J. theor. Biol. (2000) **206,** 605–619
doi:10.1006/jtbi.2000.2150, available online at http://www.idealibrary.com on IDEAL®

A Model of β-Cell Mass, Insulin, and Glucose Kinetics: Pathways to Diabetes

Brian Topp*, Keith Promislow†, Gerda deVries‡, Robert M. Miura§ and Diane T. Finegood*‖

*Diabetes Research Laboratory, School of Kinesiology, Simon Fraser University, Burnaby, BC, Canada V5A IS6,
†Department of Mathematics and Statistics, Simon Fraser University, Burnaby, BC, Canada V5A IS6,
‡Department of Mathematical Sciences, University of Alberta, Edmonton, Alberta, Canada and
§Department of Mathematics, University of British Columbia, Vancouver, BC, Canada

(*Received on* 10 *December* 1999, *Accepted in revised form on* 24 *July* 2000)

Diabetes is a disease of the glucose regulatory system that is associated with increased morbidity and early mortality. The primary variables of this system are β-cell mass, plasma insulin concentrations, and plasma glucose concentrations. Existing mathematical models of glucose regulation incorporate only glucose and/or insulin dynamics. Here we develop a novel model of β-cell mass, insulin, and glucose dynamics, which consists of a system of three nonlinear ordinary differential equations, where glucose and insulin dynamics are fast relative to β-cell mass dynamics. For normal parameter values, the model has two stable fixed points (representing physiological and pathological steady states), separated on a slow manifold by a saddle point. Mild hyperglycemia leads to the growth of the β-cell mass (negative feedback) while extreme hyperglycemia leads to the reduction of the β-cell mass (positive feedback). The model predicts that there are three pathways in prolonged hyperglycemia: (1) the physiological fixed point can be shifted to a hyperglycemic level (regulated hyperglycemia), (2) the physiological and saddle points can be eliminated (bifurcation), and (3) progressive defects in glucose and/or insulin dynamics can drive glucose levels up at a rate faster than the adaptation of the β-cell mass which can drive glucose levels down (dynamical hyperglycemia).

Introduction

Diabetes mellitus is a disease of the glucose regulatory system characterized by fasting and/or postprandial hyperglycemia (The Expert Committee, 1997). There are two major classifications of diabetes based on the etiology of the hyperglycemia. Type 1 diabetes (also referred to as juvenile onset or insulin-dependent diabetes) is due to an autoimmune attack on the insulin secreting β cells. Type 2 diabetes (also referred to as adult onset or non-insulin-dependent diabetes) is associated with a deficit in the mass of β cells (Kloppel *et al.*, 1985), reduced insulin secretion, and resistance to the action of insulin (The Expert Committee, 1997). The relative contribution and interaction of these defects in the pathogenesis of this disease remains to be clarified (Cerasi, 1995).

‖Author to whom correspondence should be addressed.
E-mail: finegood@sfu.ca

0022–5193/00/200605 + 15 $35.00/0

Blood glucose levels are regulated by two negative feedback loops. In the short term, hyperglycemia stimulates a rapid increase in insulin release from the pancreatic β cells. The associated increase in blood insulin levels causes increased glucose uptake and decreased glucose production leading to a reduction in blood glucose (Bergman *et al*., 1985). Recent evidence suggests that chronic hyperglycemia may contribute to a second negative feedback loop by increasing the mass of insulin secreting β-cells (Bonner-Weir *et al*., 1989), through changes in the rates of β-cell replication (Swenne, 1982; Hugl *et al*., 1998) and death (Efanova *et al*., 1998; Hoorens *et al*., 1996). An increased β-cell mass represents an increased capacity for insulin secretion which, in turn, would lead to a decrease in blood glucose. Type 2 diabetes has been associated with defects in components of both the short-term and chronic negative feedback loops.

Although type 2 diabetes is associated with insulin resistance, insulin secretory defects, and insufficient β-cell mass, each of these defects can also be found in people without diabetes. Insulin-stimulated glucose disposal is reduced by 50–100% in patients with type 2 diabetes as compared to non-diabetic controls (Finegood, 1997). However, insulin resistance of a similar magnitude also has been documented in many non-diabetic individuals including obese subjects, or during pregnancy, puberty, and aging (Finegood, 1997). Thus, normoglycemia can be maintained in subjects with insulin resistance via increases in blood insulin levels. Defects of insulin secretion have been demonstrated in some people with type 2 diabetes (Leahy, 1990). Even more severe defects in insulin secretion are present in patients with type 1 diabetes following islet transplantation, when normoglycemia is maintained in the absence of exogenous insulin treatment (Tobin *et al*., 1992). This suggests that glucose homeostasis can be maintained despite significant loss of β-cell function when an individual has normal insulin sensitivity. Kloppel *et al*. (1985) observed that β-cell mass is reduced by 40–50% in patients with type 2 diabetes when compared with weight matched non-diabetic subjects. In comparison, approximately 80–90% of the β-cell mass is lost before the onset of hyperglycemia in individuals who develop type 1 diabetes (Kloppel *et al*., 1985), suggesting that a greater β-cell mass is required in the presence of insulin resistance. This is also consistent with the observation of a 43% higher β-cell mass in normoglycemic subjects with insulin resistance due to obesity (Kloppel *et al*., 1985).

Although these data suggest that multiple defects are required for the onset of type 2 diabetes, it is unclear if these defects have a single causal origin or if they occur independently (Cerasi, 1995). Experimental induction of insulin resistance using either high fat feeding (Kaiyala *et al*., 1999), glucocorticoid administration (Ogawa, 1992), or genetically induced obesity (Tokuyama *et al*., 1995) has been shown to cause type 2 diabetes under certain circumstances. This supports the hypothesis that insulin resistance can cause β-cell defects, and hence diabetes, either by overworking the β cells (β-cell exhaustion) (DeFronzo *et al*., 1992) or by toxic effects of hyperglycemia on the β cells (glucose toxicity) (Unger & Grundy, 1985). However, the existence of normoglycemia in humans and animals highly resistant to insulin suggests independent defects in insulin sensitivity and β-cell function are required for type 2 diabetes (Bergman *et al*., 1985). Finally, hyperglycemia is known to induce insulin resistance (Rossetti, 1995). This supports the hypothesis that a primary insulin secretory defect that causes hyperglycemia could lead to insulin resistance and diabetes via increased glucose levels.

Mathematical modeling in diabetes research has focused predominately on the dynamics of a single variable, usually blood glucose or insulin level, on a time-scale measured in minutes (Bergman *et al*., 1979; Toffolo *et al*., 1980; Steele, 1959). Generally, these models are used as tools for measuring either rates (such as glucose production and uptake rates or insulin secretion and clearance rates) or sensitivities (such as insulin sensitivity, glucose effectiveness, or the sensitivity of insulin secretion rates to glucose). Two model-based studies have examined coupled glucose and insulin dynamics (Bergman *et al*., 1985; Matthews *et al*., 1985). In each of these studies, multiple parameter changes, representing multiple physiological defects, were required to simulate glucose and insulin dynamics observed in humans with diabetes. However, neither of these efforts incorporated β-cell mass dynamics. Here,

develop a mathematical model of β-cell mass dynamics as a component of a coupled model of β-cell mass, insulin, and glucose dynamics. This model allows us to examine the normal behavior of the system and to investigate the effects of a single defect or a combination of defects on the behavior of the whole system. In doing so, we find three distinct pathways to the diabetic state: regulated hyperglycemia, bifurcation and dynamical hyperglycemia.

Model Development

GLUCOSE DYNAMICS

In the post-absorptive state, glucose is released into the blood by the liver and kidneys, removed from the interstitial fluid by all the cells of the body, and distributed into many physiological compartments (e.g. arterial blood, venous blood, cerebral spinal fluid, interstitial fluid). In spite of the spatial complexity of the glucose distribution (Cobelli *et al.*, 1989), Steele suggested that the rates of glucose production and uptake could be calculated with isotope dilution techniques and a one-compartment model of glucose kinetics (Steele, 1959). Allsop *et al.* (1978) validated the use of Steele's single-compartment equation for near steady-state glucose dynamics. However, when glucose or tracer concentrations are changing rapidly (on a time-scale of minutes), a single-compartment model is not adequate (Finegood *et al.*, 1987). Consistent with these observations is the demonstration by Bergman *et al.* (1979) that a single compartment can be used to model glucose kinetics following an intravenous glucose bolus, provided the rapid mixing phase ($\sim$10 min) is not considered. Together, these studies suggest that a single-compartment model is appropriate when glucose kinetics are relatively slow. Since we are primarily concerned with the evolution of fasting blood glucose levels over a time-scale of days to years, glucose dynamics are modeled with a single-compartment mass balance equation

$$\mathrm{d}G/\mathrm{d}t = \text{Production} - \text{Uptake}, \qquad (1)$$

where G is the concentration of glucose in the blood (measured in $\mathrm{mg\,dl^{-1}}$), and t the time (measured in days). The rates of glucose production and uptake are normalized by the volume of glucose distribution to obtain the proper units ($\mathrm{mg\,dl^{-1}\,d^{-1}}$).

The rates of glucose production and uptake depend on blood glucose and insulin levels. These relationships have been defined experimentally with the glucose clamp technique, which allows for the measurement of glucose production and uptake rates at various steady-state blood glucose and insulin levels (Bergman *et al.*, 1985). At constant insulin levels, glucose production decreases while uptake increases, both linearly with respect to glucose levels (Best *et al.*, 1981). The slopes of these linear dependences are the "glucose effectiveness" parameters. Hyperinsulinemic clamp studies have shown that the glucose effectiveness parameters are linearly related to blood insulin levels. The slope of the glucose effectiveness vs. insulin curve is referred to as insulin sensitivity. Thus,

$$\text{Production} = P_0 - (E_{G0P} + S_{IP} I)\, G, \qquad (2)$$

$$\text{Uptake} = U_0 + (E_{G0U} + S_{IU} I) G, \qquad (3)$$

where P_0 and U_0 are the rates of glucose production and uptake at zero glucose ($\mathrm{mg\,dl^{-1}\,d^{-1}}$), E_{G0P} and E_{G0U} ($\mathrm{d^{-1}}$) are glucose effectiveness at zero insulin for production and uptake, S_{IP}, S_{IU} ($\mu\mathrm{U^{-1}\,ml\,d^{-1}}$) are insulin sensitivity for production and uptake, and I represents blood insulin concentrations ($\mu\mathrm{U\,ml^{-1}}$). Substituting eqns (2) and (3) into eqn (1), we arrive at

$$\mathrm{d}G/\mathrm{d}t = R_0 - (E_{G0} + S_I I)\, G, \qquad (4)$$

where $R_0 (= P_0 - U_0)$ is the net rate of production at zero glucose, $E_{G0} (= E_{G0P} + E_{G0U})$ is the total glucose effectiveness at zero insulin, and $S_I (= S_{IP} + S_{IU})$ is the total insulin sensitivity.

INSULIN DYNAMICS

Insulin is secreted by pancreatic β cells, cleared by the liver, kidneys, and insulin receptors, and distributed into several compartments (e.g. portal vein, peripheral blood, and interstitial fluid). Our main concern is the long-time evolution of fasting insulin levels in peripheral blood. Since the dynamics of fasting insulin levels on this time-scale

are slow, we use a single-compartment equation given by

$$dI/dt = \text{Secretion} - \text{Clearance}, \quad (5)$$

where Secretion and Clearance are rates normalized by insulin's volume of distribution ($\mu U\,ml^{-1}\,d^{-1}$).

The rate of insulin clearance is proportional to blood insulin levels when the system is near steady state (Berzins *et al*., 1986). We note that several single-compartment models which incorporate proportional insulin clearance have simulated relatively fast and complex insulin dynamics successfully (Toffolo *et al*., 1980; Cobelli & Pacini, 1988). Given the relatively slow dynamics in the present model, we assume

$$\text{Clearance} = kI, \quad (6)$$

where k is a clearance constant which represents the combined insulin uptake at the liver, kidneys, and insulin receptors (d^{-1}).

The rate of insulin secretion from pancreatic tissue is a sigmoidal function of glucose concentration (Malaisse *et al*., 1967). However, the relative contributions of β-cell recruitment and cellular insulin secretion rates have not been quantified (Salomon and Meda, 1986). Studies by Schuit *et al*. (1988) and Bosco & Meda (1991) demonstrated a sigmoidal relationship between the percentage of insulin secreting β cells and the glucose level. Schuit *et al*. (1988) demonstrated a sigmoidal relationship between glucose levels and the percentage of β cells containing newly synthesized proinsulin, while Bosco & Meda (1991) showed that the majority of insulin released from the β-cell mass comes from cells which are actively synthesizing proinsulin. Studies analysing individual β cell and islet electrical activity (Ashcroft *et al*., 1984), granulation (Leahy, 1993), and insulin secretion (Salomon & Meda, 1986) have shown that the rate of secretion from individual β cells varies with glucose, but a functional relationship has yet to be identified.

The *in vitro* relationship determined by Malaisse *et al*. (1967), as well as the successful use of sigmoidal insulin secretion rates in existing models (Cobelli *et al*., 1980; Rudenski *et al*., 1991), indicate that the net insulin secretion rate can be modeled as a sigmoidal function of glucose level. Hence, we assume

$$\text{Secretion} = \beta\sigma G^2/(\alpha + G^2), \quad (7)$$

where β is the mass of pancreatic β cells (measured in mg). All β cells are assumed to secrete insulin at the same maximal rate σ ($\mu U\,ml^{-1}\,day^{-1}$), and $G^2/(\alpha + G^2)$ is a Hill function with coefficient 2 that describes a sigmoid ranging from 0 to 1 which reaches half its maximum at $G = \alpha^{1/2}$. Substituting eqns (6) and (7) into eqn (5), we obtain the equation governing insulin kinetics,

$$dI/dt = \beta\sigma G^2/(\alpha + G^2) - kI. \quad (8)$$

β-CELL MASS DYNAMICS

Despite a complex distribution of pancreatic β cells throughout the pancreas, β-cell mass dynamics have been successfully quantified with a single-compartment model (Finegood *et al*., 1995)

$$d\beta/dt = \text{Formation} - \text{Loss}, \quad (9)$$

where Formation and Loss represent the rates at which β-cell mass is added to or removed from the population, respectively.

New β cells can be formed by the replication of existing β cells, neogenesis (replication and differentiation) from stem cells, and transdifferentiation of other cells. Presently, it is not possible to quantify rates of neogenesis and transdifferentiation. However, calculations suggest indirectly that they make a negligible contribution to β-cell mass dynamics except during development and in response to extreme physiological or chemically induced trauma (Finegood *et al*., 1995; Fernandes *et al*., 1997, Bonner-Weir *et al*., 1993). For these reasons, neogenesis and transdifferentiation are not incorporated into the present model, and Formation is assumed to be equal to Replication.

In vitro studies show that the percentage of β cells undergoing replication varies as a nonlinear function of glucose level in the medium (Swenne, 1982, Hugl *et al*., 1998). Replication rates for β cells increase with increasing glucose

TABLE 1
Normal parameter values

Parameter	Value	Units	Reference
S_I	0.72	$\mathrm{ml}\,\mu\mathrm{U}^{-1}\,\mathrm{d}^{-1}$	Finegood (1997)
E_{G0}	1.44	d^{-1}	Bergman *et al.* (1981) Finegood (1997)
R_0	864	$\mathrm{mg}\,\mathrm{dl}^{-1}\,\mathrm{d}^{-1}$	Bergman *et al.* (1981) Finegood (1997)
σ	43.2	$\mu\mathrm{U}\,\mathrm{ml}^{-1}\,\mathrm{d}^{-1}$	Bergman *et al.* (1981) Malaisse *et al.* (1967) Toffolo *et al.* (1980)
α	20000	$\mathrm{mg}^2\,\mathrm{dl}^{-2}$	Malaisse *et al.* (1967)
k	432	d^{-1}	Toffolo *et al.* (1980)
d_0	0.06	d^{-1}	Bergman *et al.* (1981) Imamura *et al.* (1988) Finegood *et al.* (1995)
r_1	0.84×10^{-3}	$\mathrm{mg}^{-1}\,\mathrm{dl}\,\mathrm{d}^{-1}$	Bergman *et al.* 1981 Imamura *et al.* (1988) Finegood *et al.* (1995)
r_2	0.24×10^{-5}	$\mathrm{mg}^{-2}\,\mathrm{dl}^2\,\mathrm{d}^{-1}$	Bergman *et al.* (1981) Imamura *et al.* (1988) Finegood *et al.* (1995)

levels; however, at extreme hyperglycemia, β cell replication may be reduced. We modeled this behavior with a simple second-degree polynomial (patterned after logistic growth),

$$\text{Replication} = (r_{1r}G - r_{2r}G^2)\beta, \qquad (10)$$

where r_{1r} ($\mathrm{mg}^{-1}\,\mathrm{dl}\,\mathrm{day}^{-1}$) and r_{2r} ($\mathrm{mg}^{-2}\,\mathrm{dl}^2\,\mathrm{day}^{-1}$) are rate constants.

Cells can be lost from the β-cell mass by apoptosis (regulated cell death), necrosis (unregulated cell death), or possibly transdifferentiation into other types of endocrine cells. For the model presented here, the rate of transdifferentiation is assumed to be negligible. *In vitro*, β-cell Death has been shown to vary nonlinearly with glucose (Hoorens *et al.*, 1996; Efanova *et al.*, 1998). Increasing the glucose level from 0 to approximately 11 mM in the medium surrounding cultured β cells reduced the rate of β-cell death. Above 11 mM glucose, the rate of β-cell death either remained low or increased. We have modeled this behavior with a simple second-degree polynomial,

$$\text{Death} = (d_0 - r_{1a}G + r_{2a}G^2)\beta, \qquad (11)$$

where d_0 (d^{-1}) is the death rate at zero glucose and r_{1a} ($\mathrm{mg}^{-1}\,\mathrm{dl}\,\mathrm{d}^{-1}$) and r_{2a} ($\mathrm{mg}^{-2}\,\mathrm{dl}^2\,\mathrm{d}^{-1}$) are constants. Substituting eqns (10) and (11) into eqn (9), we obtain the equation for β-cell mass dynamics,

$$\mathrm{d}\beta/\mathrm{d}t = (-d_0 + r_1G - r_2G^2)\beta, \qquad (12)$$

where $r_1(=r_{1r}+r_{1a})$ and $r_2(=r_{2r}+r_{2a})$ are constants.

In summary, the βIG model is comprised of eqns (4), (8), and (12). Parameter values assumed for the normal human physiological state are given in Table 1. Below, we examine both the behavior of solutions of the βIG model in the phase space of the variables using the standard parameter values (Table 1), and the change in geometrical structure of the solutions in the phase space as the parameter values are varied. Finally, we postulate some plausible pathways that lead from a normal physiological state to a pathological diabetic state.

Model Behavior

Putting eqns (4), (8), and (12) into dimensionless form (not shown), the model can be decomposed into fast (G, I) and slow (β) subsystems, and the number of parameters can be reduced from 9 to 8 (reduction occurs in the glucose equation). The fast subsystem describes acute changes in

glucose and insulin levels on a time-scale of minutes while the slow subsystem describes evolution of the β-cell mass on a time-scale of days. We will examine the behavior of these subsystems independently and then analyse the system as a whole. In the following, we will continue to use dimensional variables.

Due to its slow dynamics, β-cell mass will be treated as a parameter when analysing the fast subsystem. From the nullclines ($dG/dt = 0$ and $dI/dt = 0$) for a given normal β-cell mass value and a local and global stability analysis of the steady-state solution, we find that the fast subsystem has a single globally attracting stable spiral point [Fig. 1(a)]. The effect of β-cell mass on the behavior of the fast subsystem is demonstrated in Fig. 1(b). For each β-cell mass value, there is a single globally attracting fixed point. This fixed point is shifted to lower glucose and higher insulin level as β increases. In summary, for any initial condition, blood glucose and insulin levels will undergo a damped oscillation in time settling down to a steady state which is dependent on the β-cell mass.

To study the slow subsystem, we assume that the fast subsystem is at a steady state and changes in the β-cell mass slowly shift that steady state. The one-dimensional slow subsystem (12), has three steady-state solutions, which we index by $\beta = 0$, $G = 100$, and $G = 250$. These three steady states will be referred to as the pathological, physiological, and unstable steady states, respectively.

The behavior of the slow subsystem is best described using a plot of the β-cell replication and death rates, eqns (10) and (11), as functions of glucose levels as shown in Fig. 2. This graph can be divided into three zones of behavior. In Zone I (hypoglycemia), death rates are greater than replication rates and the β-cell mass decreases which causes glucose levels to rise. Thus, glucose is regulated back to the physiological fixed point. In Zone II (mild hyperglycemia), replication rates are greater than death rates, and the β-cell mass increases, causing glucose levels to decrease. Thus, glucose is again regulated back to the physiological steady state. Taken together, Zones I and II constitute a basin of regulation to the physiological steady state. However, in Zone III (extreme hyperglycemia), death rates exceed

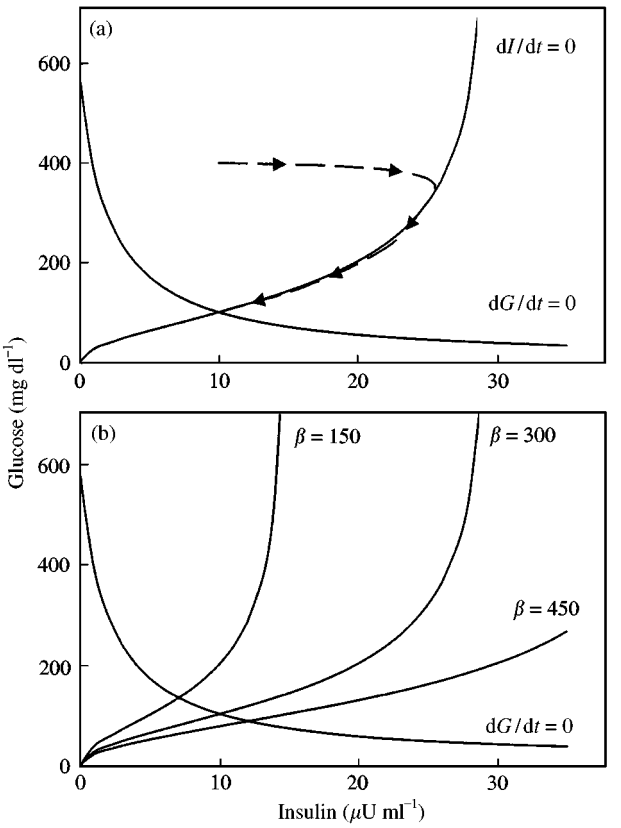

FIG. 1. Global behavior of the fast subsystem. (a) β-cell mass is fixed at 300 mg dl^{-1}. Shown are the glucose nullcline, the insulin nullcline, and a simulation of the response of the fast subsystem to a bolus injection of glucose (- - - -). Stability analysis shows that the fixed point, located at the intersection of the glucose and insulin nullclines, is a globally attractive stable spiral. (b) The glucose nullcline and three insulin nullclines are shown for $\beta = 150$, 300, and 450. As the β-cell mass increases, the fixed point of the fast subsystem moves to lower glucose and higher insulin levels, but the global behavior remains unchanged.

replication rates, thereby driving glucose levels even higher. This positive feedback loop drives the system to the pathological steady state corresponding to zero β-cell mass and extreme hyperglycemia. For this reason, Zone III is referred to as a basin of dysregulation or a basin of regulation to a pathological state. In summary, the slow subsystem has two stable steady states and an unstable steady state that divides the basins of attraction for the two stable points.

Analysing the system as a whole, the βIG model has three steady-state solutions in (β, I, G): a stable spiral (300, 10, 100), a saddle point (37, 2.8, 250), and a stable node (0, 0, 600). These points correspond to the physiological fixed

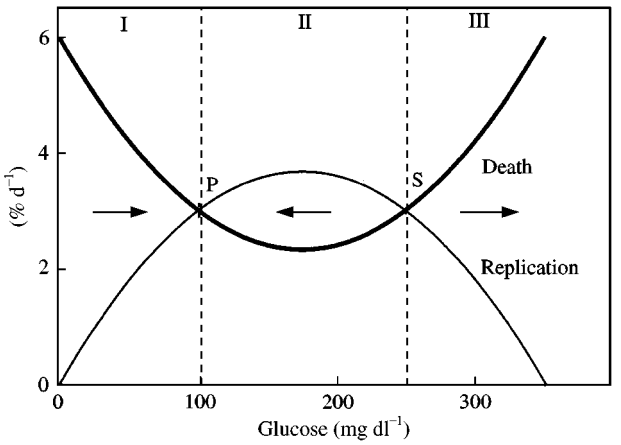

FIG. 2. Global behavior of the slow subsystem. Replication and death rates are plotted against blood glucose levels. These curves intersect at two non-trivial steady states, a physiological fixed point (P) and a saddle point (S). In addition, there is a pathological, trivial steady state at $\beta = 0$. In Zone I, death rates exceed replication rates, driving β-cell mass down and causing the steady-state glucose level to rise. In Zone II, replication rates exceed death rates driving β-cell mass up and the blood glucose level down. Zones I and II constitute a basin of attraction for the physiological fixed point (P). In Zone III, death exceeds replication, causing a decrease in β-cell mass and driving the glucose level even higher. This is a zone of pathological regulation, in which the system is driven to the trivial steady state of zero β-cell mass.

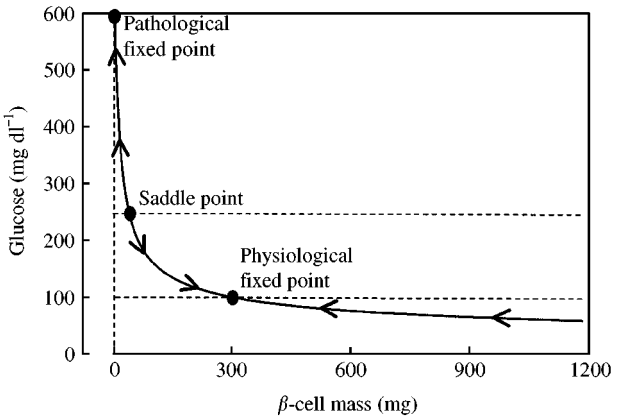

FIG. 3. Global behavior of the βIG model. Plotted here is a projection of the slow manifold and the β-cell mass null surfaces onto the G and β plane. In terms of the variables (β, I, G), the system has three steady states: physiological (300, 10, 100) and pathological (0, 0, 600) fixed points, and a saddle point at (37, 2.8, 250). All solutions travel on trajectories approximately perpendicular to the β-cell mass axis onto the slow manifold, then move along the slow manifold according to β-cell mass dynamics. The derivative $d\beta/dt$ is negative for all points below the $G = 100$ null surface, and above the $G = 250$ null surface and it is positive between the non-trivial β-cell mass null surfaces. The saddle point divides the slow manifold into two basins of attraction, one for each of the physiological and pathological fixed points: (——) slow manifold; (- - - -) β null surface.

point, the saddle point, and the pathological fixed point of the slow subsystem, respectively. The two-dimensional glucose and insulin null surfaces are attractive and their intersection forms a one-dimensional slow manifold. All solutions move quickly along trajectories which are approximately perpendicular to the β-cell mass axis onto the slow manifold, and then move along this slow manifold according to the β-cell mass dynamics. There are three β-cell mass null surfaces, namely, the planes defined by $G = 100$, $250\,\mathrm{mg\,dl^{-1}}$, and $\beta = 0$ mg. A projection of the slow manifold and the β-cell mass nullsurfaces onto the G and β plane is given in Fig. 3. For all points above $G = 250\,\mathrm{mg\,dl^{-1}}$ and below $G = 100\,\mathrm{mg\,dl^{-1}}$, the β-cell mass is decreasing and for all points between these planes, the mass is increasing. The steady-state saddle point solution divides this slow manifold into two parts. In the upper part, β is decreasing and all solutions move towards the pathological fixed point, whereas in the lower part the β-cell mass dynamics drive all solutions towards the physiological fixed point (Fig. 3). Due to the fast glucose and insulin dynamics, the global separatrix can be approximated by a plane that is perpendicular to the β-cell axis and passes through the saddle point. This plane then divides the phase space into two basins of attraction, one for the physiological fixed point and one for the pathological fixed point. The fast subsystem solution quickly comes to a steady state at the initial β-cell mass, then this steady-state solution is driven by the β-cell mass dynamics to either the physiological or pathological steady state.

The qualitative behavior of this model is consistent with observed behavior of the glucose regulatory system in response to changes in glucose concentration or β-cell mass. Increased plasma glucose, via an intravenous glucose injection, causes a rapid increase in plasma insulin followed by damped oscillations of both variables towards the pre-injection steady state (Bergman *et al.*, 1979). Reduction of the β-cell mass, via administration of the β-cell toxin streptozotocin (STZ), generates transient

hyperglycemia while the β-cell mass adapts (Ferrand *et al.*, 1995). Mild hyperglycemia, generated and maintained by a constant glucose infusion rate, also causes an increase in β-cell mass and plasma insulin that drive plasma glucose back towards pre-infusion levels (Bonner-Weir *et al.*, 1989). However, when glucose levels are maintained at or above 250 mg dl^{-1} via a variable glucose infusion rate, the plasma insulin, as well as the rate of glucose infusion required to maintain this severe hyperglycemia, decrease over time (Imamura *et al.*, 1988). All of these observations are consistent with the behavior of the βIG model.

Effects of Parameter Changes on Global Behavior

The number of non-trivial steady-state solutions is determined by the parameters of the β-cell mass equation, while the position of these fixed points is determined by all nine-dimensional parameters. The trivial steady-state solution is fixed at zero β-cell mass and insulin, but has a steady-state glucose level that is dependent on the glucose parameters (R_0, E_{G0}).

DEFECTS IN GLUCOSE DYNAMICS

Defects in insulin sensitivity, represented by a reduced S_I, decrease the curvature of the glucose nullcline and thus the slow manifold. This forces the physiological and saddle points to shift to higher β-cell mass and insulin levels, but does not affect the number of fixed points or the glucose levels at any of these fixed points. Thus, the long-term effects of non-progressive insulin resistance are hyperinsulinemia, β-cell hyperplasia, and normoglycemia. For comparison, experimental reduction of insulin sensitivity via dexamethasone causes an increase in blood insulin and β-cell mass levels (Ogawa *et al.*, 1992). Defects in glucose dynamics represented by an increase in R_0 or a decrease in E_{G0} cause the glucose nullsurface and the associated slow manifold to shift upwards. There are also shifts of the physiological and saddle fixed points to higher β-cell mass and insulin levels, as well as a shift of the pathological fixed point to a higher glucose level. Experimental augmentation of R_0, via continuous glucose infusion, has been shown to increase β-cell mass and plasma insulin levels (Bonner-Weir *et al.*, 1989; Leahy & Weir, 1988). In summary, glucose dynamics have no effect on the model's physiologically regulated glucose level, but do affect the β-cell mass and insulin levels required to maintain normal fasting glucose levels as is consistent with the experimental data.

DEFECTS IN INSULIN DYNAMICS

An increase in the insulin clearance rate or a decrease in the insulin secretion rate per active β cell, modeled by an increase in k or a decrease in σ, respectively, compresses the insulin null surface along the direction of the insulin axis. This changes the curvature of the slow manifold, thereby shifting the physiological and saddle points to higher β-cell mass values. This is similar to the observations of Curry *et al.* (1984), who have shown that the maximal insulin secretion rate per unit of β-cell mass decreases and β-cell mass increases with age in non-diabetic Fischer 344 rats. Defects in the recruitment of β cells from an inactive to an active state represented by an increase in α, stretch the insulin nullsurface along the direction of the glucose axis, changing the curvature of the slow manifold. This also shifts the physiological and saddle points to higher β-cell mass values. It is not known whether α, specifically, can be manipulated. In summary, insulin dynamics do not affect the glucose or insulin levels at the physiological fixed point, but do affect the β-cell mass required to maintain normal fasting glucose and insulin levels. These predictions are consistent with experimental data.

DEFECTS IN β-CELL MASS DYNAMICS

Defects in β-cell replication and/or death rates, represented by an increase in d_0 or r_2, or by a decrease in r_1, cause the non-trivial β-cell mass null surfaces ($G = 100$ and 250 mg dl^{-1}) to move closer together along the direction of the glucose axis. The physiological fixed point shifts to a point with higher glucose, lower insulin, and lower β-cell mass, while the saddle point shifts to a point with lower glucose, higher insulin, and higher β-cell mass, increasing the volume of the basin of pathological attraction. If a defect in the β-cell mass parameters progresses to the point

where death rates exceed replication rates at all glucose levels, then there is only one steady-state solution, namely the globally attractive pathological steady state with zero β-cell mass. Although the relationship between plasma glucose levels and mild defects in β-cell replication and/or death rates has not been determined *in vivo*, a large increase in the β-cell death rate has been shown to drive β-cell mass towards zero and generate extreme hyperglycemia (O'Brien *et al.*, 1996). In summary, the β-cell mass parameters determine the number of fixed points, as well as the glucose levels at the physiological and saddle points, when they exist.

Pathways into Diabetes

If we define diabetes as persistent hyperglycemia, then there are three general pathways into diabetes according to the βIG model: (1) move the physiological fixed point to a hyperglycemic level, (2) eliminate the physiological and saddle points, and (3) drive a trajectory across the separatrix. These pathways into diabetes will be referred to as regulated hyperglycemia, bifurcation, and dynamical hyperglycemia (or catch and pass), respectively.

REGULATED HYPERGLYCEMIA

Defects in β-cell mass regulation can shift the physiological fixed point to a hyperglycemic level. Since steady-state glucose levels are determined by β-cell mass dynamics, there are two possible ways to shift the physiological fixed point to a hyperglycemic level: (1) a defect in β-cell mass regulation, or (2) a loss of β-cell mass regulation combined with a defect in glucose and/or insulin dynamics. Small defects in any of the β-cell mass parameters, for example a decrease in the response of replication and/or an increase in the response of death rates to glucose, cause the physiological fixed point to shift to a hyperglycemic level [Fig. 4(a)]. The β-cell mass is responsive to changes in plasma glucose concentrations and has a basin of physiological attraction, but the physiologically regulated glucose level is now hyperglycemic. Bernard *et al.* (1999) showed that rats made hyperglycemic by STZ have β cells capable of increasing replication rates and decreasing death rates in response to glucose infusion; yet in the absence of glucose infusion, the rats remained hyperglycemic.

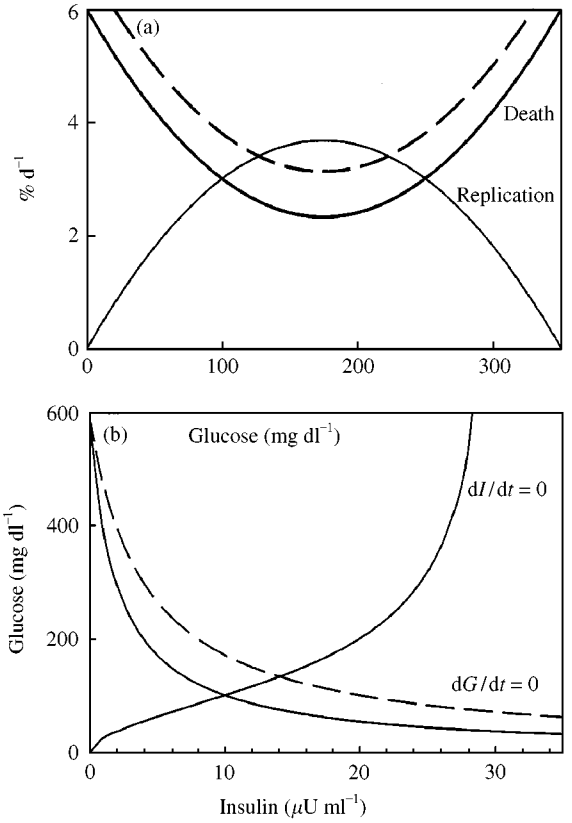

FIG. 4. Regulated hyperglycemia. There are two distinct ways of generating regulated hyperglycemia: (1) a mild β-cell mass defect, and (2) a loss of β-cell mass regulation (dβ/dt = 0 for all glucose levels) coupled to a defect in glucose and/or insulin dynamics. (a) A small upward shift of the death rate curve (- - - -) moves the physiological fixed point to a higher glucose level and shrinks the basin of physiological attraction. (b) A reduction in insulin sensitivity changes the curvature of the glucose nullcline (- - - -) and shifts the steady state of the fast subsystem to a hyperglycemic level.

A second way to shift the physiological fixed point to a hyperglycemic level is to couple a loss of β-cell mass regulation to an abnormality in glucose and/or insulin dynamics. Setting each of the β-cell mass parameters to zero yields equal replication and death rates at all glucose levels, and as a result, the β-cell mass becomes nonresponsive. In this situation, the βIG model reduces to the two-dimensional fast subsystem, and defects in any of the glucose or insulin parameters can generate hyperglycemia. Intuitively, this pathway fits the human autopsy data of Kloppel *et al.* (1985). Pancreas from obese non-diabetic

subjects had a larger than normal β-cell mass, while obese people with diabetes had normal β-cell mass levels. In the latter subjects, a coupling of insulin resistance, due to obesity, to a non-adaptive β-cell mass may have been the cause of their diabetes. Figure 4(b) shows the effects of reduced insulin sensitivity on the fasting blood glucose and insulin levels assuming a non-adaptive β-cell mass.

BIFURCATION

The bifurcation pathway consists of any combination of parameter changes that result in the elimination of the physiological and saddle fixed points. Large defects in β-cell mass dynamics cause a saddle-node bifurcation, resulting in the single globally attractive pathological fixed point at zero β-cell mass. This occurs when death rates exceed replication rates at all glucose levels (Fig. 5). A bifurcation diagram is shown in Fig. 6. The dashed line in Fig. 6 represents the saddle point while the solid lines represent the two stable fixed points. As r_1 decreases to r_{1c}, the saddle and physiological points move closer together, eventually meeting at a saddle–node bifurcation; so for values of $r_1 < r_{1c}$, there only exists the pathological fixed point. At $r_1 = 0.0015\ \mathrm{dl\,mg^{-1}}$, a transcritical bifurcation occurs, involving the saddle point and the pathological fixed point. For larger r_1, the basin of physiological attraction grows to encompass all physiological initial conditions, while the basin of pathological attraction encompasses initial conditions with negative insulin and β-cell mass. Similar bifurcation diagrams can be generated for r_2 and d_0. The saddle–node bifurcation pathway outlined above resembles type 1 diabetes mellitus. An autoimmune attack on the pancreatic β cells increases death rates above replication rates for all glucose levels, and the β-cell mass falls towards zero (O'Brien *et al.*, 1996).

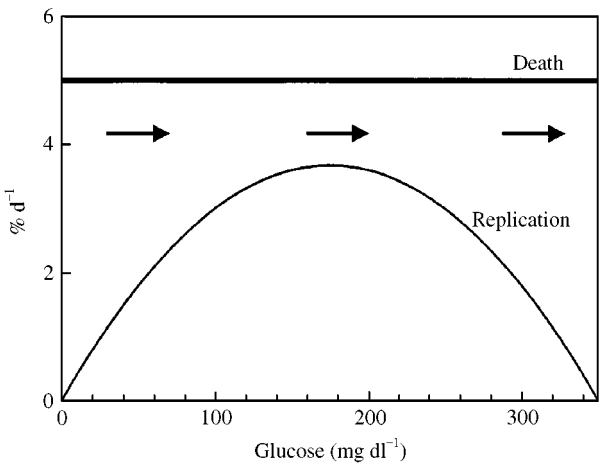

FIG. 5. Bifurcation. Large changes in the replication and/or death curves results in a situation where the death rate exceeds the replication rate for all glucose levels. Here the physiological and saddle points are eliminated. As the β-cell mass falls, insulin levels fall, and glucose levels are driven up. Under these conditions the system has a single globally attractive pathological fixed point at zero β-cell mass.

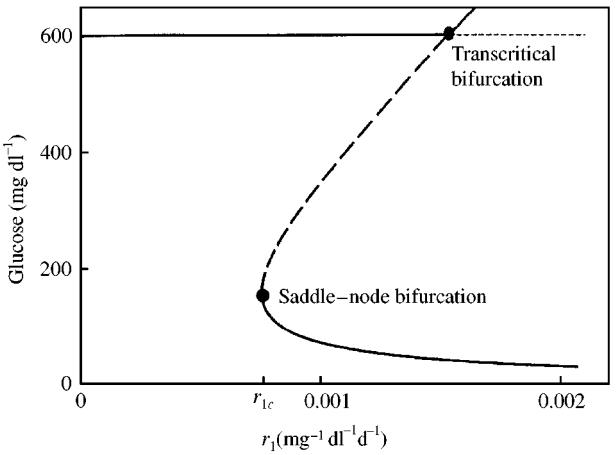

FIG. 6. Bifurcation diagram. The glucose levels of the stable fixed points (——) and the saddle point (- - - -) are plotted as functions of the parameter r_1. Keeping all other parameters fixed, a saddle–node bifurcation occurs at the critical value $r_{1C} = 0.00076$. The βIG model has a pathological fixed point for $r_1 < r_{1C}$ and three fixed points (a physiological stable spiral, a saddle, and a pathological stable node) for $r_1 > r_{1C}$. A transcritical bifurcation occurs at $r_1 = 0.0015$. To the right of this point, the fixed point with a glucose level greater than $600\ \mathrm{mg\,dl^{-1}}$ is not physiologically interesting as it has negative β-cell mass.

DYNAMICAL HYPERGLYCEMIA

Dynamical hyperglycemia, also referred to as the "catch and pass" pathway, is a race between the actual β-cell mass and the location of the β-cell mass required to maintain normal glucose levels as parameters change. Defects in glucose and/or insulin dynamics increase the β-cell mass required to maintain the glucose level at the physiological fixed point. A defect manifests itself as a change in one of these parameters which

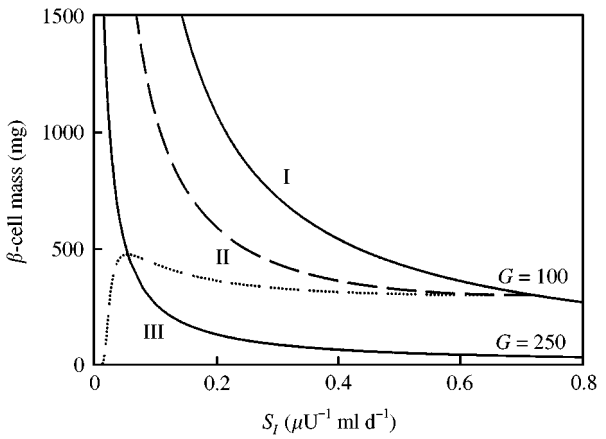

FIG. 7. Dynamical hyperglycemia. The (——) represent the β-cell mass of the physiological (upper) and saddle fixed points (lower) as functions of insulin sensitivity, S_I. The derivative $d\beta/dt$ is negative in Zone I (above the upper curve), and III (below the lower curve), but is positive in Zone II (between the solid curves). If S_I is reduced slowly ($c = 0.01$), then the β-cell mass can adapt and maintain mild hyperglycemia (- - - -). However, if S_I is reduced too quickly ($c = 0.05$), then the β-cell mass adapts for some period of time, but eventually glucose levels are driven above 250 mg dl^{-1} and the β-cell mass begins to decrease (·····).

generates a transient state where the β-cell mass is unable to secrete enough insulin to maintain normal blood glucose levels. Hyperglycemia persists while the β-cell mass adapts. A time-dependent shift in one or more of these parameters results in a race between a progressive defect, which drives glucose levels up, and β-cell mass adaptation, which drives glucose levels down.

Figure 7 shows the β-cell mass at the physiological and saddle points (solid curves) as a function of insulin sensitivity. In zone I, above the curve of physiological fixed points, glucose levels are below 100 mg dl^{-1} and the β-cell mass is decreasing. In zone II, between the solid curves, glucose levels range from 100 to 250 mg dl^{-1} and the β-cell mass increases. The points in zones I and II constitute the basin of physiological attraction. In zone III, below the lower solid curve, glucose levels are above 250 mg dl^{-1} and the β-cell mass decreases. The points below the lower solid curve constitute the basin of pathological attraction. Note that at large values of S_I, large changes in insulin sensitivity can be offset by relatively small changes in β-cell mass. However, at small values of S_I a dramatic change in β-cell mass is needed to balance any change in S_I.

We now investigate the adaptation of the β-cell mass in response to a continuous change in S_I, which is modeled by

$$dS_I/dt = -cS_I, \tag{13}$$

where c is the rate constant (d^{-1}). Two trajectories are superimposed on Fig. 7: normal adaptation ($c = 0.01$, dashed curve) and the catch and pass pathway ($c = 0.05$, dotted curve). Note that both trajectories start at the physiological steady state of $G = 100$ mg dl^{-1} for $S_I = 0.72\ \mu U^{-1}$ ml d^{-1} and that simulations are run for a period of 120 days. In the first case ($c = 0.01$), the actual β-cell mass adapts at roughly the same rate as the increase in β-cell mass of the physiological fixed point. Thus, the trajectory remains in Zone II, close to the curve of physiological fixed points and mild hyperglycemia is maintained throughout the simulation via increases in β-cell mass and blood insulin levels. In the case of the catch and pass pathway, however, the decrease in S_I is too fast. The β-cell mass and blood insulin levels for this trajectory increase too slowly, so that the trajectory moves away from the curve of physiological fixed points and towards the curve of saddle points. Eventually, the trajectory crosses the curve of saddle points into Zone III, where the β-cell mass and blood insulin levels begin to decrease, accelerating the rise in blood glucose levels. Note that the catch and pass pathway also can be realized with a slowly decreasing S_I coupled with a defect in β-cell mass dynamics. The insulin dynamics predicted by the catch and pass scenario, a period of increasing insulin level followed by a period of decreasing insulin level, is similar to that observed in the Zucker Diabetic Fatty (ZDF) rat (Cockburn *et al.*, 1997) and in cross-sectional human data (DeFronzo *et al.*, 1992). However, it is likely that the insulin dynamics observed *in vivo* also are influenced by adaptation and/or failure of β-cell secretion rates as well as changes in insulin clearance rates that are not accounted for in our model.

Discussion

In this paper, we present a novel model of coupled β-cell mass, insulin, and glucose

dynamics that we use to investigate the normal behavior of the glucose regulatory system and pathways into diabetes. The model predicts a physiological steady state with a large basin of attraction. Blood glucose levels greater than $250\,mg\,dl^{-1}$ cause β-cell death rates to exceed replication rates, driving the system towards a pathological steady state. The behavior of the model is consistent with the observed behavior of the glucose regulatory system in response to changes in blood glucose levels (Bergman *et al*., 1979; Bonner-Wier *et al*., 1989; Imamura *et al*., 1988), β-cell mass (Ferrand *et al*., 1995), insulin sensitivity (Ogawa *et al*., 1992), and β-cell insulin secretion rates (Curry *et al*., 1984). Furthermore, the model predicts three distinct pathways into diabetes: regulated hyperglycemia, bifurcation, and dynamical hyperglycemia (catch and pass). Although it is difficult to quantify the extent to which these pathways correspond to the behavior of the real system, the regulated hyperglycemia pathway seems consistent with STZ-induced diabetes (Bernard *et al*., 1999), and the bifurcation pathway has strong parallels with the pathogenesis of type 1 diabetes mellitus (O'Brien *et al*., 1996). The catch and pass pathway provides a novel theoretical explanation for observed, horseshoe-shaped insulin dynamics in longitudinal studies of the ZDF rat (Cockburn *et al*., 1997) and in cross-sectional human studies (DeFronzo *et al*., 1992).

The idea that "glucose toxicity" or some other factor leads to β-cell "exhaustion" has been around for many years (Unger & Grundy, 1985). We suggest that nonlinear β-cell mass dynamics can explain the adaptation of plasma insulin levels to insulin resistance whereas the failure of this adaptation is followed by, hyperglycemia, and reduced plasma insulin levels. The βIG model predicts that blood glucose levels are determined by the rate of change of insulin demand, compared to the rate of adaptation of the β-cell mass. Moderate levels of hyperglycemia (below the saddle point) leads to a negative feedback and adaptation of the β-cell mass. At higher plasma glucose levels (above the saddle point), adaptation fails, and positive feedback leads to a decrease in the β-cell mass, further increasing the glucose level. The βIG model provides (1) a framework for developing experimental protocols to test specific hypotheses regarding β-cell exhaustion and the pathogenesis of type 2 diabetes, and (2) a tool for investigating the behavior of β-cell mass, plasma insulin, and plasma glucose, in response to therapeutic interventions.

The equation describing β-cell mass dynamics is based on a limited number of *in vitro* experiments, which support the hypothesis that β-cell replication and apoptosis rates are nonlinear with respect to blood glucose levels. Other endogenous signals may be important in control of β-cell mass dynamics, but it remains to be determined whether these signals act independently or whether they modify the relationship between plasma glucose and β-cell turnover (Bonner-Weir, 2000). Clearly, additional experimental work is needed to refine the model further, but the fundamental relationship between β-cell mass dynamics and the plasma glucose level will probably remain.

The model does not incorporate all known physiological effects, for example, the effects of hyperglycemia on neogenesis (Bernard *et al*., 1999), insulin sensitivity (Rossetti, 1995), insulin secretion rates (Sreenan *et al*., 1998), and β-cell heterogeneity (Ling & Pipeleers, 1996). The model also does not incorporate the effects of insulin and incretin hormones on β-cell mass dynamics (Schuppin *et al*., 1998; Xu *et al*., 1999) and the possible adaptation of insulin clearance rates to insulin resistance and elevations in free fatty acids (Haffner *et al*., 1992; Wiesenthal *et al*., 1999). Although these effects have been demonstrated experimentally, they have not been sufficiently quantified experimentally to incorporate them into the βIG model without speculations on the rates and magnitudes of these events. Moreover, preliminary analyses and simulations of some speculative models (data not shown) indicate that these physiological responses do affect the quantitative behavior of the solutions, but do not affect their qualitative behavior or the predicted pathways into diabetes.

Experimental studies have indicated a potentially important role of lipid metabolism in blood glucose regulation and the pathogenesis of type 2 diabetes (Unger, 1995). Insulin dynamics are an important component of lipid metabolism (Despres, 1994), and plasma fatty acid levels have been linked to insulin resistance (Belfiore &

Iannello, 1998), insulin secretion defects (Carpentier *et al*., 1999), and abnormal β-cell mass dynamics (Shimabukuro *et al*., 1998). The incorporation of lipid dynamics into the βIG model may affect the three pathways into diabetes or reveal additional pathways not evident with the present form of the βIG model.

Finally, the βIG model may provide the theoretical basis for an indirect measurement of β-cell mass *in vivo*. Present techniques for measuring β-cell mass are time consuming and require removal of the pancreas. However, $\sigma\beta$, from eqn (12), can be calculated from *in vivo* glucose clamp data, and σ can be measured *in vitro*. Thus, utilizing the *in vitro* population mean value for σ, or developing a means of measuring σ *in vivo*, would permit the estimation of β-cell mass *in vivo*. This would eliminate the need for removing the pancreas, allow for longitudinal studies of β-cell mass dynamics, and facilitate the study of β-cell mass dynamics in humans.

B. Topp was supported by a grant from the Mathematics of Information Technology and Complex Systems Network of Centres of Excellence and SmithKline Beecham Pharmaceuticals. This work also was supported by a grant from the Medical Research Council of Canada (MT 10574) to D. T. Finegood. G. de Vries, R. M. Miura, and K. Promislow are funded by the individual research grants from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

Allsop, J. R., Wolfe, R. R. & Burke, J. F. (1978). The reliability of rates of glucose appearance *in vivo* calculated from constant tracer infusions. *Biochem. J*. **172,** 407–416.

Ashcroft, F. M., Harrison, D. E. & Ashcroft, S. J. (1984). Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. *Nature* **312,** 446–448.

Belfiore, F. & Iannello, S. (1998). Insulin resistance in obesity: metabolic mechanisms and measurment methods. *Mol. Genet. Metab*. **65,** 121–128.

Bergman, R. N., Ider, Y. Z., Bowden, C. R. & Cobelli, C. (1979). Quantitative estimation of insulin sensitivity. *Am. J. Physiol*. **236,** E667–E677.

Bergman, R. N., Phillips, L. S. & Cobelli, C. (1981). Physiologic evaluation of factors controlling glucose tolerance in man. Measurement of insulin sensitivity and β-cell glucose sensitivity from the response to intravenous glucose. *J. Clin. Invest*. **68,** 1456–1467.

Bergman, R. N., Finegood, D. T. & Ader, M. (1985). Assessment of insulin sensitivity *in vivo*. *Endocrine Rev*. **6,** 45–58.

Bernard, C., Berthault, M. F., Saulnier, C. & Ktorza, A. (1999). Neogenesis vs. apoptosis as main components of pancreatic beta-cell mass changes in glucose-infused normal and mildly diabetic adult rats. *FASEB J*. **13,** 1195–1205.

Berzins, R., Tam, Y. K., Molnar, G. D., Rajotte, R. V., Wieczorek, K. R., McGregor, J. R. & Fawcett, D. M. (1986). Pharmacokinetic approach to the estimation of hepatic removal of insulin. *Pancreas* **1,** 544–549.

Best, J. D., Taborsky Jr, J., Halter, J. B. & Port Jr, D. (1981). Glucose disposal is not proportional to plasma glucose level in man. *Diabetes* **30,** 847–850.

Bonner-Weir, S. (2000). Perspective: Postnatal pancreatic β-cell growth. *Endocrinology* **141,** 1926–1929.

Bonner-Weir, S., Deery, D., Leahy, J. L. & Weir, G. C. (1989). Compensatory growth of pancreatic β-cells in adult rats after short-term glucose infusion. *Diabetes* **38,** 49–53.

Bonner-Weir, S., Baxter, L. A., Schuppin, G. T. & Smith, F. E. (1993). A second pathway for regeneration of adult exocrine and endocrine pancreas: a possible recapitulation of embryonic development. *Diabetes* **42,** 1715–1720.

Bosco, D. & Meda, P. (1991). Actively synthesizing β-cells secrete preferentially after glucose stimulation. *Endocrinology* **129,** 3157–3166.

Carpentier, A., Mittelman, S. D., Lamarche, B., Bergman, R. N., Giacca, A. & Lewis, G. F. (1999). Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. *Am. J. Physiol*. **276,** E1055–E1066.

Cerasi, E. (1995). Insulin deficiency and insulin resistance in the pathogenesis of NIDDM: is a divorce possible? *Diabetologia* **38,** 992–997.

Cobelli, C. & Pacini, G. (1988). Insulin secretion and hepatic extraction in humans by minimal modeling of C-peptide and insulin kinetics. *Diabetes* **37,** 223–231.

Cobelli, C., Pacini, G. & Salvan, A. (1980). On a simple model of insulin secretion. *Med. Biol. Eng. Comput*. **18,** 457–463.

Cobelli, C., Saccomani, M. P., Ferrannini, E., DeFronzo, R. A., Gelfand, R. & Bonadonna, R. (1989). A compartmental model to quantitate *in vivo* glucose transport in the human forearm. *Am. J. Physiol*. **257,** E943–E958.

Cockburn, B. N., Ostrega, D. M., Sturis, J., Kubstrup, C., Polonsky, K. S. & Bell, G. I. (1997). Changes in pancreatic islet glucokinase and hexokinase activities with increasing age, obesity, and the onset of diabetes. *Diabetes* **46,** 1434–1439.

Curry, D. L., Reaven, G. & Reaven, E. (1984). Glucose-induced insulin secretion by perfused pancreas of 2- and 12-mo-old Fischer 344 rats. *Am. J. Phsiol*. **247,** E385–E388.

DeFronzo, R. A., Bonadonna, R. C. & Ferrannini, E. (1992). Pathogenesis of NIDDM. A balanced overview. *Diabetes Care* **15,** 318–368.

Despres, J. P. (1994). Dyslipidaemia and obesity. *Baillieres Clin. Endocrinol. Metab*. **8,** 629–660.

Efanova, I. B., Zaitsev, S. V., Zhivotovsky, B., Kohler, M., Efendic, S., Orrenius, S. & Berggren, P. O. (1998). Glucose and tolbutamide induce apoptosis in pancreatic β-cells. *J. Biol. Chem*. **273,** 33501–33507.

FERNANDES, A., KING, L. C., GUZ, Y., STEIN, R., WRIGHT, V. E. & TEILELMAN, G. (1997). Differentiation of new insulin-producing cells is induced by injury in adult pancreatic islets. *Endocrinology* **138, 1**750–1762.

FERRAND, N., ASTESANO, A., PHAN, H. H., LELONG, C. & ROSSELIN, G. (1995). Dynamics of pancreatic cell growth and differentiation during diabetes reversion in STZ-treated new born rats. *Am. J. Physiol.* **269,** C1250–C1265.

FINEGOOD, D. T. (1997). Application of the minimal model of glucose kinetics. In: *The Minimal Model Approach and Determinants of Glucose Tolerance* (Bergman, R. N. & Lovejoy, J. C., eds), pp. 51–122. Baton Rouge: Louisiana State University Press.

FINEGOOD, D. T., BERGMAN, R. N. & VRANIC, M. (1987). Estimation of endogenous glucose production during hyperinsulinemic–euglycemic glucose clamps. Comparison of unlabeled and labeled exogenous glucose infusates. *Diabetes* **36,** 914–924.

FINEGOOD, D. T., SCAGLIA, L. & BONNER-WEIR, S. (1995). Dynamics of β-cell mass in the growing rat pancreas estimation with a simple mathematical model. *Diabetes* **44,** 249–256.

HAFFNER, S. M., STERN, M. P., WATANABE, R. M. & BERGMAN, R. N. (1992). Relationship of insulin clearance and secretion to insulin sensitivity in non-diabetic Mexican Americans. *Eur. J. Clin. Invest.* **22,** 147–153.

HOORENS, A., VAN DE CASTEELE, M., KLOPPEL, G. & PIPELEERS, D. (1996). Glucose promotes survival of rat pancreatic β-cells by activating synthesis of proteins which suppress a constitutive apoptotic program. *J. Clin. Invest.* **98,** 1568–1574.

HUGL, S. R., WHITE, M. F. & RHODES, C. J. (1998). Insulin-like growth factor I (IGF-I)-stimulated pancreatic β-cell growth is glucose-dependent. *J. Biol. Chem.* **273,** 17771–17779.

IMAMURA, T., KOFFLER, M., HELDERMAN, J. H., PRINCE, D., THIRLBY, R., INMAN, L. & UNGER, R. H. (1988). Severe diabetes induced in subtotally depancreatized dogs by sustained hyperglycemia. *Diabetes* **37,** 600–609.

KAIYALA, K. J., PRIGEON, R. L., KAHN, S. E., WOODS, S. C., PORTE JR, D. & SCHWARTZ, M. W. (1999). Reduced β-cell function contributes to impaired glucose tolerance in dogs made obese by high-fat feeding. *Am. J. Physiol.* **277,** E659–E667.

KLOPPEL, G., LOHR, M., HABICH, K., OBERHOLZER, M. & HEITZ, P. U. (1985). Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. *Surv. Synth. Path. Res.* **4,** 110–125.

LEAHY, J. L. (1990). Natural history of β-cell dysfunction in NIDDM. *Diabetes Care* **13,** 992–1010.

LEAHY, J. L. (1993). Increased proinsulin/insulin ratio in pancreas extracts of hyperglycmic rats. *Diabetes* **42,** 22–27.

LEAHY, J. L. & WEIR, G. C. (1988). Evolution of abnormal insulin secretory responses during 48-h in vivo hyperglycemia. *Diabetes* **37,** 217–222.

LING, Z. & PIPELEERS, D. G. (1996). Prolonged exposure of human β cells to elevated glucose levels results in sustained cellular activation leading to a loss of glucose regulation. *J. Clin. Invest.* **98,** 2805–2812.

MALAISSE, W., MALAISSE-LAGAE, F. & WRIGHT, P. H. (1967). A new method for the measurement *in vitro* of pancreatic insulin secretion. *Endocrinology* **80,** 99–108.

MATTHEWS, D. R., HOSKER, J. P., RUDENSKI, A. S., NAYLOR, B. A., TREACHER, D. T. & TURNER, R. C. (1985). Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28,** 412–419.

O'BRIEN, B. A., HARMON, B. V., CAMERON, D. P. & ALLAN, D. J. (1996). Beta-cell apoptosis is responsible for the development of IDDM in the multiple low-dose streptozotocin model. *J. Pathol.* **178,** 176–181.

OGAWA, A., JOHNSON, J. H., OHNEDA, M., MCALLISTER, C. T., INMAN, L., ALAM, T. & UNGER, R. H. (1992). Roles of insulin resistance and β-cell dysfunction in dexamethasone-induced diabetes. *J. Clin. Invest.* **90,** 497–504.

ROSSETTI, L. (1995). Glucose toxicity: the implications of hyperglycemia in the pathophysiology of diabetes mellitus. *Clin. Invest. Med.* **18,** 255–260.

RUDENSKI, A. S., MATTHEWS, D. R., LEVY, J. C. & TURNER, R. C. (1991). Understanding "insulin resistance": both glucose resistance and insulin resistance are required to model human diabetes. *Metabolism* **40,** 908–917.

SALOMON, D. & MEDA, P. (1986). Heterogeneity and contact-dependent regulation of hormone secretion by individual β-cells. *Exp. Cell Res.* **162,** 507–520.

SCHUIT, F. C., VELD, P. A. & PIPELEERS, D. G. (1988). Glucose stimulates proinsulin biosynthesis by a dose-dependent recruitment of pancreatic β-cells. *Proc. Natl. Acad. Sci. U.S.A.* **85,** 3865–3869.

SCHUPPIN, G. T., PONS, S., HUGL, S., AIELLO, L. P., KING, G. L., WHITE, M. & RHODES, C. J. (1998). A specific increased expression of insulin receptor substrate 2 in pancreatic beta-cell lines is involved in mediating serum-stimulated beta-cell growth. *Diabetes* **47,** 1074–1085.

SHIMABUKURO, M., ZHOU, Y. T., LEVI, M. & UNGER, R. (1998). Fatty acid-induced β-cell apoptosis: a link between obesity and diabetes. *Proc. Natl. Acad. Sci. U.S.A.* **95,** 2498–2502.

SREENAN, S. K., COCKBURN, B. N., BALDWIN, A. C., OSTREGA, D. M., LEVISETTI, M., GRUPE, A., BELL, G. I., STEWART, T. T., ROE, M. W. & POLONSKY, K. S. (1998). Adaptation to hyperglycemia enhances insulin secretion in glucokinase mutant mice. *Diabetes* **47,** 1881–1888.

STEELE, R. (1959). Influence of glucose loading and of injected insulin on hepatic glucose output. *Ann. NY Acad. Sci.* **82,** 420–430.

SWENNE, I. (1982). The role of glucose in the *in-vitro* regulation of cell cycle kinetics and proliferation of fetal pancreatic β-cells. *Diabetes* **31,** 754–760.

THE EXPERT COMMITTEE ON THE DIAGNOSIS AND CLASSIFICATION OF DIABETES MELLITUS (1997). Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* **20,** 1183–1197.

TOBIN, B. W., LEWIS, J. T., TOBIN, B. L., RAJOTTE, R. V. & FINEGOOD, D. T. (1992). Markedly reduced β-cell function does not result in insulin resistance in islet autografted dogs. *Diabetes* **41,** 1172–1181.

TOFFOLO, G., BERGMAN, R. N., FINEGOOD, D. T., BOWDEN, C. R. & COBELLI, C. (1980). Quantitative estimation of β-cell sensitivity to glucose in the intact organism: a minimal model of insulin kinetics in the dog. *Diabetes* **29,** 979–990.

TOKUYAMA, Y., STURIS, J., DEPAOLI, A. M., TAKEDA, J., STOFFEL, M., TANG, J., SUN, X., POLONSKY, K. S., & BELL, G. I. (1995). Evolution of beta-cell dysfunction in the male Zucker diabetic fatty rat. *Diabetes* **44,** 1447–1457.

UNGER, R. H. (1995). Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* **44,** 863–870.

UNGER, R. H. & GRUNDY, S. (1985). Hyperglycemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: implications for the management of diabetes. *Diabetologia* **28,** 119–121.

WIESENTHAL, S. R., SANDHU, H., MCCALL, R. H., TCHIPASHVILI, V., YOSHII, H., POLONSKY, K., SHI, Z. Q., LEWIS, G. F., MARI, A. & GIACCA, A. (1999). Free fatty acids impair hepatic insulin extraction in vivo. *Diabetes* **48,** 766–774.

XU, G., STOFFERS, D. A., HABENER, J. F. & BONNER-WEIR, S. (1999). Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* **48,** 2270–2276.