-cells activation due to changes in blood glucose, we shall take the delay τ_g in the insulin secretion in response to glucose increase to be equal to the amount of time that elapses before insulin secretion would respond to the changes in the activated β-cells density. The model system can then be written as follows:

$$\frac{dx}{dt} = z(t-\tau_g)[r_1 y(t-\tau_g) - r_2 x + c_1], \tag{11}$$

$$\frac{dy}{dt} = \frac{R_3 N}{z} - R_4 x(t-\tau_i) + C_2 + w, \tag{12}$$

$$\frac{dz}{dt} = R_5(y-\hat{y})(T-z) + R_6 z(T-z) - R_7 z, \tag{13}$$

$$\frac{dw}{dt} = -\alpha w + \alpha p w^2, \tag{14}$$

using the same notations as in the model system (7)-(10) already defined earlier.

Since Equation (14) involves the dependent variables w only, it may be solved easily to find that $w(t)$ vanishes as $t \to \infty$ provided that the initial level of w is less than $\frac{1}{p}$. The above model then reduces to a system of 3 differential equations (11)-(13) with $w = 0$, which may be written in the form

$$\frac{dx}{dt} = f(x, y_{\tau_g}, z_{\tau_g}) \tag{15}$$

$$\frac{dy}{dt} = g(x_{\tau_i}, y, z) \tag{16}$$

$$\frac{dz}{dt} = h(x, y, z) \tag{17}$$

where

$$f(x,y,z) \equiv z[r_1 y - r_2 x + c_1] \tag{18}$$

$$g(x,y,z) \equiv \frac{R_3 N}{z} - R_4 x + C_2 \tag{19}$$

$$h(x,y,z) \equiv R_5(y-\hat{y})(T-z) + R_6 z(T-z) - R_7 z \tag{20}$$

with $x_{\tau_i} \equiv x(t-\tau_i)$, $y_{\tau_g} \equiv y(t-\tau_g)$, and $z_{\tau_g} \equiv z(t-\tau_g)$.

First, we prove a result on the existence and uniqueness of the steady state solution (x_s, y_s, z_s), where $f(x_s, y_s, z_s) = g(x_s, y_s, z_s) = h(x_s, y_s, z_s) = 0$ for the system (15)-(17) with (18)-(20).

Lemma 1

The system of equations (15)-(17) with (18)-(20) has a unique non-washout steady state solution (x_s, y_s, z_s), $x_s > 0$, $y_s > 0$, $z_s > 0$, provided that

$$R_7 - R_6 T > 0 \tag{21}$$

Proof

We first observe that f = 0 when $z = 0$, or

$$r_1 y = r_2 x - c_1 \tag{22}$$

Since $g \to \infty$ as $z \to 0$, any steady state (x_s, y_s, z_s) of the system must not lie on the (x,y)-plane where $z = 0$, and has to lie on the plane described by Equation (22) which is parallel to the z-axis. On this cylindrical surface given by Equation (22), we can see that x increases with y.

On the other hand, g = 0 on the cylindrical surface

$$x = \frac{R_3 N}{R_4 z} + \frac{C_2}{R_4} \tag{23}$$

on which x decreases with increasing z.

Thus, on the curve where the surfaces given by (22) and (23) intersect, z decreases with increasing y.

Now, turning our attention to the surface h = 0, on which

$$y = \hat{y} + \frac{R_7 z}{R_5(T-z)} - \frac{R_6 z}{R_5} \tag{24}$$

we keep in mind that $z \leq T$. Since, Equation (24) does not involve x, this is a cylindrical surface which is parallel to the x-axis on which

$$\frac{dy}{dz} = \frac{R_7 T}{R_5(T-z)^2} - \frac{R_6}{R_5} \tag{25}$$

Thus, if inequality (21) holds then the surface given by (24) intersects the (x,y)-plane ($z = 0$) along the line $y = \hat{y}$, at which point

$$\left.\frac{dy}{dz}\right|_{y=\hat{y}} = \frac{R_7 - R_6 T}{R_5 T} > 0 \quad (26)$$

Since the slope given by (25) decreases as $z \to T$, we see that, if (21) holds, then y will be increasing at $y = \hat{y}$ (or $z = 0$) and continues to rise as $z \to T$ from below. Thus, along the curve where the surfaces given by (22) and (24) intersect, z increases with increasing y for $y \geq \hat{y}$ and $0 \leq z < T$. Note that the 2 curves {f = g = 0} and {f = h = 0} both lie on the surface {f = 0} which is a 2 dimensional plane. On {f = g = 0}, $z \to 0$ as $y \to 0$ and $z \to 0$ as $y \to \infty$, while on {f = h = 0}, $z < 0$ at $y = 0$ and $z \to T > 0$ as $y \to \infty$.

By the intermediate value theorem, we therefore conclude that the curve {f = g = 0}and {f = h = 0} must intersect at some point (x_s, y_s, z_s) where f = g = h = 0 in the upper hemisphere of the (x, y, z)-space with $x_s > 0$, $y_s > 0$, and $z_s > 0$. Moreover, by the Rolle's theorem, the intersection point is unique, which completes our proof.

Existence of Sustained Oscillation Dependent on Delays

In order to investigate the effect of delays on the possibility of periodic dynamics in our system, we now assume that (x_s, y_s, z_s) is a non-washout steady state of our model system. Letting $X = x - x_s$, $Y = y - y_s$, $Z = z - z_s$, we will be led to the following linearized system of (15)-(17)

$$\begin{pmatrix} \dot{X} \\ \dot{Y} \\ \dot{Z} \end{pmatrix} = A \begin{pmatrix} X \\ Y \\ Z \end{pmatrix} \quad (27)$$

where A is the corresponding Jacobian matrix evaluated at (x_s, y_s, z_s) namely

$$A = \begin{pmatrix} -r_2 z_s & r_1 z_s e^{-\lambda\tau_g} & (r_1 y_s - r_2 x_s + c_1)e^{-\lambda\tau_g} \\ -R_4 e^{-\lambda\tau_i} & 0 & \frac{-R_3 N}{z_s^2} \\ 0 & R_5(T - z_s) & \frac{-R_5(T - z_s)}{z_s} \end{pmatrix} \quad (28)$$

For simplicity, we introduce new parameters by letting

$a = A_6 - A_5$
$b = -A_1 A_3 - A_5 A_6$
$c = A_7$
$d = -A_1 A_2$
$e = -A_1 A_4 - A_5 A_7$
where
$A_1 = -R_5(T - z_s)$
$A_2 = \frac{r_2 R_3 N}{z_s}$
$A_3 = \frac{R_3 N}{z_s^2}$
$A_4 = R_4(r_1 y_s - r_2 x_s + c_1)$
$A_5 = \frac{A_1}{z_S}$
$A_6 = r_2 z_s$
$A_7 = R_4 r_1 z_s$

If we also let $\tau = \tau_i + \tau_g$ be the composite lag-time, we may then write the characteristic equation of A as

$$F(\lambda) \equiv \lambda^3 + a\lambda^2 + b\lambda + d + (c\lambda + e)e^{-\lambda\tau} = 0 \quad (29)$$

According to the Hopf bifurcation theory, for a periodic solution to exist, it is necessary that Equation (29) has a pair of pure imaginary complex roots $\lambda = \pm i(\omega)$for some value of τ. In order that such a pair can be found, one must have $F(i\omega) = 0$, that is,

$$(i\omega)^3 + a(i\omega)^2 + [b + ce^{-i\omega\tau}](i\omega) + [d + ee^{-i\omega\tau}] = 0 \quad (30)$$

Equating real and imaginary parts on the right of Equation (30) to zero, we obtain the following 2 equations

$$a\omega^2 - d = e\cos(\omega\tau) + c\omega\sin(\omega\tau) \quad (31)$$

$$\omega^3 - b\omega = c\omega\cos(\omega\tau) - e\sin(\omega\tau) \quad (32)$$

Squaring both sides of Equations (31) and (32) then adding, one is led to

$$\varphi(\omega) \equiv \omega^6 + (a^2 - 2b)\omega^4 + (b^2 - 2ad - c^2)\omega^2 + (d^2 - e^2) = 0 \quad (33)$$

Setting ω^2 in Equation (33), we arrive at the following equation

$$\psi(z) \equiv z^3 + pz^2 + qz + r = 0 \quad (34)$$

where
$p = a^2 - 2b$,
$q = b^2 - 2ad - c^2$,
$r = d^2 - e^2$

We see that Equation (29) will have a pair of complex solutions $\lambda = \pm i(\omega)$ if Equation (34) has a positive real solution $z = \omega^2 > 0$.

For such a polynomial Equation (34), the following results have been proved by Ruan and Wei[17], and so we state them in the following 3 lemmas without proofs.

Lemma 2

If $r < 0$, then Equation (34) has at least one positive root.

Lemma 3

If $r \geq 0$, then the necessary condition for Equation

(34) to have positive real root is that $\Delta \equiv p^2 - 3q \geq 0$.

Lemma 4

If

$$r \geq 0 \tag{35}$$

and

$$\Delta \geq 0, \tag{36}$$

then Equation (34) has a positive root if and only if

$$z_1 > 0 \text{ and } \psi(z_1) \leq 0 \tag{37}$$

where $z_1 = \dfrac{-p + \sqrt{\Delta}}{3}$.

Proof

We note that $\psi'(z) = 0$ at $z = z_1$ and z_2 such that $z_{1,2} = \dfrac{-p \pm \sqrt{p^2 - 3q}}{3}$. The proof of this lemma can be seen in the work of Ruan and Wei[17].

Thus, by the above lemmas, we now suppose that Equation (34) has positive roots. Without loss of generality, we suppose that it has three positive roots denoted by z_1, z_2 and z_3. Then the followings will be positive roots of Equation (33).

$\omega_1 = \sqrt{z_1}$, $\omega_2 = \sqrt{z_2}$ and $\omega_3 = \sqrt{z_3}$, or $\omega_k = \sqrt{z_k}, k = 1,2,3$, which again leads us to Equations (31) and (32) with ω substituted by ω_k.

Dividing Equation (31) by (32) when $\omega = \omega_k$ and rearranging, we then arrive at the following expression for $\tan(\omega_k \tau)$, provided $\cos(\omega_k \tau) \neq 0$:

$$\tan(\omega_k \tau) = \frac{-e(\omega_k^3 - b\omega_k) + c\omega_k(a\omega_k^2 - d)}{e(a\omega_k^2 - d) + c\omega_k(\omega_k^3 - b\omega_k)}, k = 1,2,3. \tag{38}$$

We are now in the position to state and prove the following theorem.

Theorem 1.

Suppose

$$a > 0,\ d + e > 0 \text{ and } ab + ac - d - e > 0 \tag{39}$$

(a) If $r \geq 0$ and $\Delta < 0$, then all roots of Equation (29) have nonzero real parts for all $\tau \geq 0$.

(b) If either

i) $r < 0$, or

ii) $r \geq 0$, $\Delta \geq 0$, $z_1 > 0$ and $h(z_1) \leq 0$,

then all roots of Equation (29) have negative real parts when $\tau \in [0, \tau_0)$, where

$$\tau_0 = \min_{1 \leq k \leq 3,\, j \geq 1} \{\tau_k^{(j)}, \tau_k^{(j)} > 0\} \tag{40}$$

with

$$\tau_k^{(j)} = \frac{1}{\omega_k} \tan^{-1}\left[\frac{-e(\omega_k^3 - b\omega_k) + c\omega_k(a\omega_k^2 - d)}{e(a\omega_k^2 - d) + c\omega_k(\omega_k^3 - b\omega_k)}\right] + \frac{2\pi(j-1)}{\omega_k} \tag{41}$$

where k = 1,2,3, j = 1,2,3,….

Proof

(a) By contradiction, if Equation (29) has a root with zero real part for some $\tau \geq 0$, then this means that Equation (34) has a positive real root. By Lemma 3, the necessary condition of this is then that $\Delta \geq 0$ which contradicts the fact that $\Delta < 0$. Therefore all roots of Equation (29) have nonzero real parts for all $\tau \geq 0$.

(b) First, we observe that when $\tau = 0$, Equation (29) reduces to

$$\lambda^3 + a\lambda^2 + (b + c)\lambda + (d + e) = 0 \tag{42}$$

Then, by the Routh-Hurwitz criterion, all roots of Equation (42) have negative real parts since the conditions in (39) hold. This therefore implies that all roots $\lambda(\tau)$ of Equation (29) have negative real parts at the point $\tau = 0$. We can deduce then, from the continuity of $\lambda(\tau)$, that all roots of Equation (29) will have negative real parts for values of τ in some open interval containing $\tau = 0$. This means that all roots of Equation (29) have negative real parts for positive values of $\tau \in [0, \tau_c)$ for some $\tau_c > 0$.

However, τ_0 is defined by (40) to be the minimum of all the positive $\tau = \tau_k^{(j)}$ that solve Equation (38). Thus, τ_0 is the minimum of such positive τ's for which the real parts of some roots of Equation (29) vanish, provided i) or ii) holds. Therefore $\tau_c = \tau_0$, which completes the proof.

Finally, for a Hopf bifurcation to occur, leading to a limit cycle surrounding the non-washout steady state (x_s, y_s, z_s), we also need to show that

$$\left.\frac{d\,\mathrm{Re}\,\lambda(\tau)}{d\tau}\right|_{\tau=\tau_0} \neq 0 \tag{43}$$

This is done in the next theorem.

Theorem 2

Suppose conditions i) or ii) in Theorem 1 hold, then $\lambda = \pm i\omega_0$ is a pair of purely imaginary roots of Equation (29). Moreover, if

$$\psi'(z_0) \neq 0 \tag{44}$$

where

$$z_0 = \omega_0^2,\ \omega_0 = \omega_k\big|_{\tau=\tau_0}, \tag{45}$$

then Equation (43) holds.

Proof

The first part of this theorem is an immediate consequence of Theorem 1 and the definition of τ_0. To prove that (43) holds, we begin by writing $F(\lambda)$ in the form

$$F(\lambda) = F_1(\lambda) - F_2(\lambda)e^{-\lambda\tau} \tag{46}$$

where $F_1 \equiv \lambda^3 + a\lambda^2 + b\lambda + d$ and $F_2 \equiv e - c\lambda$. Then, on $F = 0$, the total derivative of F with respect to τ is

$$\frac{dF}{d\tau} = [\dot{F}_1 - (\dot{F}_2 e^{-\lambda\tau} - \tau F_2 e^{-\lambda\tau})]\frac{d\lambda}{d\tau} + \lambda F_2 e^{-\lambda\tau} = 0 \tag{47}$$

where $\dot{F}_i \equiv \dfrac{dF_i}{d\lambda}$, i = 1,2

Solving (47) for $\lambda' = \dfrac{d\lambda}{d\tau}$, we obtain

$$(\lambda')^{-1} = -\frac{\dot{F}_1 - (\dot{F}_2 - \tau F_2)e^{-\lambda\tau}}{\lambda F_2 e^{-\lambda\tau}} \tag{48}$$

Also, on $F(i\omega) = 0$, we must have $F_1 = F_2 e^{-\lambda\tau}$, and thus

$$\mathrm{Re}(\lambda')^{-1}\Big|_{\tau=\tau_0} = \mathrm{Re}\left(-\frac{\dot{F}_1}{\lambda F_1} + \frac{\dot{F}_2}{\lambda F_2}\right)_{\tau=\tau_0}$$

That is,

$$\mathrm{Re}(\lambda')^{-1}\Big|_{\tau=\tau_0} = \mathrm{Re}\left(-\frac{\dot{F}_1\overline{F}_1}{\lambda\|F_1\|^2} + \frac{\dot{F}_2\overline{F}_2}{\lambda\|F_2\|^2}\right)_{\tau=\tau_0} \tag{49}$$

Now, letting
$R_1 = Re(F_1)$, $R_2 = Re(F_2)$
$I_1 = Im(F_1)$, $I_2 = Im(F_2)$
at $\tau = \tau_0$, we see that for $F(i\omega) = 0$, we need to have

$$\phi(\omega) = R_1^2 + I_1^2 - R_2^2 - I_2^2 = 0 \tag{50}$$

or, equivalently, (33) holds, which means

$$\|F_1\|^2 = R_1^2 + I_1^2 = R_2^2 + I_2^2 = \|F_2\|^2$$

Equation (49) then becomes

$$\mathrm{Re}(\lambda')^{-1}\Big|_{\tau=\tau_0} = \frac{\left[\left(R_1\frac{dR_1}{d\omega} + I_1\frac{dI_1}{d\omega}\right) - \left(R_2\frac{dR_2}{d\omega} + I_2\frac{dI_2}{d\omega}\right)\right]_{\omega=\omega_0}}{\omega_0\|F_1\|^2}$$

$$= \frac{\left[\frac{d}{d\omega}\left(R_1^2 + I_1^2\right) - \frac{d}{d\omega}\left(R_2^2 + I_2^2\right)\right]_{\omega=\omega_0}}{2\omega_0\|F_1\|^2}$$

That is,

$$\mathrm{Re}(\lambda')^{-1}\Big|_{\tau=\tau_0} = \frac{\left.\frac{d\phi}{d\omega}\right|_{\omega=\omega_0}}{2\omega\|F_1\|^2} \tag{51}$$

Equivalently,

$$\left(\frac{d\,\mathrm{Re}\,\lambda}{d\tau}\right)^{-1}\Bigg|_{\tau=\tau_0} = \frac{\psi'(z_0)}{\|F_1\|^2} \tag{52}$$

Hence,

$$sign\left\{\left.\frac{d\,\mathrm{Re}\,\lambda}{d\tau}\right|_{\tau=\tau_0}\right\} = sign\left\{\left(\frac{d\,\mathrm{Re}\,\lambda}{d\tau}\right)^{-1}\Bigg|_{\tau=\tau_0}\right\} = sign\{\psi'(z_0)\}$$

Since $\psi'(z_0) \neq 0$, it is either positive or negative. Therefore, $\left.\frac{d\,\mathrm{Re}\,\lambda}{d\tau}\right|_{\tau=\tau_0}$ is either positive or negative as well. That is,

$\left.\frac{d\,\mathrm{Re}\,\lambda}{d\tau}\right|_{\tau=\tau_0} \neq 0$ which completes our proof.

In summary, the above analysis provides the proof of the following.

Theorem 3

If conditions (35), (36), (37), (39), and (44) hold, then a Hopf bifurcation occurs in our model system (15)-(17) for a positive composite delay $\tau = \tau_0$ given by Equations (39) and (40). The non-washout steady state (x_s, y_s, z_s) is stable for $\tau < \tau_0$, and loses its stability at $\tau = \tau_0$. Further more, there will be a positive number ε such that the model system under study possesses periodic solutions for values of $\tau \in (\tau_0, \tau_0 + \varepsilon)$.

In such a case that $\tau \in (\tau_0, \tau_0 + \varepsilon)$, the periodic solution is a limit cycle, that bifurcates from the steady state (x_s, y_s, z_s), whose radius increases with increasing τ.[23,24] Figure 2 shows numerical simulations of the model system under study when the parametric values, given in the figure caption, have been chosen so that the conditions listed in Theorem 3 are satisfied. Here, $\tau_0 = 0.30275$. In 2a) and 2c), $\tau < \tau_0$ so that the steady state is stable. The solution trajectories are seen, projected onto the (x, y)-plane to spiral towards the steady state. In 2b) and 2d), $\tau > \tau_0$ and so, according to Theorem 3, the solution trajectories are seen here, projected onto the (x,y)-plane, to tend towards limit cycles surrounding the steady states, as theoretically predicted. These figures demonstrate the independence of the two delays, and that each time delay does not have a unique

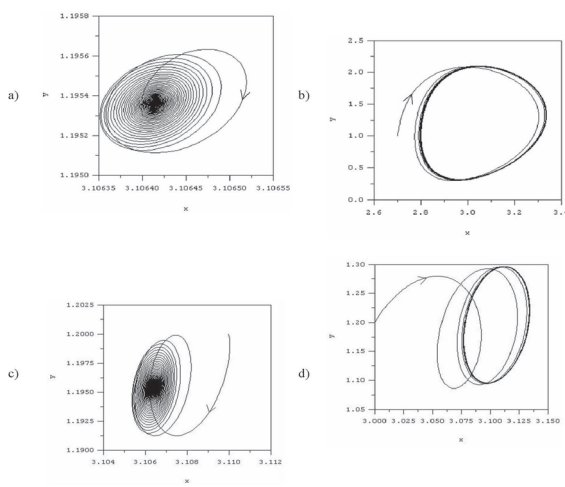

Fig 2. Computer simulation of the model system equations (15)-(20).Here, $r_1 = 0.211321$, $r_2 = 0.21430$, $R_3 = 0.3241469$, $R_4 = 0.2627777$, $R_5 = 0.033341$, $R_6 = 0.031531$, $R_7 = 0.022$, $c_1 = 0.4131$, $C_2 = 0.08125$, $\hat{y} = 1.424$, T = 1.94, $N = 1.026667$, $x_s = 3.1064140393$, $y_s = 1.1953593284$, $z_s = 0.4527482267$, and $\tau_0 = 0.30275$.
a) $(\tau_i, \tau_g) = (0.043, 0.07)$Thus, $\tau < \tau_0$ and the solution trajectory spirals towards a stable steady state.
b) $(\tau_i, \tau_g) = (0.043, 0.3525)$ Thus, $\tau_0 < \tau$ and the steady state is unstable. The solution trajectory tends towards a limit cycle.
c) $(\tau_i, \tau_g) = (0.0029, 0.08)$ Thus, $\tau < \tau_0$ and the solution trajectory spirals towards a stable steady state.
d) $(\tau_0, \tau_g) = (0.25, 0.08)$ Thus, $\tau_0 < \tau$ and the steady state is unstable. The solution trajectory tends towards a limit cycle.

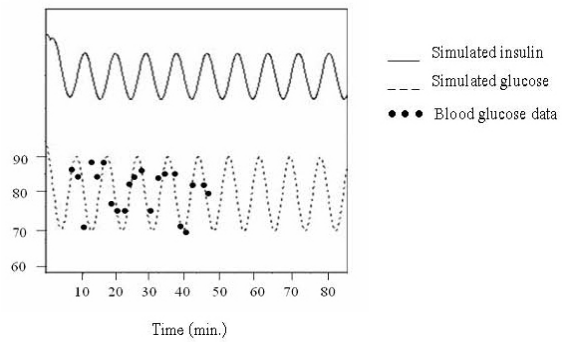

Fig 3. The simulated time series of insulin and glucose concentrations above basal levels are shown together with blood glucose data, provided by the UCSC Metabolic Diseases Department, Italy. The solid lines represent the output generated by the model with τ_0 = 0.210146, while the dots represent the experimental data points. An intravenous cannula was inserted into a left anticubital vein of a subject, after overnight fasting, and blood samples (3 ml each, in lithium heparin, every 2 minutes) were obtained. On each blood sample, glucose was determined by spectrophotometry.

value at which the steady state becomes unstable.

Finally, in Figure 3 a numerical simulation of the model system is shown as time series of insulin and glucose levels. We observe that as time progresses, the patterns of both insulin and glucose concentrations exhibit sustained oscillation which have been experimentally reported in various literatures.[5,16,18-22] We also show in this figure a collection of blood glucose measurements carried out at the UCSC Metabolic Diseases Department, Italy. The measurements were done on a subject having negative family and personal history for diabetes mellitus or other endocrine diseases, not on any medications and no reported current illness. The subject reported a constant body weight for the six months preceding the study, and underwent three days of standard composition diet (55% carbohydrate, 30% fat, 15% protein) ad libitum with at least 250 g carbohydrates per day. After overnight fasting, an intravenous cannula was inserted into a left anticubital vein and blood samples (3 ml each, in lithium heparin) were obtained every 2 minutes. On each blood sample, glucose was determined by spectrophotometry. After appropriate rescaling and translation to take into account the units of measurements and the fact that the glucose level in our model is measured above the basal level, the theoretical curve is seen here to be capable of simulating real experimental data quite well in the 40 minutes period shown.

Discussion and Conclusion

We have investigated the effect of delays in the glucose-insulin feedback mechanism involving β-cells. It is found that the system is stable for sufficiently small composite delay $\tau = \tau_i + \tau_g$. As τ becomes large enough, the system can exhibit oscillatory behavior.

In selecting a model, it is no longer deemed reliable to simply choose that in which the parameters may be chosen so that the model solution best fits the experimental data. Rather, a more appropriate choice should be capable of simulating those dynamic behavior observed experimentally. Specifically, if sustained oscillations have been clinically observed, a model which can not simulate such behavior should be discarded.

In this work, we have chosen to modify the model proposed by Lenbury *et al.*[9] which has been reported to give good qualitative agreement with the experimental observations. Incorporating the delays τ_i and τ_g, and also taking into account the delay in the action of changing activated β-cells density, we have been able to investigate the possibility of different dynamic behavior permitted by our model dependent on the delays. Although other factors may take part in this control mechanism, their effects are mostly considered less significant, or indirect, so that their inclusion has been deemed not warranted. As can be seen in a number of works that reported on different models of this system, they only involved no more than 3 components, namely glucose, insulin and β-cells.[3,5,12,13,15] Many workers only modelled the interaction between plasma glucose and insulin concentrations.[3,10,15,25,26] Some contended that since the β-cell density changes very slowly compared to glucose and insulin concentrations, β-cell density may be assumed to be essentially constant. However, our model considers rather the activated β-cells whose dynamics could be fast even while the total number of β-cells remains relatively constant.

It has also been contended that the compartment of incretins, which include glucagon, and glucagon-like peptides, plays an important role in glucose-stimulated insulin secretion and should be incorporated in a model that describes the glycemic and hormonal responses to glucose load. However, incretins represent gastrointestinal factors released in response to nutrient ingestion that stimulates oral glucose-dependent insulin secretion[27], while our study considers the situation when the gastrointestinal absorption term $w(t)$ has already tended to zero. Thus, our model only describes the glycemic and hormonal responses to an intravenous glucose load, and the role of incretins has therefore not been explicitly incorporated.

As seen in Figure 3, our model yields simulated curve which fits satisfactorily well with real experimental data. It is clearly an improvement on the model considered by Lenbury *et al*. since the current model has incorporated the delays τ_i and τ_g. The curve

for plasma glucose in Figure 3 is seen to significantly lag that of insulin concentration, due to such delays, a realistic characteristics that cannot be captured by the earlier models without delays. Moreover, although such delays have been incorporated in the model by Li *et al*.[26], our model also considers the role of β-cells and the delayed hormonal response to its number of proliferative population. The resulting model then consists of 3 nonlinear equations which we believe is capable of capturing more diversified nonlinear behavior, including chaotic dynamics, than the earlier models.

Specifically, in our simulation presented in Figures 3, we used $\tau_g = 2.0$ and $\tau_i = 0.4$. On the other hand, the critical composite delay is $\tau_0 = 0.210146$, below which we may expect stability. We note that our analysis appears to indicate that it is the combined effect of the delays (composite delay), rather than each individual delay τ_i or τ_g, that influences the dynamics of the feedback mechanism in the insulin-glucose control system under study. This means that if, for example, the hepatic glucose delay τ_i becomes large, the feedback regulation system will be able to maintain stability by shortening the insulin production delay τ_g to keep the composite delay τ smaller than τ_0. In a healthy subject, the adjustment of the lag time τ_g in insulin production in response to glucose variation, and the corresponding delay τ_i, is supposed to be functioning normally. A malfunction of such ability could, in light of our modelling and analysis, lead to loss of stability, a situation that is medically harder to control. Here, the analysis has been done in the event that intestinal absorption (w) has vanished which would correspond, say, to the sleep-period during which the subject is not being fed. During the wake-period, food digestion would lead to nonzero w and more chaotic secretion patterns can be expected. The incorporation into our model of an appropriate absorption term w should shed lights onto how the system may be controlled with proper prescription of drugs protocol in combination with suitably designed feeding regimens. The role of incretins should then be incorporated into the model. Further experimental work is also needed to accurately calibrate the parametric values in our system model. These are subjects for our future endeavor.

Acknowledgement

This work was supported by the National Center for Genetic Engineering and Biotechnology, and a scholarship under the Staff Development Project of the Commission on Higher Education. Appreciation is also extended to the UCSC Metabolic Diseases Department for providing us with the experimental data.

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