



Modeling the Insulin–Glucose Feedback System: The Significance of Pulsatile Insulin Secretion

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(Received on 18 November 1999, Accepted in revised form on 24 August 2000)

A mathematical model of the insulin–glucose feedback regulation in man is used to examine the effects of an oscillatory supply of insulin compared to a constant supply at the same average rate. We show that interactions between the oscillatory insulin supply and the receptor dynamics can be of minute significance only. It is possible, however, to interpret seemingly conflicting results of clinical studies in terms of their different experimental conditions with respect to the hepatic glucose release. If this release is operating near an upper limit, an oscillatory insulin supply will be more efficient in lowering the blood glucose level than a constant supply. If the insulin level is high enough for the hepatic release of glucose to nearly vanish, the opposite effect is observed. For insulin concentrations close to the point of inflection of the insulin–glucose dose–response curve an oscillatory and a constant insulin infusion produce similar effects.

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1. Introduction

Endocrine systems are often characterized by a pulsatile secretion of hormones (Crowley & Hofler, 1987; Leng, 1988). Examples are the release of growth hormone and gonadotropins, which have been observed to occur with characteristic intervals of 1–3 h. It has been suggested (Goldbeter, 1989; Li & Goldbeter, 1989) that periodically modulated signals are more effective than constant, stochastic or chaotic stimuli in producing a sustained physiological response in the target cells. It has also been suggested (Hesch, 1989) that information associated with the temporal variation in the concentrations of

hormones plays an important role in the regulatory processes.

Experiments (Simon *et al.*, 1987; Shapiro *et al.*, 1988) have revealed that the release of insulin from the pancreas occurs in an oscillatory fashion with a typical period of 80–150 min. Hence, in healthy subjects the concentration of insulin in the plasma oscillates, and these oscillations are accompanied by variations in the plasma glucose concentration. The physiological significance and pharmacological implications of the pulsatile secretion of insulin have been discussed, e.g. by Lefèbvre *et al.* (1987).

Superimposed on the slow (ultradian) oscillations, one can also observe more rapid pulses in the release of insulin with a period of 8–15 min (Lang *et al.*, 1979; Hansen *et al.*, 1982). These oscillations are particularly visible in the portal vein, and they are assumed to have an important

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influence on several hepatic processes. These oscillations are smoothed out to a certain degree in the main blood compartment because of the relatively large volume of this compartment.

The mechanisms that generate both types of oscillations are not yet fully understood. However, the existence of rapid pulses in isolated animal pancreases (Stagner *et al.*, 1980) suggests that these rapid pulses result from an intrapancreatic pacemaker mechanism.

The case of slow oscillations is less clear. Slow oscillations could arise from an intermittent uptake of glucose in the gastrointestinal tract. Yet, the fact that these oscillations persist during constant intravenous glucose infusion (Shapiro *et al.*, 1988) indicates that a different type of mechanism must be at work. Since slow oscillations are present in patients who have had a segmental pancreas transplantation (Polonsky *et al.*, 1990), it seems that the central nervous system is not the site of origin for the variations in insulin secretion. Moreover, lack of correlation between insulin oscillations and oscillations of glucagon and cortisol (Shapiro *et al.*, 1988) suggests that the generative processes for the oscillations in insulin secretion do not involve interactions with counterregulatory hormones.

Hence, we are left with two possible explanations of the underlying mechanisms for the ultradian oscillations in insulin secretion: like the rapid oscillations they could reflect the activity of an intrapancreatic pacemaker, or they could result from an instability in the insulin–glucose feedback system. The latter hypothesis has been pursued in a number of works (Sturis, 1991; Sturis *et al.*, 1991a, b) in which we have developed a mathematical model of the insulin–glucose feedback system.

A parallel line of research focuses on the effect of an oscillatory supply of insulin. Experiments (Matthews *et al.*, 1983; Paolisso *et al.*, 1991) have shown that a pulsatile supply of insulin can have a higher hypoglycemic effect than a constant supply at the same average rate. The purpose of the present study is to use the model of the insulin–glucose feedback regulation in order to identify a possible mechanism behind the higher efficiency of oscillatory insulin. We show that the interaction of the oscillatory insulin supply with the receptor dynamics of the glucose utilizing

cells can be of minute significance only. The reason for this is that the amplitude of the oscillations in the insulin concentration in the intercellular space is small, and changes in the average glucose utilization depend only weakly on this amplitude. However, it is possible to interpret seemingly conflicting results of clinical studies in terms of the position of the working point relative to the point of inflection of the hepatic glucose production vs. plasma insulin concentration.

2. Description of the Original Insulin–Glucose Feedback Model

The insulin–glucose model that is considered in the present paper was originally developed by Sturis *et al.* (Sturis, 1991; Sturis *et al.*, 1991a; see also Mosekilde, 1996; Keener & Sneyd, 1998). The purpose of the model was to provide a possible mechanism for the origin of the slow oscillations. Analysis of the model suggests that the slow oscillations of insulin secretion and plasma glucose concentration could originate from a Hopf bifurcation in the insulin–glucose feedback mechanism (see, e.g. Thompson & Stewart, 1986).

The following feedback loops are included in the model: glucose stimulates pancreatic insulin secretion, insulin stimulates glucose uptake and inhibits hepatic glucose production, and glucose enhances its own uptake. The system contains two significant delays. One delay is related to the fact that the physiological action of insulin on the utilization of glucose is correlated with the concentration of insulin in a slowly equilibrating intercellular compartment rather than with the concentration of insulin in the plasma (Yang *et al.*, 1989; Poulin *et al.*, 1994). The other delay is associated with the time lag between the appearance of insulin in the plasma and its inhibitory effect on the hepatic glucose production (Prager *et al.*, 1986; Bradley *et al.*, 1993).

The model has three main variables: the amount of glucose in the plasma and intercellular space, G , the amount of insulin in the plasma, I_p , and the amount of insulin in the intercellular space, I_i . In addition, there are three variables, x_1 , x_2 , and x_3 , that represent the above-mentioned delay between insulin in plasma and its effect on the hepatic glucose production.

The equations describing the dynamics of the model are

$$\frac{dI_p}{dt} = f_1(G) - E \left(\frac{I_p}{V_p} - \frac{I_i}{V_i} \right) - \frac{I_p}{t_p}, \quad (1)$$

$$\frac{dI_i}{dt} = E \left(\frac{I_p}{V_p} - \frac{I_i}{V_i} \right) - \frac{I_i}{t_i}, \quad (2)$$

$$\frac{dG}{dt} = G_{in} - f_2(G) - f_3(G)f_4(I_i) + f_5(x_3), \quad (3)$$

$$\frac{dx_1}{dt} = \frac{3}{t_d}(I_p - x_1), \quad (4)$$

$$\frac{dx_2}{dt} = \frac{3}{t_d}(x_1 - x_2), \quad (5)$$

$$\frac{dx_3}{dt} = \frac{3}{t_d}(x_2 - x_3). \quad (6)$$

Note that the equations are written in terms of total amount of glucose (in mg) and insulin (in mU, 1 mU insulin \cong 6.67 pmol). Glucose and insulin amounts are converted to concentration units in the figures. All the parameters and functional relations in the model are based on results of independent experiments (see below). Values of the parameters are shown in Table 1.

Insulin is secreted by the pancreas into the plasma, where it is either degraded or transported into the intercellular space [eqn (1)]. V_p is the distribution volume for insulin in plasma, and V_i the effective volume of the intercellular space.

TABLE 1
Parameters of the original model

Parameter	Value	Parameter	Value
V_p (l)	3	U_b (mg min ⁻¹)	72
V_i (l)	11	C_2 (mg l ⁻¹)	144
V_g (l)	10	C_3 (mg l ⁻¹)	1000
E (l min ⁻¹)	0.2	U_0 (mg min ⁻¹)	40
t_p (min)	6	U_m (mg min ⁻¹)	940
t_i (min)	100	β	1.77
t_d (min)	36	C_4 (mU l ⁻¹)	80
R_m (mU min ⁻¹)	210	R_g (mg min ⁻¹)	180
a_1 (mg l ⁻¹)	300	α (l mU ⁻¹)	0.29
C_1 (mg l ⁻¹)	2000	C_5 (mU l ⁻¹)	26

The transport of insulin between plasma and intercellular space is assumed to be a passive diffusion process driven by the difference in insulin concentration between the two compartments, with transfer rate E (Polonsky *et al.*, 1986). Insulin degradation is assumed to be exponential, with time constant t_p for insulin in plasma and t_i for insulin in the intercellular space [eqns (1) and (2)].

The pancreatic insulin production controlled by the glucose concentration is specified by the function

$$f_1(G) = \frac{R_m}{1 + \exp((C_1 - G/V_g)/a_1)}. \quad (7)$$

This function is fitted to independent experimental results involving a deconvolution of measured rates of C-peptide release (Polonsky *et al.*, 1988; Shapiro *et al.*, 1988).

Glucose is supplied to the plasma at an exogenously controlled rate G_{in} . This rate can represent either the rate of glucose uptake from food or the rate of intravenous glucose infusion. Glucose is removed from its distribution space through uptake by the cells in different body tissues [eqn (3)].

Insulin-independent glucose utilization (glucose uptake by the brain and nerve cells) is described by the function

$$f_2(G) = U_b(1 - \exp(-G/(C_2V_g))), \quad (8)$$

which is fitted to the experimental data from the work of Verdonk *et al.* (1981). Glucose utilization by the muscle and fat cells depends on both insulin and glucose concentration. The glucose-dependent term in the function describing glucose utilization is assumed to be

$$f_3(G) = \frac{G}{C_3V_g}, \quad (9)$$

which agrees with experimental results (Rizza *et al.*, 1981; Verdonk *et al.*, 1981). The insulin-dependent term is given by

$$f_4(I_i) = U_0 + \frac{U_m - U_0}{1 + \exp(-\beta \ln(I_i/C_4(1/V_i + 1/Et_i)))}. \quad (10)$$

The construction of this functional relation involves the assumption that the effect of insulin in the intercellular space can be expressed in terms of the experimentally determined relation between the plasma insulin concentration and the cellular glucose uptake (Rizza *et al.*, 1981). To account for the concentration gradient between the two compartments, the function $f_4(I_i)$ is adjusted so that at steady state it matches the experimental findings.

The influence of insulin on the hepatic glucose production, as determined by Rizza *et al.* (1981), is well described by the function

$$f_5(x_3) = \frac{R_g}{1 + \exp(\alpha(x_3/V_p - C_5))}. \quad (11)$$

As mentioned above, the response of the hepatic glucose production to changes in the plasma insulin concentration involves a time delay. This delay is assumed to be of third order with a total time t_d (Prager *et al.*, 1986).

The differential equations of the model [eqns (1)–(6)], are solved numerically using a Runge–Kutta (4, 5) integration routine. In Fig. 1, we show the result of a simulation in MATLAB, with the glucose infusion rate of 216 mg min^{-1} .

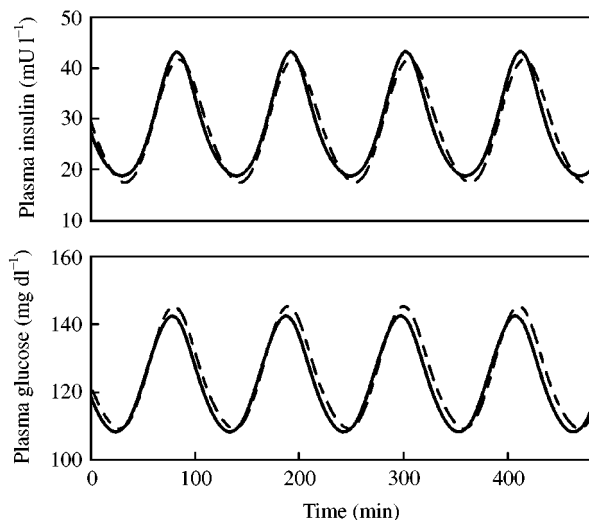


FIG. 1. Results from the original insulin–glucose feedback model (—) and the simplified model (---). Plasma glucose and plasma insulin concentrations during a simulated constant glucose infusion with the rate of 216 mg min^{-1} .

The model exhibits self-sustained oscillations when a constant glucose infusion is simulated for a large range of parameters (Sturis *et al.*, 1991a). It can account for several experimental observations: periods of oscillations obtained from the model are within the range of the experimentally observed values (110–120 min); increase of the rate of glucose infusion results in increased amplitudes of the oscillations, but does not affect the frequency (VanCauter *et al.*, 1989); there is a high correlation between time evolution of the glucose and insulin concentration (Simon *et al.*, 1987; Polonsky *et al.*, 1988; Shapiro *et al.*, 1988; VanCauter *et al.*, 1989); and glucose peaks precede insulin peaks by a few minutes (VanCauter *et al.*, 1989).

Furthermore, simulation of a meal produces decreasing oscillations that resemble well the patterns seen in the experimental studies (Polonsky *et al.*, 1988). The model also shows that the periodicity of the oscillations can be entrained to a periodic exogenous glucose infusion (Sturis *et al.*, 1995b). Entrainment of the oscillations has also been experimentally observed (Sturis, 1991; Sturis *et al.*, 1991b). This is a way to validate the significance of nonlinear interactions in the model.

The next step in testing the model is to examine the hypothesis that oscillatory insulin supply is more efficient in reducing blood glucose concentration than is continuous delivery. It was shown experimentally (Sturis *et al.*, 1995a) that slow insulin oscillations with periods in the range 100–150 min promote more efficient glucose utilization. In that study subjects were given somatostatin to temporarily suppress endogenous insulin secretion. A constant intravenous glucose infusion was applied, and exogenous insulin was infused either at a constant rate or in a sinusoidal manner with a period of 120 min and an average value equal to the constant rate value. The mean glucose concentrations over a period of 28 h was $13 \pm 6 \text{ mg dl}^{-1}$ lower when insulin was infused in an oscillatory way than when the rate of infusion was constant.

In order to simulate the exogenous insulin infusion, the term describing the endogenous insulin secretion in the model, $f_1(G)$, was replaced by a term representing an exogenous sinusoidal insulin infusion. Equation (1) was thus

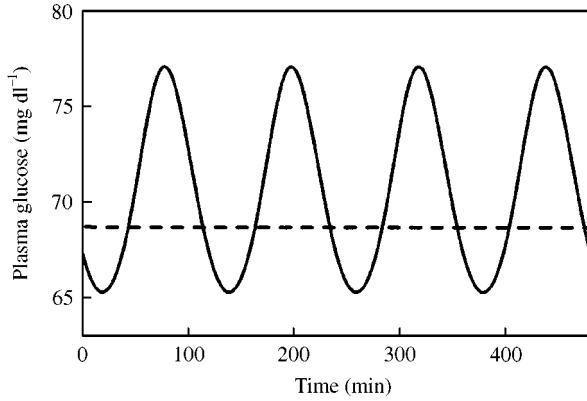


FIG. 2. Results from a simulation of exogenous insulin infusion instead of the endogenous insulin secretion in the original model. Plasma glucose concentrations for the oscillatory (—) and constant (----) insulin infusion. A lower mean value of glucose is obtained for the constant insulin infusion. Mean rate of the insulin infusion is 21 mU min^{-1} , rate of the glucose infusion is 216 mg min^{-1} .

replaced by

$$\frac{dI_p}{dt} = m(1 + A \sin(2\pi t/T)) - E \left(\frac{I_p}{V_p} - \frac{I_i}{V_i} \right) - \frac{I_p}{t_p}, \quad (12)$$

where m represents the mean rate of the insulin infusion, which in the experiments by Sturis *et al.* (1995a) equals 21 mU min^{-1} . The relative amplitude of the insulin infusion, A , was set to zero for the constant infusion, and to 0.3 for the oscillatory infusion. The period T was set to 120 min.

Figure 2 shows the result of a simulation of exogenous insulin infusion. In contrast to the experiments, the model predicts a lower mean value of the plasma glucose when insulin is infused at a constant rate than when the infusion rate oscillates. In the following we will: (i) analyse the model; (ii) point to the cause of the discrepancies between simulations and experiments; (iii) suggest possible ways to improve the model; and (iv) propose an explanation for higher efficacy of oscillatory insulin.

3. Analysis of the Insulin-Glucose Feedback Model

In order to gain insight into certain features of the model, we have simplified the model. The

simplified version of the model has two important properties: it is analytically tractable, and it shows the same main characteristics as the original model, i.e., self-sustained oscillations and values of the state variables in the same range as the original model.

In the range of values of plasma glucose realized by a simulation of glucose infusion with a rate of 216 mg min^{-1} ($105\text{--}145 \text{ mg dl}^{-1}$, see Fig. 1), the function $f_2(G)$ is well approximated by a constant, and the function $f_1(G)$ by a first-order polynomial. The function $f_4(I_i)$ is also well approximated by a first-order polynomial in the range of obtained values of intercellular insulin ($15\text{--}23 \text{ mU l}^{-1}$). In the physiologically relevant range of glucose and insulin concentrations, the function $f_5(x_3)$ shows significantly greater variation in its second derivative than all other functions in the model. For values of x_3 in the range $65\text{--}115 \text{ mU}$ the function $f_5(x_3)$ changes from concave to convex, and is well approximated by a third-order polynomial.

We replaced $f_1(G)$ with the first-order Taylor expansion around the mean value of G , $f_2(G)$ with a constant, $f_4(I_i)$ with the first-order expansion around the mean value of I_i , and $f_5(x_3)$ with the third-order expansion around the mean value of x_3 . Mean values were obtained by a simulation of glucose infusion at the rate of 216 mg min^{-1} using the original model.

The simplified model takes the form:

$$\frac{dI_p}{dt} = aI_p + bI_i + cG + d, \quad (13)$$

$$\frac{dI_i}{dt} = eI_p + fI_i, \quad (14)$$

$$\begin{aligned} \frac{dG}{dt} = & gI_iG + hG + kx_3 + lx_3^2 \\ & + nx_3^3 + p, \end{aligned} \quad (15)$$

$$\frac{dx_1}{dt} = rI_p - rx_1, \quad (16)$$

$$\frac{dx_2}{dt} = rx_1 - rx_2, \quad (17)$$

$$\frac{dx_3}{dt} = rx_2 - rx_3. \quad (18)$$

TABLE 2
Parameters of the simplified model

Parameter	Value	Parameter	Value
$a(\text{min}^{-1})$	-0.233	$h(\text{min}^{-1})$	2.64×10^{-3}
$b(\text{min}^{-1})$	0.0182	$k(\text{mg mU}^{-1} \text{min}^{-1})$	17.5
$c(\text{mU mg}^{-1} \text{min}^{-1})$	4.79×10^{-3}	$l(\text{mg mU}^{-2} \text{min}^{-1})$	-0.315
$d(\text{mU min}^{-1})$	-43.9	$n(\text{mg mU}^{-3} \text{min}^{-1})$	1.48×10^{-3}
$e(\text{min}^{-1})$	0.0667	$p(\text{mg min}^{-1})$	80.5
$f(\text{min}^{-1})$	-0.0282	$r(\text{min}^{-1})$	0.0833
$g(\text{mU}^{-1} \text{min}^{-1})$	-9.44×10^{-5}		

Values of the parameters are summarized in Table 2.

As illustrated in Fig. 1, the time evolution of plasma glucose and insulin concentration that results from the simplified model does not differ significantly from the results of the original model. Based on this we assume that the analysis of the simplified model will provide insight into certain specific characteristics of the original model in the relevant range of the state variables.

In the simulation of an exogenous insulin infusion, eqn (13) was replaced by

$$\frac{dI_p}{dt} = m(1 + A \sin(2\pi t/T)) + aI_p + bI_i, \quad (19)$$

where $m = 21 \text{ mU min}^{-1}$. A was set to zero for the constant infusion and to 0.3 for the oscillatory infusion. The period $T = 120 \text{ min}$. Furthermore, since the glucose infusion rate in the experiments by Sturis *et al.* (1995a) equals 420 mg min^{-1} , the parameter p in eqn (15) was set to 285 mg min^{-1} to compensate for the difference between the rate of the glucose infusion of 420 and 216 mg min^{-1} .

When the amplitude of the insulin infusion is zero, the system has a steady-state solution which can easily be found analytically. In response to the oscillatory insulin infusion the system shows oscillatory behavior. We found the exact solutions to a system consisting of the two first-order differential equations, eqns (19) and (14). We next found the solution to eqns (16)–(18). For large t , the solutions are periodic functions with period T and may be expressed in the form

$$y_j = Y_j + A_j \sin((2\pi t/T) + \phi_j), \quad j = 1, \dots, 5, \quad (20)$$

where $y_j = I_p, I_i, x_1, x_2$, or x_3 , and Y_j and A_j do not depend on time. The value of Y_j depends in a linear manner on the mean rate of insulin infusion and does not depend on the amplitude or the period of the rate of insulin infusion. Therefore, the time average of the quantities I_p, I_i, x_1, x_2 , and x_3 is the same in the case of an oscillatory insulin infusion as in the case of a constant infusion.

The only equation in the simplified model with nonlinear terms is eqn (15). It can be expressed in the form

$$\frac{dG(t)}{dt} + P(t)G(t) = Q(t), \quad (21)$$

where

$$P(t) = -gI_i(t) - h, \quad (22)$$

and

$$Q(t) = kx_3(t) + lx_3^2(t) + nx_3^3(t) + p. \quad (23)$$

Equation (21) has the general solution

$$G(t) = \frac{1}{\mu(t)} \left(\mu(t_0)G_0 + \int_{t_0}^t \mu(\xi)Q(\xi) d\xi \right), \quad (24)$$

where

$$\mu(t) = \exp(H(\cos(2\pi t/T) + \phi_i) - \cos(\phi_i)) \exp(-Jt), \quad (25)$$

$$J = g\langle I_i \rangle + h, \quad (26)$$

and

$$H = gA_i \frac{T}{2\pi}. \quad (27)$$

A_i is the amplitude and ϕ_i the phase shift of the oscillations of $I_i(t)$, and $\langle \cdot \rangle$ denotes an average with respect to time.

Since $|H| \ll 1$, we expand the first exponential in eqn (25) to first order

$$\mu(t) \approx (1 + H(\cos(2\pi t/T + \phi_i) - \cos(\phi_i))) \exp(-Jt), \quad (28)$$

and insert this into eqn (24). The expression for $x_3(t)$ from eqn (20) is substituted into eqn (23), and the square and cube terms expanded. Keeping only the dominant oscillatory terms in the integral in eqn (24), we obtain (for $t \gg T$)

$$\begin{aligned} G(t) = & C_0 + C_1 \cos(2\pi t/T) + C_2 \sin(2\pi t/T) \\ & + C_3 \cos(4\pi t/T) + C_4 \sin(4\pi t/T) \\ & + C_5 \cos(6\pi t/T) + C_6 \sin(6\pi t/T) \\ & + C_7 \cos(8\pi t/T) + C_8 \sin(8\pi t/T), \quad (29) \end{aligned}$$

where C_0, \dots, C_8 are constants. Figure 3 shows the analytical solution for $G(t)$, eqn (29), as well as the numerical solution of the equations of the simplified model, eqns (14)–(19). As in the original model (see Fig. 2), the simplified model predicts

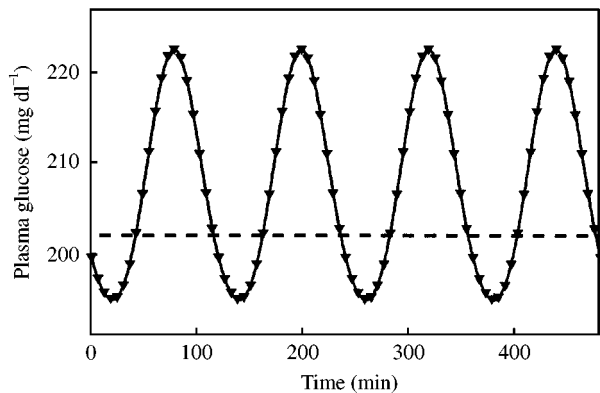


FIG. 3. Plasma glucose concentrations obtained by the simplified model in the case of an oscillatory insulin infusion: the analytical solution, eqn (29) (—), and the numerical solution, eqns (14)–(19) (▼). Steady-state value of glucose for a constant insulin infusion in the simplified model (----). Note a lower mean value of the plasma glucose when insulin is infused at a constant rate than when the infusion rate oscillates. Mean rate of the insulin infusion is 18 mU min^{-1} , rate of the glucose infusion is 420 mg min^{-1} .

a lower mean value of the plasma glucose when insulin is infused at a constant rate than when the infusion is oscillatory.

In eqn (29), the mean value of $G(t)$ is C_0 , which equals

$$C_0 = -\frac{2K_0 + K_2}{2J} + \varepsilon, \quad (30)$$

where

$$K_0 = k\langle x_3 \rangle + l\langle x_3 \rangle^2 + n\langle x_3 \rangle^3 + p, \quad (31)$$

and

$$K_2 = A_3^2(l + 3n\langle x_3 \rangle). \quad (32)$$

A_3 is the amplitude of the oscillations of $x_3(t)$, and ε is a small term which is a function of the amplitude and phase of the oscillations of $I_i(t)$ and $x_3(t)$ (see Fig. 4).

When the rate of insulin infusion is constant, the system reaches a steady state. The steady-state value of $G(t)$ is denoted by G_{ss} and equal to $-K_0/J$. Because of the nonlinear terms in eqn (15), the time average of $G(t)$ in the case of an oscillatory insulin infusion differs from the steady-state value G_{ss} .

The relative difference between the mean value and the steady-state value of $G(t)$ is

$$\frac{\langle G \rangle - G_{ss}}{G_{ss}} = \frac{K_2}{2K_0} - \frac{\varepsilon J}{K_0}. \quad (33)$$

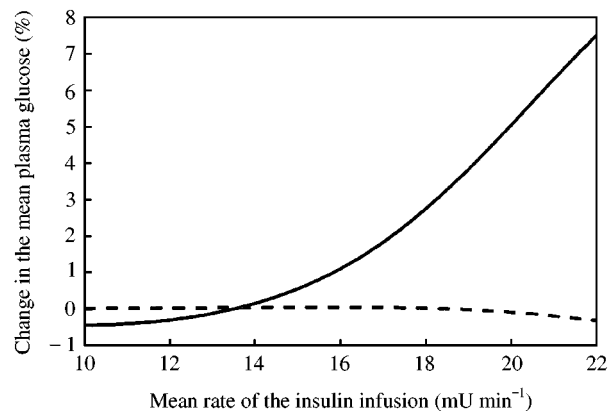


FIG. 4. Relative difference between the mean value of the plasma glucose concentration for an oscillatory and a constant insulin infusion. Difference caused by the change in the hepatic glucose production, $K_2/2K_0$ in eqn (33) (—). Difference caused by the change in the cellular glucose consumption, $-\varepsilon J/K_0$ in eqn (33) (----). Rate of the glucose infusion is 420 mg min^{-1} .

The first term on the right-hand side describes the effects of the hepatic glucose production, $Q(t)$. The second term is smaller, and includes also the effects of the cellular glucose consumption, $P(t)$. Figure 4 shows the values of $K_2/2K_0$ and $-\varepsilon J/K_0$ vs. the mean rate of insulin infusion.

It is readily seen that the main contribution to the change of the mean value of plasma glucose comes from the hepatic glucose production. When the mean rate of insulin infusion is low, the hepatic glucose production is near saturation, and the effect of an oscillatory component in the insulin infusion is to reduce the average plasma glucose concentration. On the other hand, when the mean rate of insulin infusion is high, the hepatic glucose production is near total suppression, and the effect of an oscillatory component in the insulin infusion is to increase the average plasma glucose concentration. In mathematical terms, increase or decrease of the mean plasma glucose concentration depends on the sign of the second derivative of the function describing the hepatic glucose production. At the point of inflection the mean value of plasma glucose concentration does not depend on the amplitude of insulin oscillations.

We now examine in greater detail the effect of the hepatic glucose production, $Q(t)$, on the relative change of the mean plasma glucose concentration. Since K_0 is always positive, $K_2/2K_0$ is negative when $K_2 < 0$, which holds for values of $\langle x_3 \rangle$ such that

$$\left. \frac{\partial^2 f_5(x_3)}{\partial x_3^2} \right|_{\langle x_3 \rangle} < 0, \quad (34)$$

as can be seen from eqns (23) and (32). In the simplified model this implies

$$\langle x_3 \rangle < -\frac{l}{3n} = 70.9 \text{ mU}, \quad (35)$$

or expressed in terms of the mean rate of insulin infusion,

$$m < 13.5 \text{ mU min}^{-1}. \quad (36)$$

In the original model, eqn (34) is satisfied, implying a decrease of the mean value of the plasma glucose for an oscillatory insulin infusion, when

$$\langle x_3 \rangle < V_p C_5 = 78.0 \text{ mU}. \quad (37)$$

The reason for the disagreement between the model and the experiments by Sturis *et al.* (1995a) is now obvious: the model shows higher mean value of the plasma glucose for an oscillatory insulin infusion compared to a constant one because the model gives rather high values of the plasma insulin, and thus also of x_3 : $\langle x_3 \rangle > V_p C_5$. The parameter $V_p C_5$ represents the amount of insulin in the plasma (in mU) at the inflection point of the insulin–glucose dose–response curve.

On the other hand, the experiments show that the plasma insulin concentration lies in the range 15–29 mU l^{-1} . The mean plasma insulin concentration was $24.03 \pm 1.08 \text{ mU l}^{-1}$ during constant infusion and $22.98 \pm 0.95 \text{ mU l}^{-1}$ during oscillatory infusion. From the perspective of the model, this means that $\langle x_3 \rangle \cong 70 \text{ mU} < V_p C_5$. Thus, the condition for the lower mean value of plasma glucose caused by oscillatory insulin infusion was satisfied in the experiments.

3.1. PULSATILE DELIVERY OF INSULIN—THE EFFECT OF FREQUENCY

In the experiments by Sturis *et al.* (1995a) discussed above, exogenous insulin was supplied in a sinusoidal pattern. A slightly different experimental procedure has been applied in order to investigate the effect of pulsatile insulin delivery, meaning that exogenous insulin was infused in pulses of a few minutes with an interval between the pulses (Matthews *et al.*, 1983; Verdin *et al.*, 1984; Paolisso *et al.*, 1991). The period was chosen to be of the order of 15 min, and the total amount of insulin given was kept the same in the case of a constant and a pulsatile insulin infusion.

The simplest way to model this kind of insulin delivery is to represent insulin concentrations by square waves. To keep the total amount of insulin infused the same for a pulsatile and a constant delivery, we must have

$$I_{puls}(\xi) = \frac{I_{const}}{\xi}, \quad (38)$$

where I_{puls} is the concentration of insulin obtained in a pulse, I_{const} is the concentration of insulin obtained by a constant infusion, and ξ is the duration of a pulse/period of pulses.

Let us compare the response for the case of a pulsatile input and a constant one. If the response function is linear, there is no difference in the total response between those two cases:

$$(1 - \xi)f_r(I_{basal}) + \xi f_r(I_{puls}(\xi)) = f_r(I_{const}), \quad (39)$$

where f_r is the response function (in this case the hepatic glucose production), and I_{basal} is the concentration of plasma insulin that induces the basal response. The response function from our model, $f_5(x_3)$, is a nonlinear function. Pulsatile insulin delivery will give a lower mean glucose concentration for

$$(1 - \xi)f_5(I_{basal}) + \xi f_5(I_{puls}(\xi)) < f_5(I_{const}). \quad (40)$$

The minimum of the left-hand side is obtained for $I_{const}/\xi \cong 36 \text{ mU l}^{-1}$. This relation defines the optimal ratio of the amount of insulin infused in a constant manner and the duration of the pulse for the maximal lowering of the plasma glucose concentration. For $I_{const} = 26 \text{ mU l}^{-1}$, which is the point of inflection of $f_5(x_3)$, there is no difference between the effect of a constant and a pulsatile insulin infusion when $\xi = 0.5$, or for the pulses that last for half of the period.

Experiments show seemingly contradictory data. While Matthews *et al.* (1983) and Paolisso *et al.* (1991) observed greater hypoglycemic effect for the pulsatile insulin delivery, Verdin *et al.* (1984) reported similar metabolic effects for the two modes of insulin infusion. All these results are in agreement with the predictions of the above model. For the experiments done by Verdin *et al.* (1984), the model predicts similar effects for different ways of infusing insulin because the mean value of the plasma insulin was close to the inflection point of the function $f_5(x_3)$ (28 and 29 mU l^{-1}), and ξ was equal to 0.5 in those experiments.

4. Insulin Receptor Dynamics

Let us now consider the effect of the cellular glucose consumption, $P(t)$, on the change in the

mean value of the plasma glucose concentration. The original model could be made more realistic by introducing new state variables representing insulin receptors. That way it might be possible to change $P(t)$ so that it produces a decrease in the mean value of plasma glucose.

The number of receptors may be measured in milliunits corresponding to the amount of insulin they can bind. The effect of insulin on glucose utilization will depend on one of the variables representing insulin receptors in a particular state (e.g. receptors with bound insulin, or internalized receptors). Thus, the function describing insulin-dependent utilization of glucose, $f_4(I_i)$, has to be rescaled into $f_4(R_a)$, where R_a denotes the active state of receptors.

Changes caused by introducing receptors into the model would affect the change in the mean value of plasma glucose through a change in the characteristics of the oscillations of the function f_4 . In the simplified model without receptors, the mean value of plasma glucose changes by less than 0.5% via glucose uptake by peripheral tissues (see Fig. 4). Introducing the receptors into the model changes g , A_i , and $\sin \phi_i$, but since g is of the order of $10^{-4} \text{ mU}^{-1} \text{ min}^{-1}$, the decrease of the mean value of $G(t)$ produced by $P(t)$ can be at most 1–2%.

In order to test possible effects of the receptor dynamics on the behavior of the original model, we constructed two detailed models of insulin receptor dynamics and connected them to the original model. These modifications are discussed in Appendix A.

None of the two proposed receptor models differs significantly from the original model in their response to an oscillatory and a constant insulin infusion. Based on our analysis of the simplified model and the results of the numerical simulations, we submit that the change of the mean value of plasma glucose is primarily caused by the change of the hepatic glucose production.

5. Adjusting the Model Parameters

In view of the results presented above, it is necessary to adjust the parameters in the original model so that the model gives the values of $\langle I_p \rangle$ and $\langle G \rangle$ in agreement with the experiments from Sturis *et al.* (1995a). To get the observed values of

$\langle I_p \rangle$ for the relatively large value of the insulin infusion rate used in the experiments (21 mU min^{-1}), we set the value of the parameter t_p to 4 min. This change means a faster degradation of plasma insulin with a rate that is still physiologically realistic, and gives values of plasma insulin in agreement with the experiments. The change of the value of t_p is sufficient to obtain the result that oscillatory insulin infusion is more effective than a steady infusion.

To get the observed values of $\langle G \rangle$, we lowered the glucose infusion rate to 120 mg min^{-1} . Furthermore, we changed the value of two parameters in the function $f_5(x_3)$. The function $f_5(x_3)$ from the original model was obtained by fitting to the data shown in fig. 3 in Rizza *et al.* (1981). Since it is stated in the same reference that the concentration of insulin required for half-maximal suppression of glucose production is $29 \pm 2 \text{ mU l}^{-1}$, we set the parameter C_5 to 29 mU l^{-1} . Using this value of C_5 , we changed the value of α from 0.29 to 0.4 l mU^{-1} in order to fit to the data in fig. 3 in Rizza *et al.* (1981). The original and the new version of the function $f_5(x_3)$, together with the relevant experimental data (Rizza *et al.*, 1981) are shown in Fig. 5. This change of the function f_5 is not necessary for the model to show higher efficacy of oscillatory insulin, but does enhance the effect.

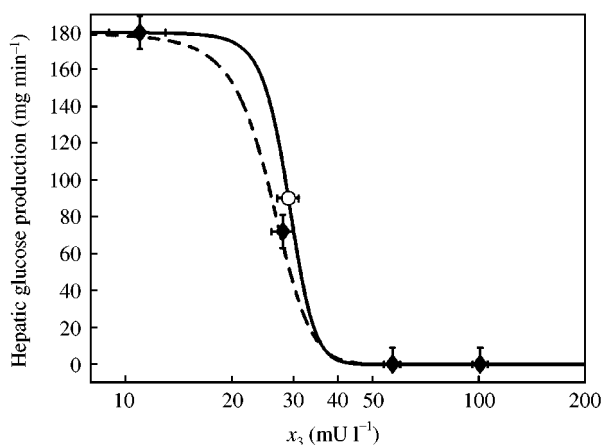


FIG. 5. Hepatic glucose production as a function of plasma insulin. The adjusted function $f_5(x_3)$ (—). The function $f_5(x_3)$ from the original model (---). Experimental data (\blacklozenge) from Rizza *et al.* (1981), and the value of the half-maximal suppression of glucose production (\circ) reported by the same authors.

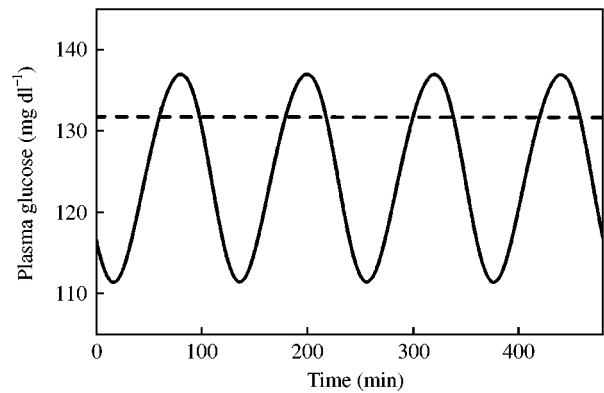


FIG. 6. Results from the model with adjusted parameters: $t_p = 4 \text{ min}$, $\alpha = 0.4 \text{ l mU}^{-1}$, and $C_5 = 29 \text{ mU l}^{-1}$. Glucose concentrations for an oscillatory (—) and a constant (---) insulin infusion. A significantly lower mean value of glucose is obtained for the oscillatory insulin infusion. Mean rate of the insulin infusion is 21 mU min^{-1} , rate of the glucose infusion is 120 mg min^{-1} .

Figure 6 shows the results of a simulation of an oscillatory and a constant insulin infusion obtained using the original model with the changed parameters t_p , C_5 , and α . Since the model gives the values of the plasma insulin consistent with the experiments, it also shows the lower mean value of plasma glucose for the oscillatory insulin infusion compared to a constant infusion, as predicted from the analysis of the simplified model.

To make the original model more realistic under conditions of high glucose concentration, we introduced two new expressions. These additional functions representing the effect of hyperglycemia on the hepatic glucose production and on the splanchnic glucose uptake are discussed in Appendix A.

The time evolution of the plasma glucose and insulin concentration resulting from the improved model is shown in Fig. 7. It does not differ significantly from the results of the original model (Fig. 1). However, in the simulations of a very high rate of glucose infusion, plasma glucose concentration is kept at a physiological level by the decreased hepatic glucose production, and by the induced glucose uptake by the splanchnic tissue.

The improved model keeps the successful features of the original model, but, additionally, it accounts for: (i) the inhibition of the hepatic

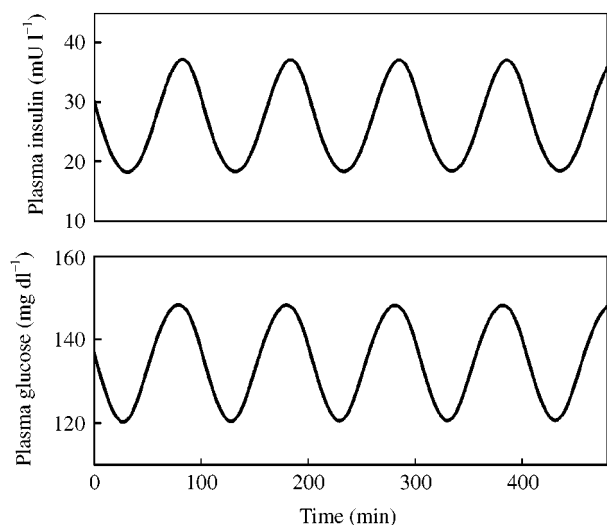


FIG. 7. Results from the improved model. Plasma glucose and plasma insulin concentrations during a simulated constant glucose infusion with the rate of 216 mg min^{-1} . Parameters different from the original model: $t_p = 4 \text{ min}$, $\alpha = 0.41 \text{ mU}^{-1}$, and $C_5 = 29 \text{ mU l}^{-1}$. Rate of change of plasma glucose includes new functions $f_6(G)$ and $f_7(G)$ (see Appendix A).

glucose production by high concentrations of plasma glucose; (ii) the stimulation of the splanchnic glucose uptake by plasma glucose; (iii) the higher efficiency of the oscillatory insulin infusion compared to a constant supply.

6. Discussion

Several *in vivo* and *in vitro* studies have shown that pulsatile insulin has greater hypoglycemic effect than continuous delivery (Matthews *et al.*, 1983; Bratusch-Marrain *et al.*, 1986; Marsh *et al.*, 1986; Lefèbvre *et al.*, 1987; Paolisso *et al.*, 1991; Sturis *et al.*, 1995a). The exact mechanism of this phenomenon has so far not been well explained. Here we present a model that agrees with experimental data, and offer a possible explanation why oscillatory insulin secretion is more effective than a constant one.

The overall effect of insulin on the body glucose metabolism is the sum of the effects of insulin on suppression of glucose production and stimulation of glucose utilization. The more pronounced hypoglycemic effect observed during oscillatory insulin infusion could result from a stronger inhibition of hepatic glucose production, or an increased peripheral glucose uptake,

or both. The model presented here and its analysis suggest that the primary reason for a greater hypoglycemic effect of the oscillatory insulin is the higher inhibition of the hepatic glucose production. Enhanced glucose utilization seems not to be able to produce a substantial change in the mean value of the plasma glucose.

Our model predicts that oscillatory insulin would have a greater hypoglycemic effect when the mean value of the plasma insulin is less than the value at the point of inflection of the insulin dose-response curve for the hepatic glucose production (approx. 30 mU l^{-1}).

Indeed, this hypothesis seems to explain most of the experimental results from the literature. Sturis *et al.* (1995a) reported greater effect for the oscillatory insulin at the mean plasma insulin concentrations of $23\text{--}24 \text{ mU l}^{-1}$. Matthews *et al.* (1983) reported the same effect at the insulin concentrations of $5\text{--}19 \text{ mU l}^{-1}$. On the other hand, Verdin *et al.* (1984) showed that pulsatile insulin has the same effect as constant insulin at the mean insulin concentrations of $28\text{--}29 \text{ mU l}^{-1}$, which is exactly what our model predicts for the mean values of insulin near the inflection point of the insulin-glucose dose-response curve.

Results of several experiments suggest that oscillatory insulin infusion has greater effect than a constant infusion for a number of different frequencies of oscillations. We hypothesize that the enhanced efficacy of oscillatory insulin is primarily due to effects on the hepatic glucose production. This holds for both rapid and slow (ultradian) insulin oscillations. The effect of the frequency of the oscillations on the extent of the inhibition of the hepatic glucose production needs further experimental investigation. Such experiments should be designed so that only the frequency is varied, while the mean value of the plasma insulin remains constant.

On the other hand, the ability of the oscillatory insulin to enhance glucose uptake should not be left out of consideration. Greater biological effect of the pulsatile insulin was usually the most pronounced after a few hours (Matthews *et al.*, 1983; Sturis *et al.*, 1995a). This fact suggests that the pulsatile insulin might reverse down-regulation of insulin receptors on the time-scale of several hours. Such a process could be included in our model.

Furthermore, it may be desirable to replace the variables for the time delay between the insulin in plasma and its effect on the hepatic glucose production by physiologically meaningful state variables. There are indications that insulin suppresses endogenous glucose production at least partly by an indirect mechanism. Such a mechanism could result from effects of insulin on the pancreatic alpha cells, adipocytes and muscle cells (Cherrington *et al.*, 1998). In alpha cells insulin inhibits glucagon secretion, which can reduce hepatic glycogenolysis. In adipocytes insulin inhibits lipolysis, thus decreasing the level of glycerol and free fatty acids that reach the liver. The decreased concentration of glycerol reduces gluconeogenesis, while the lower concentration of free fatty acids suppresses the glycogen degradation into glucose (Rebrin *et al.*, 1995, 1996; Mittelman *et al.*, 1997). In muscle cells insulin reduces the release of gluconeogenic amino acids, thereby reducing hepatic gluconeogenesis. On the other hand, there is evidence that insulin inhibits hepatic glucose production primarily by a direct mechanism (Maheux *et al.*, 1997). The relative importance of the direct and indirect inhibition has yet to be investigated. If an indirect mechanism proves to be dominant, then the effect of insulin oscillations might be on some intermediate step in the signaling pathway to the liver.

Finally, since the liver is the most prominent organ involved in the control of blood glucose concentration, it is important to determine the hepatic glucose balance accurately. A promising way of determining the hepatic glucose balance is to express it in terms of fluxes of liver enzymes glucokinase and glucose-6-phosphatase. The inhibition of the hepatic glucose release by high glucose levels may reflect an increase in glucokinase flux, or a decrease in liver glucose-6-phosphatase, or a combination of these (Rognstad, 1994). Furthermore, glucose, as well as insulin, induces the inhibition of glycogen phosphorylase, and the activation of glycogen synthase (Cárdenas & Goldbeter, 1996). We plan to extend our model to represent the regulatory processes in the liver on the molecular level.

I.M.T. thanks S.F. Nørrelykke and H. Flyvbjerg for inspiring discussions. I.M.T. received support from

the Danish Research Academy through its Graduate School in Nonlinear Science.

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APPENDIX A

The Receptor Down-regulation Model

Insulin action in cells starts with binding of the insulin molecule to the receptor in the cell membrane. This activates a cascade of reactions. One of the final effects is the activation of glucose transporters which then facilitate glucose uptake into the cell (Di Guglielmo *et al.*, 1998; Hunter & Garvey, 1998).

Although most of the receptors are recycled from the endosomes back into the plasma membrane, a proportion of them are degraded. Thus, with continuous exposure to high concentrations of insulin, the number of receptors in the cell membrane decreases. By this type of mechanism, known as receptor down-regulation, a cell can slowly, over a few hours, adjust its sensitivity to the insulin concentration.

The equations of the proposed model are the following:

$$\frac{dR_{bound}}{dt} = k_{bind}I_{i,free}R_{free} - k_{dis}R_{bound} - k_{int,b}R_{bound}, \quad (A.1)$$

$$\frac{dR_{int}}{dt} = k_{int,b}R_{bound} + k_{int,f}R_{free} + k_{syn} - k_{re}R_{int} - k_{deg}R_{int}, \quad (A.2)$$

$$\frac{dR_{free}}{dt} = k_{re}R_{int} + k_{dis}R_{bound} - k_{bind}I_{i,free}R_{free} - k_{int,f}R_{free}, \quad (A.3)$$

where R_{bound} is the amount of bound receptors in the membrane (mU), R_{int} the amount of internalized receptors (mU), R_{free} the amount of free receptors in the membrane (mU), $I_{i,free}$ the amount of free insulin in the intercellular space (mU), k_{bind} the binding rate constant ($\text{mU}^{-1} \text{min}^{-1}$), k_{dis} the dissociation rate constant (min^{-1}), $k_{int,b}$ the bound receptor internalization rate constant (min^{-1}), $k_{int,f}$ the free receptor internalization rate constant (min^{-1}), k_{syn} the receptor synthesis rate constant (mU min^{-1}), k_{deg} the receptor degradation rate constant (min^{-1}), and k_{re} the receptor recycling rate constant (min^{-1}).

We extended the original model by coupling it with the model for insulin receptor down-regulation. Besides, the variable representing insulin in the intercellular space was divided into two: $I_{i,bound}$, which represents the amount of insulin bound to receptors and equals R_{bound} , and $I_{i,free}$, the amount of free insulin in the intercellular space. The effect of insulin on glucose utilization was supposed to occur when insulin is bound to receptors, thus the function $f_4(I_i)$ was rescaled into $f_4(R_{bound})$.

Values of the parameters are chosen in the following way: $k_{bind} = 6.7 \times 10^{-5} \text{mU}^{-1} \text{min}^{-1}$ and $k_{dis} = 0.02 \text{min}^{-1}$ are taken from the work of Jones *et al.* (1984), while the rest is taken and adapted from the work of Quon & Campfield (1991): $k_{int,b} = 2.2 \times 10^{-3} \text{min}^{-1}$, $k_{int,f} = 2 \times 10^{-4} \text{min}^{-1}$, $k_{syn} = 5 \times 10^{-3} \text{mU min}^{-1}$, $k_{deg} = 5 \times 10^{-5} \text{min}^{-1}$, and $k_{re} = 1.8 \times 10^{-3} \text{min}^{-1}$.

Simulation based on the insulin–glucose feedback model including receptor down-regulation shows a slight increase of the mean value of the plasma glucose over time because the number of insulin receptors in the cell membrane is decreasing, and thus also the cellular response to insulin. This occurs both in the case of a constant insulin supply and for the oscillatory infusion, while constant infusion is more effective for the glucose uptake. The model of receptor down-regulation thus does not improve the original model with respect to the effects of a constant and an oscillatory insulin infusion.

The Receptor Modification Model

A different way that insulin receptor adaptation could occur is via receptor modification. This process does not involve the change of the total number of receptors in the membrane, but rather a conformational change (Schäffer, 1994) or reversible covalent modifications of receptors (Di Guglielmo *et al.*, 1998).

In this model, only the bound active receptors induce glucose uptake. The model equations are adapted from the general model of receptor desensitization (Li & Goldbeter, 1989):

$$\frac{dR_{b,a}}{dt} = k_{bind,a}I_{i,free}R_{f,a} + k_{act,b}R_{b,i} - k_{dis,a}R_{b,a} - k_{in,b}R_{b,a}, \quad (A.4)$$

$$\frac{dR_{b,i}}{dt} = k_{bind,i}I_{i,free}R_{f,i} + k_{in,b}R_{b,a} - k_{dis,i}R_{b,i} - k_{act,b}R_{b,i}, \quad (A.5)$$

$$\frac{dR_{f,a}}{dt} = k_{dis,a}R_{b,a} + k_{act,f}R_{f,i} - k_{bind,a}I_{i,free}R_{f,a} - k_{in,f}R_{f,a}, \quad (A.6)$$

$$\begin{aligned} \frac{dR_{f,i}}{dt} = & k_{dis,i}R_{b,i} + k_{in,f}R_{f,a} \\ & - k_{bind,i}I_{i,free}R_{f,i} - k_{act,f}R_{f,i}, \end{aligned} \quad (A.7)$$

where $R_{b,a}$ is the amount of bound active receptors (mU), $R_{b,i}$ the amount of bound inactive receptors (mU), $R_{f,a}$ the amount of free active receptors (mU), $R_{f,i}$ the amount of free inactive receptors (mU), $I_{i,free}$ the amount of free insulin in the intercellular space (mU), $k_{bind,a}$ the active receptor binding rate constant ($\text{mU}^{-1} \text{min}^{-1}$), $k_{bind,i}$ the inactive receptor binding rate constant ($\text{mU}^{-1} \text{min}^{-1}$), $k_{dis,a}$ the active receptor dissociation rate constant (min^{-1}), $k_{dis,i}$ the inactive receptor dissociation rate constant (min^{-1}), $k_{act,b}$ the bound receptor activation rate constant (min^{-1}), $k_{act,f}$ the free receptor activation rate constant (min^{-1}), $k_{in,b}$ the bound receptor inactivation rate constant (min^{-1}), and $k_{in,f}$ the free receptor inactivation rate constant (min^{-1}).

The parameter values $k_{bind,a} = 6.7 \times 10^{-5} \text{mU}^{-1} \text{min}^{-1}$ and $k_{dis,a} = 0.02 \text{min}^{-1}$ are taken from the work of Jones *et al.* (1984). The binding and dissociation rate constants for the inactive receptors are assumed to be the same as for the active receptors ($k_{bind,i} = 6.7 \times 10^{-5} \text{mU}^{-1} \text{min}^{-1}$ and $k_{dis,i} = 0.02 \text{min}^{-1}$).

For this model the key relations among parameters are $k_{in,b} \gg k_{act,b}$ and $k_{act,f} \gg k_{in,f}$, meaning that the equilibrium between bound receptors is shifted towards the inactive form, while the equilibrium between free receptors is shifted towards the active form. The values used in the computer simulation, $k_{act,f} = 0.03$, $k_{in,f} = 0.003$, $k_{act,b} = 0.002$, and $k_{in,b} = 0.02 \text{min}^{-1}$, are taken from the work of Li & Goldbeter (1989).

As the original model and the model that includes receptor down-regulation, this model also shows a feature that contradicts experimental

results: a higher efficiency of a constant than an oscillatory insulin infusion with respect to glucose uptake.

Effects of Hyperglycemia

Hyperglycemia is a powerful inhibitor of the hepatic glucose production (Moore *et al.*, 1998). It is capable of suppressing hepatic glucose production by approximately 80% (DeFronzo & Ferrannini, 1987). In order to account for this effect, we introduced a functional relation:

$$f_6(G) = \frac{1}{1 + \exp(\gamma(G/C_3V_g - C_6))}, \quad (A.8)$$

where $\gamma = 5.0$ and $C_6 = 2.0$, in accordance with the experimental data from the work of DeFronzo & Ferrannini (1987). The hepatic glucose production is now represented by $f_5(x_3) \cdot f_6(G)$.

Furthermore, high glucose concentrations enhance glucose uptake by the splanchnic (liver and gut) tissue. To account for this we introduced an expression that describes glucose uptake by the splanchnic tissue:

$$f_7(G) = S_b + \frac{S_m - S_b}{1 + \exp(\delta(G/C_3V_g - C_7))}, \quad (A.9)$$

where $S_b = 20 \text{mg min}^{-1}$, $S_m = 140 \text{mg min}^{-1}$, $\delta = -2.4$ and $C_7 = 2.0$, in agreement with the experiments from the work of DeFronzo & Ferrannini (1987).

The overall rate of change of the amount of glucose in the system thus takes the form

$$\begin{aligned} \frac{dG}{dt} = & G_{in} - f_2(G) - f_3(G)f_4(I_i) \\ & + f_5(x_3)f_6(G) - f_7(G). \end{aligned} \quad (A.10)$$