



Review

Mathematical modeling of the glucose–insulin system: A review

Pasquale Palumbo^a, Susanne Ditlevsen^{b,*}, Alessandro Bertuzzi^c, Andrea De Gaetano^a^a BioMatLab, IASI “A. Ruberti”, National Council of Researches, UCSC Largo A. Gemelli 8, 00168 Rome, Italy^b Department of Mathematical Sciences, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark^c IASI “A. Ruberti”, National Council of Researches, Viale Manzoni 30, 00185 Rome, Italy

ARTICLE INFO

Article history:

Received 3 December 2012

Received in revised form 10 May 2013

Accepted 16 May 2013

Available online 1 June 2013

Keywords:

Diabetes

Minimal Model

IVGTT and OGTT

Insulin secretion and oscillations

Euglycemic Hyperinsulinemic Clamp

Long-term diabetes

ABSTRACT

Mathematical modeling of the glucose–insulin feedback system is necessary to the understanding of the homeostatic control, to analyze experimental data, to identify and quantify relevant biophysical parameters, to design clinical trials and to evaluate diabetes prevention or disease modification therapies. Much work has been made over the last 30 years, and the time now seems ripe to provide a comprehensive review. The one here proposed is focused on the most important clinical/experimental tests performed to understand the mechanism of glucose homeostasis. The review proceeds from models of pancreatic insulin production, with a coarser/finer level of detail ranging over cellular and subcellular scales, to short-term organ/tissue models accounting for the intra-venous and the oral glucose tolerance tests as well as for the euglycemic hyperinsulinemic clamp, to total-body, long-term diabetes models aiming to represent disease progression in terms of β -cell population dynamics over a long period of years.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

The glucose–insulin system offers one of the clearest and simplest examples of homeostatic control in the organism. The level of glucose in blood needs to be kept within a narrow range. Since it represents the main metabolic substrate, or energy source, for brain tissue, abnormally low glucose concentrations give rise to anxiety, tremors, aggressiveness, obfuscation, coma and eventually death. On the other hand, excessive plasma glucose concentrations produce microvascular damages (notably in the retina and kidney) and neural damages, leading among others to blindness and chronic renal insufficiency. The way the body controls glycemia seems deceptively simple. Essentially a single hormone (insulin) is secreted by the β -cells of the pancreas in response to rising glucose concentrations (hyperglycemia). Insulin effects include increasing peripheral tissue glucose uptake (mainly by the muscle and fat tissues) and decreasing spontaneous glucose output by the liver. When insulin secretion by the pancreas is insufficient or absent, due to (autoimmune) destruction of β -cells, the clinical picture of Type 1 Diabetes Mellitus (T1DM) results; when insulin is secreted in normal, or supranormal amounts, but it is ineffective in lowering glycemia to normal levels, Type 2 Diabetes Mellitus (T2DM) is said to be present. A number of hormones contribute to rescuing the organism from hypoglycemia (adrenalin, glucagon, growth hormone, cortisol): however, since in clinical practice the

situation of interest is normally inappropriately high glycemia, concentrating attention on the response to hyperglycemia by insulin seems justified, at least as a first modeling approach. We may therefore consider, as a first approximation, a simplified system in which a single metabolite (glucose) is controlled by a single hormone (insulin). This system will have to maintain glycemia in the absence of food intake, and will have to suppress hyperglycemia rapidly after meals, without incurring in dangerous hypoglycemia. We see therefore that the glucose–insulin system could be viewed, at least approximately, as a feedback control with a controller (the pancreas) and multiple effectors (muscle, liver, fat tissue), but where the only state variables of interest are glycemia and insulinemia.

The present review has the goal of highlighting the biomedical problem of the glucose–insulin homeostasis from a physiological and clinical viewpoint, then describing the main combined experimental-modeling tools which are currently employed in investigating the behavior of the control system in individuals or populations. The review is structured as follows. The next section focuses on one specific biological function played by the pancreas, the organ responsible for glucose homeostasis by means of glucose-stimulated insulin production. In this case the range of the available models for insulin release spans a subcellular (models dealing with the molecular mechanisms leading to the ejection of the insulin packet from a beta-cell), and a cellular/organ level (models treating the pancreas as a population of secretory units). In these cases the loop is often (but not always) closed with the action of remote organs and tissues (explaining the occurrence of

* Corresponding author. Tel.: +45 35320785.

E-mail address: susanne@math.ku.dk (S. Ditlevsen).

sustained insulin oscillations, apparent in long-term clinical experiments). The time scale corresponding to these secretion phenomena is of milliseconds to seconds (or minutes in the case of potentiation).

Section 3 deals with glucose–insulin models aiming to describe glucose homeostasis from a top-down point of view, at the organ and tissue levels without getting too deep into the molecular/sub-cellular details (e.g. the ones related to insulin-dependent glucose uptake or to glucose-induced insulin production). These models describe the glucose/insulin dynamics from a phenomenological viewpoint, typically employ compartmental formalization and are strongly related to specific clinical protocols for which they provide information on “how good” are pancreatic insulin secretion and peripheral glucose uptake in the subject under investigation.

Finally, the last section addresses total-body models developed to offer the physician some glimpse on the possible long-term evolution of the disease Diabetes, depending on long-term (months to years) evolution of the beta-cell population and its replicating ability in the face of potential glucose toxicity. The following sections, therefore, cover multiple size and time scales, from subcellular and cellular phenomena of seconds, to organ/tissue functional interplay over minutes to hours, to cell replication and long-term total-body adaptation over months to years.

2. Insulin secretion and oscillations

Mathematical modeling of glucose-stimulated pancreatic insulin secretion is a challenging research topic that has involved much work in the last decades. Among the first references we find the pioneering work of Grodsky [37], who first distinguished a first phase of insulin release, due to the insulin packets immediately releasable as a consequence of a rapid increase in glycemia, from the second phase related to the potentiation effect that occurs during a sustained glucose stimulation. The models reported in Section 2.1 take place over a fine biological scale, detailing the subcellular mechanism (the insulin granule dynamics) that eventually determines insulin release from the β -cell. Phenomena at a subcellular scale could be invoked to explain the occurrence of sustained oscillations in pancreatic insulin release (the topic of Section 2.2): however the different kinds of oscillations may be better explained according to a coarser, larger scale population model of the secretory units, that is also able to reproduce a large collection of clinical experiments.

2.1. Insulin granule dynamics

Insulin granule trafficking in pancreatic β -cells has been investigated in recent years by using fluorescent proteins that are targeted to secretory granules and allow the real-time imaging of granules in living cells [70]. A β -cell contains 10,000–13,000 granules of diameter ~ 350 nm, each containing ~ 1.6 amol of insulin plus other polypeptides and smaller molecules. Most granules belong to a large “reserve pool”, around 600 are docked with the plasma membrane, with 50–100 granules in a pool of immediate release, and other ~ 1500 are located close to cell surface [70,30]. Granule exocytosis requires the fusion of granule membrane with plasma membrane and the formation of a pore that connects granule lumen to extracellular space. Incomplete fusion and kiss-and-run exocytosis may also occur.

As found by early observations *in vitro* and *in vivo*, stimulation by a rapid and large increase in the extracellular glucose concentration induces a biphasic time course of the insulin secretion rate (ISR) with a 5–10 min peak, the first phase, followed by a more prolonged second phase. These features were modeled by the early

mathematical models [37,16]. Recent experimental data from mouse and rat β -cells showed that only 1–2 granules/min per β -cell are released at a glucose concentration around 3 mM, and 20–30 granules/min per cell are released at the peak of the first-phase insulin secretion after a step increase in glucose, with a total secretion of about 680 granules in 50 min (for instance, see [79]).

In a first pathway of stimulus-secretion coupling (triggering pathway), the glucose stimulus increases the ATP/ADP ratio, which induces closing of the K_{ATP} channels and depolarization of cell membrane. The resulting Ca^{2+} influx through the voltage-sensitive Ca^{2+} channels raises the cytosolic free calcium concentration, $[Ca^{2+}]_c$, so promoting the exocytosis of insulin granules. Still not completely characterized are the mediators of the amplifying pathway, that augments the efficacy of Ca^{2+} in stimulating the insulin secretion [38,30,39].

2.1.1. Mathematical models

Shibasaki et al. [74] used a model based on a three-dimensional random walk process to simulate the movement of insulin granules in an idealized β -cell. The introduction of a bias, that represented the recruitment of granules to plasma membrane, produced the second-phase response. The first phase was simulated by assuming that the randomly distributed granules had an increased density near the plasma membrane, so representing a pool of immediately releasable granules. Simulation results were in agreement with the observation that cAMP promotes exocytosis by both increasing the size and accelerating the refilling of the pool of immediate release.

The model proposed in [11] defines a pool, I , of free proinsulin and a pool, V , of free granule membrane material, not yet enclosing proinsulin. Moreover, four pools of insulin granules were considered: the reserve pool (R), the docked granules (D), the immediately releasable granules (D_{IR}), and the granules fused with cell membrane (F). Model equations are as follows:

$$\frac{dI}{dt} = -kI(t)V(t) - \alpha_I I(t) + b_I, \quad (1)$$

$$\frac{dV}{dt} = -kI(t)V(t) - \alpha_V V(t) + b_V + \sigma F(t - \tau_V), \quad (2)$$

$$\frac{dR}{dt} = kI(t)V(t) - \gamma(t)R(t), \quad (3)$$

$$\frac{dD}{dt} = \gamma(t)R(t) - k_1^+[C_T - D_{IR}(t)]D(t) + k_{-1}^- D_{IR}(t), \quad (4)$$

$$\frac{dD_{IR}}{dt} = k_1^+[C_T - D_{IR}(t)]D(t) - k_{-1}^- D_{IR}(t) - \rho(t)D_{IR}(t), \quad (5)$$

$$\frac{dF}{dt} = \rho(t)D_{IR}(t) - \sigma F(t). \quad (6)$$

In (1) and (2), k is an aggregation rate constant, α_I, α_V are degradation rate constants, and b_I, b_V denote, respectively, the rate of proinsulin biosynthesis and the production rate of granule membranes. The last term in the right-hand side of (2) accounts for the contribution to granule membrane formation of the recycling of membrane material, with σ the rate constant of the fusion process and τ_V the time interval required for recycling. The variable γ in (3) represents the processes by which granules are irreversibly translocated from trans-Golgi network to plasma membrane, and it is dependent on the glucose concentration, G , according to (7). The pool D_{IR} is formed through the binding of docked granules to L-type Ca^{2+} channels, with C_T denoting the pool of total Ca^{2+} channels and k_1^+, k_{-1}^- the rate constants of association and, respectively, dissociation. The factors that promote granule fusion are represented by ρ , and $\sigma F(t)$ in (6) is the number of granules releasing insulin in the unit time at time t in a single β -cell.

The equations that relate glucose stimulus to the quantities, γ and ρ , that govern granule trafficking are as follows:

$$\frac{d\gamma}{dt} = \eta[-\gamma(t) + \gamma_b + \psi(t) + h_\gamma(G(t - \tau_G))], \quad (7)$$

$$\frac{d\rho}{dt} = \zeta[-\rho(t) + \rho_b + h_\rho(\gamma(t))], \quad (8)$$

where basal values are indicated by the subscript b , η and ζ are rate constants, and ψ represents factors that induce ATP and calcium oscillations. Glucose activation is modeled by the saturating function h_γ , with τ_G the time required by glucose metabolism. So γ regulates granule translocation and priming, and through h_ρ regulates granule fusion and the ISR. Glucose thus controls the material flux from b_i , b_v to σF .

Pedersen et al. [58] proposed a model that reconciles the Grodsky's threshold distribution hypothesis, concerning the different responsiveness of β -cells to glucose stimulus, with recent findings on insulin granule dynamics. The following granule pools were defined: mobilized and docked granules are merged into an intermediate pool, I ; the insulin amount in the readily releasable granules in β -cells with activation threshold between g and $g + dg$ is defined as $h(g, t)dg$ where h is a density with respect to threshold; F is the size of the fused pool. The equations are

$$\frac{dI}{dt} = M(G, t) - rI(t) - p^+I(t) + p^- \int_0^\infty h(g, t) dg, \quad (9)$$

$$\frac{d}{dt} h(g, t) = p^+I(t)\phi(g) - p^-h(g, t) - f^+h(g, t)\theta(G - g), \quad (10)$$

$$\frac{dF}{dt} = f^+ \int_0^G h(g, t) dg - kF(t) - mF(t). \quad (11)$$

$M(G, t)$ in (9) is the rate of granule mobilization from the reserve pool to plasma membrane, and is given by

$$\frac{d}{dt} M(G, t) = [M(G, t) - M_\infty(G)]/\tau, \quad (12)$$

where τ is a time constant. $M_\infty(G)$ is an increasing function of glucose concentration regulating granule inflow into the system, and the quantity $mF(t)$ in (11) is the ISR. The parameters r, f^+, k , and m are the rate constants of granule re-internalization, granule fusion, failed release because of the kiss-and-run exocytosis and insulin release. The parameters p^+ and p^- regulate the processes of granule priming and de-priming, $\phi(g)$ is the threshold distribution, and θ the Heaviside function.

Pedersen and Sherman [59] considered the following granule pools: almost docked (AP), docked (DP), primed (PP), and located close to L-type Ca^{2+} channels, immediately releasable granules (IRP). A recently described highly calcium-sensitive pool (HCSP [96]), that also contains releasable granules, was included in the model. Granules in IRP and HCSP may fuse with the plasma membrane, so entering “fused” pools (FIP or FHP), and then enter the “releasing” pools (RIP or RHP). Model equations are as follows:

$$\frac{d}{dt} \text{IRP} = r_1 \text{PP}(t) - r_{-1} \text{IRP}(t) - f_1(C_{md}(t)) \text{IRP}(t), \quad (13)$$

$$\frac{d}{dt} \text{PP} = r_2 \text{DP}(t) + r_{-1} \text{IRP}(t) - (r_{-2} + r_1) \text{PP}(t), \quad (14)$$

$$\frac{d}{dt} \text{DP} = r_3 \text{HCSP}(t) + r_{-2} \text{PP}(t) - (r_{-3} + r_2) \text{DP}(t), \quad (15)$$

$$\frac{d}{dt} \text{HCSP} = r_4 \text{AP}(t) + r_{-3} \text{DP}(t) - (r_{-4} + r_3) \text{HCSP}(t) - f_H(C_i(t)) \text{HCSP}(t), \quad (16)$$

$$\frac{d}{dt} \text{AP} = r_5 + r_{-4} \text{HCSP}(t) - (r_{-5} + r_4) \text{AP}(t), \quad (17)$$

$$\frac{d}{dt} \text{FIP} = f_1(C_{md}(t)) \text{IRP}(t) - u_2 \text{FIP}(t), \quad (18)$$

$$\frac{d}{dt} \text{RIP} = u_2 \text{FIP}(t) - u_3 \text{RIP}(t), \quad (19)$$

$$\frac{d}{dt} \text{FHP} = f_H(C_i(t)) \text{HCSP}(t) - u_2 \text{FHP}(t), \quad (20)$$

$$\frac{d}{dt} \text{RHP} = u_2 \text{FHP}(t) - u_3 \text{RHP}(t), \quad (21)$$

where r_i and r_{-i} , $i = 1, \dots, 5$, are rate constants of reversible transition between pools, and r_5 represents a granule inflow from a reserve pool into the almost docked pool. The parameters u_2 and u_3 represent irreversible transitions, and $u_3(\text{RIP} + \text{RHP})$ is the number of granules per β -cell releasing insulin in the unit time.

The Hill functions, f_i in (13) with Michaelis constant equal to 22 μM , and f_H in (16) with Michaelis constant equal to 2.5 μM , represent the fusion rates from IRP and, respectively, HCSP, as determined by the Ca^{2+} concentrations in the sub-plasma membrane microdomains (C_{md}) and in the cytosol (C_i). These concentrations obey the two-compartment system equations:

$$\frac{dC_{md}}{dt} = -f_{md} J_L(V(t)) - f_{md} B(C_{md}(t) - C_i(t)), \quad (22)$$

$$\frac{dC_i}{dt} = -f_J J_R(V(t)) + f_v f_i B(C_{md}(t) - C_i(t)) - f_i L(C_i(t)), \quad (23)$$

where f_{md} and f_i are constants representing the ratio of free to bound Ca^{2+} in the microdomain and, respectively, the cytosol; B is the transport rate constant between the compartments; f_v is the ratio of microdomain to cytosol compartment volume. $L(C_i)$ represents the processes that regulate calcium clearance out of cytosolic compartment. J_L and J_R represent the Ca^{2+} fluxes through the L-type and, respectively, R-type calcium channels, expressed as a function of the plasma membrane potential, V , which is the input variable. The two-compartment calcium model captures the presence of the large calcium gradients in the β -cell.

2.2. Modeling glucose–insulin oscillations

In the last few decades, many papers have been published detailing the occurrence of several modes of oscillation of the glucose–insulin system. These papers have attempted to construct a theoretical description of the mechanisms responsible for this behavior, considering also whether some external (neural) influence is actually needed to coordinate pancreatic islet activity in order to generate macroscopically evident oscillations [35,65,78]. What is usually found experimentally are high-frequency pulsatile variations of glycemia and insulinemia with a power spectrum occupying the 5–15 min interval [3,36,41] and low-frequency spontaneous oscillations (known as *ultradian* oscillations in the literature), with a period of approximately 50–150 min, with their amplitude enhanced upon exogenous glucose administration, from meals [64,76], continuous enteral nutrition [75] or constant intravenous infusions [73,91].

The reference literature usually deals with the *pulsatile insulin secretion* as the apparent occurrence, at the level of the portal vein, of a series of partially overlapping insulin pulses, [67,68], whose period ranges from 5 to 15 min. In fact, at the level of a single β -cell, insulin is released as discrete granules (see also Section 2.1), leading to the characteristic behavior known as *bursting*, whose period from spike to spike takes tens of seconds (see [57] and references therein). These *simple bursts* cluster in *compound bursts*, with a period of several minutes [9] and intra-islet synchronization leads to consider these compound bursts as produced at the level of a “homogeneous secretory unit”. Compound bursting has been supposed to be responsible for overall insulin pulsatility [9]; however, some additional mechanism seems to be required in order to justify the occurrence of apparent oscillations, since completely

de-synchronized compound bursts from a very large number of secretory units would provide a generally flat insulin secretion profile.

Models published so far are not able to describe, with a unique mathematical construct, both *ultradian* and rapid pulsatile oscillations. These models may offer a high-level mathematical synthetic description of (as yet) unknown or imperfectly understood mechanisms, offering simplified deterministic descriptions of the time course of observed insulinemia, directly relating it to other whole-body state variables (like glycemia), without considering in detail the individual effect of the secretory units (pancreatic β -cells, collected in Langerhans islets) or the actual molecular mechanisms which act within the secretory units themselves. Among the first mathematical models aiming to describe the occurrence of ultradian insulin oscillations is the nonlinear ordinary differential equation (ODE) model of Sturis et al. [80]. According to the different shapes of the exogenous glucose infusion, different *in silico* experiments have been carried on, using constant glucose infusions as well as oral glucose administration or multiple meal ingestion. The aim of these papers was to provide a plausible mechanism for the genesis of the oscillations. Indeed, the authors suggested that the *ultradian* oscillations in insulin and glucose concentrations could originate from their reciprocal interaction without postulating an intrapancreatic pacemaker for their existence. The model of Sturis et al. has been the starting point for many delay differential equation (DDE) models, aiming to replicate the occurrence of long-period glucose–insulin oscillations by means of a Hopf bifurcation (see, e.g. [5,31,43,44]). It has to be stressed that the model of Sturis et al. and its DDE versions have been used, especially in the recent years, with the aim of testing the action of pulsatile insulin profiles in (pre)-diabetic patients [17,18,31,85,93,94,97].

A different approach to mathematical modeling attempts to include, in as much detail as possible, known biochemical mechanisms operating at the β -cell level. These models entail different biochemical assessments combining electrical and calcium dynamics with the glycolytic oscillator [9,10,77], managing to describe the synchronization of intra/inter-islets β -cells and providing the oscillatory response of insulin secretion [57,88].

In the above mentioned models (the ODE as well as the DDE models), the oscillatory regime of their solutions is obtained, depending on the appropriate identification of the model parameters by means of a (typically numerical) bifurcation study. As a matter of fact, each model proposed can reproduce only one oscillatory regime (either fast or slow oscillations), according to the model structure and to the relative bifurcation assessments.

A recent work [53] proposes a different type of theoretical model for pancreatic insulin secretion, consisting of the aggregation of a large collection of insulin secretory units. This model incorporates the secretory effect of the known physiological bursting response of aggregates of β -cells to varying glucose concentrations, without detailing the chain of subcellular events giving rise to this response. Indeed, in spite of a rather elementary mathematical structure for the single secretory unit, the model is able to describe both rapid pulsatile and *ultradian* insulin oscillatory regimens, as well as their response to experimental maneuvers.

Unlike the previously published models, the single-unit model is not required to produce any oscillatory behavior *per se*. It is the aggregated response of the entire population of secretory units that reproduces the apparent whole-body insulinemia oscillations. Such a population of secretory units is naturally heterogeneous in terms of reaction thresholds, recovery times and insulin packet size. The pancreatic insulin release is provided by the aggregate of as many independent secretory units as are simultaneously considered (about one million of different single-unit models corresponding to one million Langerhans islets in humans [40]).

According to the well documented phenomenon of β -cell synchronization within an islet of Langerhans (likely due to the electrical activity of the β -cell membrane, [29,48,69,71,90]) the number N of independent secretory units is intended to represent the number of islets instead of the number of β -cells (about 1,000 β -cells per islet [27], that is 1 billion total). No coordination among units is required: by *independent units* it is meant that no direct control is exerted on a secretory unit either by other units or by neural or endocrine mechanisms, the only connection among the units being the common input signal represented by blood glucose concentration, as sensed by each secretory unit. This is consistent with the experimental literature, which stresses the crucial role of glucose feedback in governing pulsatile insulin secretion [19,66,81,57].

Entering into more mathematical detail, each single secretory unit (say, unit n) reacts to circulating glycemia $G(t)$ by ejecting a discrete packet of insulin D_n , whenever plasma glucose concentration exceeds that unit's secretion threshold B_n . As soon as a secretory unit releases its insulin packet, it enters a (relative) refractory state, where further stimulation prevents the release of new hormone. This refractory state is represented by instantaneously increasing that unit's secretion glycemia threshold to a very high level R_n whence, with time, it exponentially decreases towards its *resting threshold* value G_n , $G_n < R_n$. B_n obeys, therefore, the following ODE:

$$\frac{dB_n}{dt} = -\alpha_n B_n(t) + \alpha_n G_n + (R_n - B_n(t))\delta(G(t) - B_n(t)), \quad (24)$$

where the term containing the Dirac delta function $\delta(\cdot)$ specifies the instantaneous increase of the threshold to the refractory level R_n upon discharge of insulin.

A high value of G_n , greater than the circulating glycemia, corresponds to a secretory unit that seldom fires (possibly never), while a low value of G_n corresponds to a secretory unit that fires frequently, as soon as it recovers from its refractory state, according to its individual rate of recovery α_n . Of course, an increase of prevailing glycemia $G(t)$ (e.g. during the post-prandial state) induces the recruitment of more secretory units from the population of seldom firing units, with a corresponding increase in the amount of the insulin secreted.

A further aspect that is properly addressed in the secretory unit model is the phenomenon known as *potentiation* [46,47,87]. Potentiation indicates the phenomenon by which β -cells which are often stimulated will progressively increase the size of their synthetic enzymatic machinery (in the medium term and up to a limit), so as to deliver larger amounts of insulin. This effect is here represented by assigning a dynamics to the size of the secretory unit's insulin packet:

$$\frac{dD_n}{dt} = -k_n D_n(t) + \rho_n \frac{G'^n(t)}{G'^n(t) + \Gamma_n^{\gamma_n}}, \quad (25)$$

with k_n being the insulin packet natural decrease rate, ρ_n the maximal saturating potentiation charging rate, Γ_n the glycemia at which the islet potentiation is half of its maximal rate and γ_n the progression with which the potentiation reacts to circulating glucose concentrations (for small γ_n , a moderate potentiation occurs over a wide glycemic range, while for large γ_n , abrupt potentiation occurs over a restricted glycemic range). Notice that, if plasma glycemia is kept at a high value, the unit is forced to react to such persistent hyperglycemic condition by increasing its insulin packet size, according to (25). It is reasonable to assume that for a typical unit, Γ_n is greater than the basal value G_b : in this way, small increases of plasma glycemia do not substantially affect potentiation; on the contrary, when $G(t)$ approaches Γ_n , a faster increase of potentiation occurs, up to a glucose toxicity level, for which a saturating steady state potentiation level is maintained.

By setting once and for all the (hyper-) parameter values, i.e. parameters identifying the distribution from which single-unit structural parameters are sampled, this population model is able to replicate both the *ultradian* and rapid pulsatile oscillatory regime, in the absence of exogenous periodic stimuli, like the clinical experiments detailed in [75,82]. Moreover, it is also able to replicate the synchronization of both *ultradian* and rapid pulsatile oscillations to the frequency of exogenous periodic glucose infusion, phenomenon known as *entrainment* [66,82].

It is of interest to notice that this kind of model produces oscillations independently of the number of secretory units considered, provided that this number is sufficiently large: oscillations are not produced as a result of stochastic noise, nor do they require an oscillating input function to be present (as it has been postulated for some models of oscillatory insulin release by a single β -cell, where oscillating glycolysis provides such input). These oscillations are rather the effect of the interplay of delays in actuator function (glucose consumption at the periphery) and distributed controller population structure.

3. Short term glucose–insulin models

Short term modeling concerns the glucose–insulin dynamics after an external perturbation such as a glucose bolus injection (intra-venous glucose tolerance test, IVGTT), an oral glucose consumption (oral glucose tolerance test, OGTT) or continuous glucose and insulin infusions like the Euglycemic Hyperinsulinemic Clamp (EHC), within a relatively short time period of a few hours. These clinical experiments, and the mathematical models aimed at their interpretations, have generated much interest in the last decades since they offered the possibility to estimate a set of key markers of T2DM development. Besides generally allowing a more accurate knowledge of the regulatory mechanisms underlying glucose–insulin homeostasis, short term mathematical models may be fruitfully linked to clinical protocols in order to compute the *insulin sensitivity* of a given subject. Besides their time scale of a few hours, the common denominator of these models is the fact that they are top-down compartmental models, representing the observable features of glucose–insulin homeostasis without detailing the molecular events leading to such features.

3.1. The intra-venous glucose tolerance test

The IVGTT is a clinical experiment where a glucose bolus is rapidly injected intra-venously into a subject. Glucose and insulin samples are acquired in the following 3 h during which glycemia and insulinemia return to their basal values. The glucose injection is modeled as an instantaneous change in the plasma glucose concentration. In healthy subjects the glucose induced pancreatic response of insulin release consists of two contributions: a first phase release, which is a quick response to a sudden change in glycemia, and a second phase release, which occurs some ten minutes after the bolus injection. The first phase is usually modeled as an instantaneous change in the plasma insulin concentration. The second phase is described by the model equations, and the difference among the many existing mathematical models are evaluated by their ability to capture correctly the observed dynamics.

3.1.1. The Minimal Model

The early linear models of the glucose–insulin homeostasis, which have been validated by means of an IVGTT, date back to Bollen [12] and the Ackerman's research group [1,2,34] in the sixties. However, the most famous and still greatly widespread model used in clinical assessments, such as the estimate of the insulin sensitivity index, is the so-called *Minimal Model* (MM), proposed by Berg-

man and collaborators in the late seventies [6]. For a historical review see [7,8]. The MM is composed of two separate parts: one describing the dynamics of the glucose uptake after the external stimulus, regarding the insulin concentration as a known forcing function; the other describing the dynamics of the pancreatic insulin release in response to the glucose stimulus, with the glucose concentration regarded as a known forcing function. The model equations for the glucose dynamics are:

$$\begin{aligned} \frac{dG}{dt} &= -(p_1 + X(t))G(t) + p_1 G_b, & G(0) &= G_b + \Delta_G, \\ \frac{dX}{dt} &= -p_2 X(t) + p_3 (I(t) - I_b), & X(0) &= 0. \end{aligned} \quad (26)$$

This is a two-compartment model: the first equation refers to the plasma glucose concentration $G(t)$, the second refers to a remote compartment for the insulin. The physiological assumption is that the insulin-dependent glucose uptake does not directly depend on the plasma insulin concentration $I(t)$, but on the insulin concentration in the remote compartment, through the auxiliary function $X(t)$, whose dynamics depends on the plasma insulinemia. Δ_G is the instantaneous change of glycemia due to the glucose bolus injection.

The glucose kinetics of the MM allows to compute some important markers of insulin efficacy, which are widely used to diagnose. Among these markers the most important is the *insulin sensitivity index*, defined as the quantitative influence of basal insulin concentration to increase the glucose effectiveness at steady state, [6]:

$$S_I = \frac{\partial}{\partial I_b} \left[-\frac{\partial}{\partial G} \frac{dG}{dt} \right]_{\text{steady state}} = \frac{p_3}{p_2}. \quad (27)$$

The success of the MM in clinical applications is mostly due to the ability of providing these important markers as a by-product of the model identification procedure.

The second part of the MM concerns the insulin kinetics and consists of a single compartment model, [84]:

$$\frac{dI}{dt} = -n(I(t) - I_b) + \gamma t[G(t) - h]^+, \quad I(0) = I_b + \Delta_I. \quad (28)$$

The insulin kinetics exhibits a linear clearance rate n , and an insulin secretion rate modeled by a time-varying forcing function, which is motivated by the hypothesis that the effect of circulating hyperglycemia on the rate of pancreatic insulin secretion is proportional both to the hyperglycemia attained and to the time elapsed from the glucose stimulus. The insulin kinetics of the MM is thus necessarily associated to the IVGTT procedure, since the initial experimental time plays a crucial role in assessing the ISR. Therefore the model cannot be used for other purposes, such as a multi-boli experiment, or during glucose infusions. Parameter h is the *target glycemia* that the actual plasma glucose concentration needs to exceed to stimulate the second-phase pancreatic insulin production. The first phase insulin release is modeled by Δ_I . The identification procedure thus has to estimate 4 parameters (n, γ, h, Δ_I).

3.1.2. Beyond the Minimal Model

The MM has played a crucial role in modeling the glucose–insulin system and it still is of practical use in many clinical settings. Nevertheless, many criticisms have been raised in the last decade [26,56,55]. Some questionable physiological assumptions are that the pancreas is able to linearly increase its rate of insulin secretion with time, or the introduction of a non-observable remote compartment to model the insulin effect on the insulin-dependent glucose uptake. The main drawbacks are the lack of mathematical coherence of the model and the lack of robustness in the parameter identification procedure. Both drawbacks are strongly related to the assumption of two distinct parts: the glucose–insulin system is an integrated physiological system whose model should be meaningful as a whole. From a mathematical point of view, it

has been proved in [26] that the MM is not coherent, since it does not admit a steady-state solution (corresponding to the basal glycemia/insulinemia), but allows an unbounded increase of the state variables for a reasonable set of model parameters. This is mainly due to the time-varying term and the values the target glycemia h assumes according to data, which are smaller than the measured basal glycemia. This drawback clearly affects the estimate of the insulin sensitivity index also, since it is defined as a steady-state index, but is estimated by use of a mathematical model which does not admit a steady-state solution.

The identification procedure is split into two steps: first the recorded insulin concentration is used as input data to derive the glucose kinetics parameters, then the recorded glucose is used as input data to derive the insulin kinetics parameters. According to this double step identification procedure, one state variable, say glucose, is fitted by using the *noisy* insulin measurements as a *true* input function, and the opposite for the insulin system. This strategy decouples the glucose–insulin regulatory system, disregarding the feedback effects of the state variables. By splitting the two subsystems we may estimate coefficients for one segment (by optimally fitting the relative data), which are not the ones which would generate an optimal approximation to the whole data set if the interaction between the two subsystems were allowed.

In 2000 the following DDE model was proposed, [26]:

$$\begin{aligned} \frac{dG}{dt} &= -b_1 G(t) - b_4 I(t) G(t) + b_7, \quad G(0) = G_b + b_0, \\ \frac{dI}{dt} &= -b_2 I(t) + \frac{b_6}{b_5} \int_{t-b_5}^t G(\tau) d\tau, \quad I(0) = I_b + b_3 b_0, \end{aligned} \quad (29)$$

with $G(t) \equiv G_b$, $t \in [-b_5, 0)$, and b_3 the first-phase insulin concentration increase per mM increase in glucose concentration at time zero due to the injected bolus. The following elements are different from the MM:

- the glucose–insulin system is considered as a whole, with the glucose–insulin dynamics coupled together; accordingly, there is a single step identification scheme;
- no remote compartments mediating the insulin-dependent glucose uptake are present. An insulin-dependent glucose uptake like in (29) was suggested also in an early paper [49];
- the DDE model is no longer time-varying. The pancreatic ISR is proportional to the average value of the glucose concentration in the b_5 minutes preceding time t : Thus, it can be validated by an IVGTT, but it is not constrained to that clinical framework. The model is suitable also for multi-boli injections or glucose/insulin infusions.

This model has positive bounded solutions for any physically admissible set of parameters and initial conditions. Moreover, it has a unique positive equilibrium point, corresponding to the basal glycemia/insulinemia, and this point is asymptotically stable, globally within the set of positive initial conditions. The model has also been fitted and validated by means of a standard IVGTT procedure on real data.

It is remarkable that removing the remote (unobservable) compartment, the insulin sensitivity index may be computed without referring to steady state values:

$$S_I = \frac{\partial}{\partial I} \left[-\frac{\partial}{\partial G} \frac{dG}{dt} \right] = b_4; \quad (30)$$

thus, S_I is provided by the estimate of a single parameter (b_4).

After [26], a great deal of DDE models followed (see, e.g. [5,31,42–44,50,54,56,94]), most of them referring to long-term frameworks.

Model (29) has been further modified and validated by data [54,56,55]. In [54] a family of DDE models is presented, close to the one published in [42]. It introduces general delays, both in

the insulin action on tissue glucose uptake and in the glucose action on pancreatic insulin secretion. A further important difference with most of other previous models is that the insulin-independent glucose uptake may be trivially reduced to a constant term. Of course, this is an approximation, since the brain and the nerve cells which rule the insulin-independent glucose uptake cannot use glucose in case of severe deficiency of glucose. Nevertheless, such a starvation situation is rare and not relevant in an IVGTT experimental protocol. This idea dates back to the pioneering work of [49], but it has been forgotten up to the early nineties, where [80] described the long-term glucose–insulin oscillations.

In [54] it is shown that the whole family of models has positive bounded solutions and a unique steady-state solution for any physically admissible setting of both parameters and initial conditions. Stability analysis has been carried out for a pair of significative models from the family: one with discrete delays, the other with continuous delays. In both cases necessary and sufficient conditions are given for the local asymptotic stability of the steady-state solution, which are easily verified over a wide range of realistic parameter values. In [45] further results relating the range of time delay to the global stability of the equilibrium point are reported.

Different discrete-delay models from the family have been validated in [56] by data from 40 healthy subjects undergoing an IVGTT. The best fitting model according to the Akaike Criterion resulted to be a Single Delay Model (SDM), with a constant insulin-independent glucose uptake:

$$\begin{aligned} \frac{dG}{dt} &= -K_{xgl} G(t) I(t) + \frac{T_{gh}}{V_G}, \\ \frac{dI}{dt} &= -K_{xi} I(t) + \frac{T_{IGmax}}{V_I} f(G(t - \tau_g)), \quad f(G) = \frac{(\frac{G}{G^*})^\gamma}{1 + (\frac{G}{G^*})^\gamma}. \end{aligned} \quad (31)$$

Here V_G, V_I are the glucose and insulin distribution volumes, respectively. A single explicit delay τ_g , with a range of values in [0,30] minutes, refers to the control action exerted by glucose on pancreatic insulin secretion, which is expressed by means of a saturable sigmoidal function. No explicit delay in the insulin action on the glucose uptake seemed necessary to explain the observed time series. Initial conditions are:

$$\begin{aligned} G(t) &\equiv G_b, \quad t \in [-\tau_g, 0), \\ G(0) &= G_b + \Delta_G, \quad \Delta_G = \frac{D_g}{V_G}, \quad I(0) = I_b + \Delta_I, \quad \Delta_I = I_{\Delta G} \Delta_G. \end{aligned} \quad (32)$$

The SDM has been fitted and compared to the MM. The free parameters to be estimated are $K_{xgl}, K_{xi}, V_G, \gamma, \tau_g, I_{\Delta G}$, since G^* and V_I are set *a priori*; D_g, G_b, I_b are measured and T_{gh}, T_{IGmax} come out from steady-state computation.

Besides a more accurate precision in the parameter estimates in terms of a smaller Coefficient of Variation (CV), the SDM results to be particularly suitable to estimate the insulin sensitivity index which is provided by a single parameter related to the insulin-dependent glucose uptake, $S_I = K_{xgl}$. Indeed, 40 out of 40 subjects showed identifiable K_{xgl} ($CV < 52\%$) from the SDM, whereas only 20 out of 40 in the MM. Therefore, the SDM appears to be theoretically sound and robust in practice, and can routinely be considered for the determination of insulin sensitivity from the IVGTT. Free software for estimating the SDM parameters is available from the authors' website: <http://www.biomatematica.it/bibif/bibif.html>.

3.2. The oral glucose tolerance test

In the OGTT, plasma glucose and insulin concentrations are measured at time 0, 30, 60, 90, and 120 min, following an oral glucose load of 75 g. C-peptide must also be measured to com-

pute indexes of the insulin secretion. The OGTT is a simple clinical test currently used to aid diagnosis of glucose intolerance and T2DM.

The OGTT mimics the physiological conditions of the glucose/insulin system more closely than the EHC or the IVGTT. However, the analysis of the OGTT data by a mathematical model is affected by the complication that the time course of the delivery to plasma of exogenous glucose and even the total amount of glucose delivered are unknown. In fact, the rate of appearance, Ra , of exogenous glucose in plasma is influenced by several factors: the rate of gastric emptying of ingested glucose, the extent of absorption during the intestinal transit, glucose amount used by gut as energy substrate, and hepatic uptake. Moreover, gut-derived hormones, secreted in response to glucose delivery to the small intestine, markedly increase the insulin secretion with respect to that observed after an intravenous glucose infusion that produces the same elevation of plasma glucose (incretin effect).

Experimental determinations of the rate of appearance of ingested glucose in plasma have been obtained using a double tracer technique by Ferrannini et al. [32,33]. The Ra was found to have a similar profile in healthy subjects and diabetic patients, and 73–76% of the oral load was recovered in the circulation in the 3.5-h study period. Ra data during an OGTT or a meal test (MTT) were reported by other groups (for instance, see [23,52,95]).

The rate of gastric emptying and the small intestine transit time appear to be main factors in determining glucose Ra [95]. Data of gastric retention of glucose (dose fraction retained vs. time) were fitted by a power exponential function and the rate of glucose delivery into the duodenum was computed [72]. A 5% retention of the glucose load was found to depend on interdigestive phase and estimated to occur at about 130 min and 193 min for a load of 50 and, respectively, 100 g.

Mathematical models, developed with the aim of describing the kinetics of the glucose/insulin system during an OGTT or an MTT, and estimating parameters of clinical interest, are reviewed in the following. Attention is focused on some of the more recent contributions. Methods and models used for the analysis of tracer data have not been considered in the present review.

3.2.1. The oral glucose minimal model

The OGTT minimal model [15] extends to the oral test the basic model proposed for the IVGTT, with the modification that glucose administration does no longer appear as a bolus dose D in the initial condition of the equation for glucose kinetics, but as an input function that specifies the rate of appearance of oral glucose in plasma. The OGTT minimal model has also been used in conjunction with models of C-peptide secretion (see next section).

The model describes the kinetics of the plasma glucose concentration G (basal value, G_b), and of the insulin action, X . Model equations are as follows:

$$\begin{aligned} \frac{dG}{dt} &= -(p_1 + X(t))G(t) + p_1 G_b + \frac{Ra(t)}{V_G}, \quad G(0) = G_b, \\ \frac{dX}{dt} &= -p_2 X(t) + p_3 (I(t) - I_b), \quad X(0) = 0, \end{aligned} \quad (33)$$

where I is the plasma insulin concentration (basal value, I_b). Insulin concentration data, assumed to be error-free, are used to construct an input function of the system. Ra is the rate of appearance of oral glucose in plasma, and p_1, p_2, p_3 , and V_G are parameters with the same meaning as in the IVGTT minimal model. Using the definition of insulin sensitivity given in (27), in [15] it is proposed to compute the S_I by suitably exploiting the area-under-the-curve (AUC) from time $t = 0$ to $t = \infty$, so that:

$$S_I = \frac{p_3}{p_2} = \frac{AUC(X)}{AUC(I - I_b)}. \quad (34)$$

The integrals in the AUCs are computed by taking into account the OGTT measurements throughout the test. Computations make use of a trivial linear relationship where Ra forces the glucose excursion above its basal value, and of the assumption that glucose and insulin concentrations achieve the pretest basal values at the end of the test.

As this last requirement is not usually satisfied in the clinical setting, parametric descriptions of the rate of appearance were evaluated [21]. The approach chosen was that of representing the Ra in (33) by a piecewise linear function with a given number (n) of break points:

$$Ra(t) = \begin{cases} \alpha_{i-1} + \frac{\alpha_i - \alpha_{i-1}}{t_i - t_{i-1}}(t - t_{i-1}) & t_{i-1} \leq t \leq t_i, \quad i = 1, \dots, n, \\ 0 & \text{otherwise,} \end{cases} \quad (35)$$

with $t_0 = 0$ and $\alpha_0 = 0$ ($Ra(0) = 0$). The α_i values are to be estimated from the glucose concentration data. The *a priori* identifiability of model parameters was shown to be guaranteed provided that p_1 and V_G are assumed to be known. Taking $V_G = 1.7 \text{ dl kg}^{-1}$ and $p_1 V_G = 0.024 \text{ dl kg}^{-1} \text{ min}^{-1}$, the method was applied to data from a MTT on normal subjects [21]. To achieve a reasonable *a posteriori* identifiability, the AUC of the reconstructed Ra was constrained to be equal to fD (with $f = 0.86$). Moreover, adopting a Bayesian estimation procedure, the rate constant p_2 that governs the kinetics of insulin action was taken to be normally distributed with mean 0.03 min^{-1} and $CV = 20\%$. In this way, for each subject, the insulin sensitivity, the parameter p_2 , and the piecewise-linear reconstruction of the Ra were estimated. The S_I estimates, derived from a long (420 min) and a short (120 min) oral test were compared with those obtained from an intravenous test.

The performance of the model proposed in [21] in estimating insulin sensitivity and Ra time course was evaluated by comparing the estimates with those obtained by a reference tracer technique [22]. The tracer and the non-tracer models were applied to a mixed meal test on normal subjects with data measured up to 420 min. The tracer method provided data on the distribution over the subject population of the model parameters, so allowing to refine the *a priori* fixed parameters. Substantially similar assumptions were done to guarantee the actual identifiability of the model. The estimates obtained by the non-tracer method were compared by correlation with those obtained by the tracer method ($r = 0.86$ for the S_I), whereas lower values of the correlation were found with other indexes of insulin sensitivity, such as HOMA and QUICKI.

To further assess the ability of the oral glucose minimal model in estimating the insulin sensitivity, the OGTT estimates were compared with those provided by the gold standard test, the EHC [23], see Section 3.3. Subjects with *normal glucose tolerance* (NGT) and *impaired glucose tolerance* (IGT) underwent both a labeled EHC and a labeled OGTT (OGTT data collected up to 360 min). The data were analyzed by the labeled and the non-labeled OGTT minimal models. Correlation between clamp-derived and OGTT-derived insulin sensitivity was found to give $r = 0.81$ with the non-labeled data and $r = 0.70$ with the labeled data. As reported by Basu et al. [4], the insulin sensitivity estimated by a mixed-meal test in type 2 diabetic subjects was less than half compared with the value in controls.

3.2.2. OGTT models that include minimal models of insulin secretion

A minimal model of C-peptide secretion was proposed by Breda et al. [13]. As found in a later study, it can also be derived from a more complex model at the cellular/organ level [60]. C-peptide or insulin secretion rate are represented as the sum of two components related to the glucose concentration (static component) and, respectively, to its rate of change (dynamic component):

$$ISR(t) = V_1(SR_s(t) + SR_d(t)), \quad (36)$$

where V_1 is the volume of C-peptide central (accessible) compartment. SR_s and SR_d , that may be expressed for instance as $\text{pM} \cdot \text{min}^{-1}$, are modeled as follows. The static component, related essentially to the “provision” of new insulin according to the Grodsky model [37], is defined by setting $SR_s(t) = Y(t)$, where Y obeys the equation:

$$\frac{dY}{dt} = \alpha(-Y(t) + \beta[G(t) - h]), \quad Y(0) = 0, \quad (37)$$

with α , β and h constant parameters. The dynamic component is related to the insulin stored in the β -cells in a readily releasable form [37], and is expressed as:

$$SR_d(t) = \begin{cases} k(G(t))\dot{G}(t) & \dot{G}(t) > 0, \\ 0 & \dot{G}(t) \leq 0, \end{cases} \quad (38)$$

where

$$k(G) = \begin{cases} K_d \left(1 - \frac{G - G_b}{G_t - G_b}\right) & G_b \leq G \leq G_t, \\ 0 & \text{otherwise,} \end{cases} \quad (39)$$

with K_d and G_t model parameters. The parameters in (36)–(39), that provide the input for the two-compartment model of C-peptide kinetics by Van Cauter et al. [92], were estimated from the data of C-peptide concentration and provide sensitivity indexes for the β -cell function. The static sensitivity index, Φ_s , and the dynamic index, Φ_d , have the following expressions:

$$\Phi_s = \beta, \quad \Phi_d = K_d \left(1 - \frac{G_{max} - G_b}{G_t - G_b}\right), \quad (40)$$

where G_{max} , assumed to be smaller than G_t , is the maximal glucose concentration observed during the experiment. A global index of β -cell sensitivity to glucose was also defined as

$$\Phi = \Phi_s + \frac{\Phi_d(G_{max} - G_b)}{\text{AUC}(G - h)} \simeq \frac{k_{01}\text{AUC}(CP_1)}{\text{AUC}(\Delta G)}, \quad (41)$$

where CP_1 is the C-peptide concentration in the central compartment and k_{01} is the rate constant of elimination from this compartment [92].

Using the above model, different OGTT protocols extended over five or four hours and with a different number of samples were compared on NGT and IGT subjects with respect to a reference protocol with 22 samples [13]. In a more recent review paper [20], the insulin sensitivity, the β -cell function as measured by the sensitivity indexes defined in (40) and (41), the disposition index $DI = \Phi \cdot S_i$, and the hepatic insulin extraction, were determined on young and elderly individuals and compared with the indexes evaluated by the IVGTT. As reported in [4], the index Φ was found to be less than half in diabetic patients compared with controls.

A different approach to derive parameters that characterize the β -cell function was proposed by Mari et al. [47]. The model represents the relationship between glucose concentration and C-peptide or ISR as the sum of two components. The first component, $S_g(t)$, incorporates a β -cell dose–response relationship, $f(G)$, which is a nonlinear function of glucose concentration, and a modulating factor that is a function of time and accounts for a possible enhancing effect (denoted *potentiation* in [47] and depending for instance by entero-insular factors) upon insulin secretion:

$$S_g(t) = \exp[Q(t)]f(G(t)). \quad (42)$$

$Q(t)$ is modeled as a generic piecewise linear function of time constrained to have zero mean in the time interval of the data, and the quantity $P(t) = \exp[Q(t)]$ is denoted as the potentiation factor. The function $f(G)$, defined in such a way that $f(G) > 0$ for $G > 0$ and $f(0) = 0$, was proposed to have the form

$$f(G) = p_3 G + (p_4 - p_3) \times \frac{[\log(\cosh[p_1(G - p_2)]) - \log(\cosh(p_1 p_2))]/p_1 + \tanh(p_1 p_2)G}{1 + \tanh(p_1 p_2)}, \quad (43)$$

where the parameter p_2 is a “threshold” glucose level at which the slope of $f(G)$ changes from the initial value p_3 to the final value p_4 , and p_1 determines the curvature around p_2 . The second component of the ISR is defined as $S_d(t) = p_d dG/dt$ for $dG/dt > 0$ and zero if $dG/dt \leq 0$, similarly to the model by Breda et al. [13], so the total ISR is given by $S(t) = S_g(t) + S_d(t)$. The secretion model was coupled to the C-peptide kinetic model [92].

The time course of plasma glucose concentration, to be given as input function to the insulin secretion model, is provided by a simple model of glucose kinetics,

$$\frac{dG}{dt} = -kG(t) + R(t), \quad (44)$$

where the rate constant k was set to 0.012 min^{-1} , and the function $R(t)$ was represented as a piecewise linear function to be estimated from the data. The unknown quantities are thus the function $R(t)$ of the glucose kinetic equation (44), plus the function $Q(t)$ and the parameters $p_1 - p_4$ and p_d of the insulin secretion model.

The estimation of the unknown quantities required a rather complex approach based on a regularization procedure that, using the glucose and C-peptide concentration data, predicted the glucose and C-peptide time courses together with a regularized profile of $R(t)$ and $Q(t)$. The β -cell glucose sensitivity is determined as the mean slope of the dose–response function, $f(G)$, whereas p_d represents a rate sensitivity. In [47], the model was used to analyze data from a multiple-meal test over 14–15 h on control subjects and T2DM patients. The β -cell glucose sensitivity, the rate sensitivity, and the excursion of the potentiation factor were markedly reduced in the diabetic patients with respect to controls.

To evaluate the effects of incretins on the β -cell function, an OGTT and an isoglycemic intravenous test were performed on subjects with normal and impaired glucose tolerance [51]. In the NGT group, the β -cell glucose sensitivity was found to increase from 99 ± 20 in the intravenous test to $156 \pm 28 \text{ pmol min}^{-1} \text{ m}^{-2} \text{ mM}^{-1}$ in the oral test, whereas the rate sensitivity was not significantly changed. The same behavior was found in the IGT group. The time course of the potentiation factor was significantly delayed in the IGT with respect to NGT subjects. A similar approach was followed to quantify separately the impairment in the incretin effect related to obesity, and to the impaired glucose tolerance or T2DM [52].

An extension of the C-peptide minimal model of Eqs. (36)–(39), which accounts for the action of GLP-1 on β -cell secretion, was recently proposed [24]. This new model allows the simultaneous estimation of parameters that characterize the β -cell response to glucose and the ability of GLP-1 to enhance the insulin secretion.

By using the hepatic catheterization technique, the hepatic blood flow and the insulin and C-peptide concentrations were measured in the femoral artery and the hepatic vein of healthy controls, obese, and T2DM subjects [89]. A circulatory model for the whole-body kinetics was used. For the hepatic compartment, the equations were written in terms of the steady-state mass flux through the liver, with the inputs from the portal vein (pancreatic secretion) and the hepatic artery, and the output in the hepatic vein. The estimates of ISR showed an hypersecretion in obese subjects and a reduced secretion in the diabetic subjects. The hepatic insulin extraction, found to be higher in diabetic than in control subjects, was also estimated. Data were used to validate a previously proposed mathematical model of β -cell insulin secretion during an OGTT [83].

3.2.3. OGTT Models that explicitly include the effect of incretins

Brubaker et al. [14] proposed a model that incorporates several factors that contribute to the observed response to the oral glucose load. The model parameters are indicated as $k_i, i = 1, \dots, 16$. The rate of glucose delivery to duodenum, D_G , was taken to decline linearly with time:

$$D_G(t) = \begin{cases} 0 & t < t_0, \\ k_1 - k_2 t & t_0 \leq t \leq t_{max} \end{cases} \quad (45)$$

and $D_G(t) = 0$ for $t > t_{max}$, the time such that $k_1 - k_2 t_{max} = 0$ (t_0 set to 5 min). The intercept k_1 and the slope k_2 were chosen in order to have an AUC of D_G from 5 min to t_{max} smaller but close to the oral dose of glucose. The rate of glucose delivery to duodenum was assumed to control the release of the incretins, represented by a single plasma concentration Inc that obeys the equation

$$\frac{d}{dt} Inc = -k_3 [Inc(t) - Inc_b] + k_4 D_G(t), \quad (46)$$

where the subscript “b” denotes basal values, k_3 was set to 0.1 min^{-1} , and k_4 was estimated from experimental data [72].

The time course of the rate of oral glucose appearance, Ra , was established on the basis of the data reported by Ferrannini et al. [32,33] and has the form

$$Ra(t) = \begin{cases} 0 & t < t_0, \\ k_5(t - t_0)^{k_6} \exp[-k_7(t - t_0)] & t \geq t_0, \end{cases} \quad (47)$$

where the coefficients k_5 – k_7 were chosen in order to have an AUC of Ra from 5 to 300 min smaller but close to the AUC of D_G .

The hepatic glucose balance H_G , that represents the difference between hepatic glucose production and glucose uptake from the mesenteric circulation, was expressed as

$$H_G(t) = H_{G,b} + k_8(G_b - G(t))I(t) + k_9(1/(G(t)I(t)) + 1/(G_b I_b)). \quad (48)$$

The second term in the right hand side of (48) represents a primary regulatory factor that reduces hepatic glucose release when glycemia increases. If a severe hypoinsulinemia occurs, the regulatory action represented by the third term intervenes to increase hepatic glucose release. The plasma insulin concentration, I , obeys the equation

$$\frac{dI}{dt} = -k_{10}I(t) + k_{11}Inc(t) + k_{12}G^{1.3}(t) + k_{13}, \quad (49)$$

where the stimulatory effect of incretins is modeled by the linear term, whereas the coefficient k_{13} represents additional stimulators or inhibitors of the insulin secretion.

The kinetics of plasma glucose concentration is modeled as

$$\frac{dG}{dt} = -k_{14}G^{1.3}(t) - k_{15}I(t) + \frac{1}{V_G}H_G(t) + \frac{1}{V_G}Ra(t) + k_{16}\frac{dI}{dt}I(t), \quad (50)$$

where the negative terms in the right hand side specify the dependence on glucose and insulin of the rate of glucose uptake, whereas the last term was empirically introduced to sharpen the peak in glucose concentration following glucose entry into the circulation. Model parameters were adjusted to fit literature OGTT data with doses of 50 g and 100 g, and the model prediction of an IVGTT was also obtained.

3.3. Euglycemic hyperinsulinemic clamp

The gold standard for investigating and quantifying insulin resistance is the EHC, and was proposed in [25]. It measures the amount of glucose necessary to compensate for an increased insulin level without causing hypoglycemia, i.e. it intends to “clamp” the glucose concentration at a prespecified level. It is the most

widely used experimental procedure for the determination of insulin sensitivity, in spite of its labor-intensive execution, due to the simple interpretation which is usually attributed to the obtained results. The favor with which the EHC is viewed stems in part from the belief that while mathematical models of the glucose insulin system make untenable assumptions, the EHC approach is relatively assumption-free, or model independent.

In its usual form the patient is followed under insulinization for two hours, but often the EHC is continued for 4–6 h. Through a peripheral vein a priming dose of short-acting human insulin is given during the initial 10 min in a logarithmically decreasing manner, in order to raise acutely the plasma insulin to the desired level. Thereafter, insulin is infused at 10–120 mU per m^2 per minute. To compensate for the insulin infusion, glucose is infused to maintain blood glucose levels between 5 and 5.5 mmol/l. The glucose and insulin levels are monitored every 5 min and every 20 min, respectively, and the rate of glucose infusion is adjusted following the algorithm in [25].

The rate of glucose infusion during the last 30–60 min of the test determines insulin sensitivity. If high levels (7.5 mg/min or higher) are acquired, the patient is diagnosed insulin-sensitive. Low levels (4.0 mg/min or lower) indicate that the body is insulin-resistant. Levels between 4.0 and 7.5 mg/min are not definitive and suggest impaired glucose tolerance, an early sign of insulin resistance.

In general, insulin resistance is an expression of the imbalance between the amount of pancreatic insulin, delivered in response to a glucose load, and the levels of plasma glucose reached. To obtain the same plasma glucose concentration, higher levels of plasma insulin are necessary in insulin-resistant subjects than in normal controls. The clamp, as usually employed, yields easy-to-compute indices, which are commonly used as measures of insulin resistance. The M value is defined as the average glucose infusion rate over the period of the last 30–60 min of the insulin infusion. The ratio M/I is the ratio of the M value to the average plasma insulin concentration during the same period. If a two-step clamp is performed, i.e. the insulin infusion is increased at some point during the procedure, the $\Delta M/\Delta I$ ratio is defined as the increment of M observed over the corresponding increment of I produced by raising the insulin infusion rate. The use of these indices, however, does make two fundamental assumptions: first, that at the end of the insulin infusion the experimental subject is at steady state with regard to glucose uptake rate; and second, that glucose uptake rate is linearly increasing with increasing insulinemia, either throughout the insulin concentration range (when using the M/I index) or between successive insulin concentrations reached in the two-step clamp (when using the $\Delta M/\Delta I$ index). These assumptions are however only a first approximation of the real state of things.

3.3.1. A mathematical model

To explain the oscillations of glycemia $G(t)$ occurring in response to the hyperinsulinization $I(t)$ and to the continuous glucose infusion at varying speeds, the following model was proposed in [61],

$$\begin{aligned} \frac{dG}{dt} &= \frac{T_{gx}(t - \tau) + T_{gh}(t)}{V_g} - T_{xg} \frac{G(t)}{0.1 + G(t)} - \left(K_{xgl} \int_0^\infty \omega(s)I(t-s)ds \right) G(t), \\ \frac{dI}{dt} &= \frac{T_{ig}G(t) + T_{ix}(t)}{V_i} - K_{xi}I(t), \end{aligned} \quad (51)$$

where

$$\begin{aligned} T_{gh}(t) &= T_{gh}^* \exp \left(-\lambda G(t) \int_0^\infty \omega(s)I(t-s)ds \right), \\ T_{gh}(0) &= T_{ghb} = T_{gh}^* e^{-\lambda G_b I_b} \end{aligned} \quad (52)$$

and

$$\omega(s) = \alpha^2 s e^{-\alpha s}; \quad T_{gx}(s) = 0 \quad \forall s \in [-\tau, 0]; \quad T_{ix}(0) = T_{ixb}. \quad (53)$$

Initial conditions are: $G(0) = G_b$ and $I(t) = I_b \quad \forall t \leq 0$. The input functions $T_{gx}(t)$ and $T_{ix}(t)$ are the glucose and the insulin infusion rates, and V_g and V_i are the volume of distribution for glucose and insulin, respectively. K_{xgl} is the insulin-dependent first-order rate constant for glucose tissue uptake, and K_{xi} is the first-order rate constant for insulin removal from plasma.

The variation of glucose concentration is due to the external glucose infusion rate, to liver glucose output $T_{gh}(t)$, and to delayed-insulin-dependent as well as insulin-independent glucose tissue uptake. Here, T_{xg} is the maximal insulin-independent rate constant for glucose tissue uptake, and the constant 0.1 in the denominator is scaled to the units of glycemia, mM. Infused glucose raises glycemia after a delay τ due to the necessity of equilibrating the intravenously infused quantity throughout the distribution space. The net hepatic glucose output is assumed to be equal to T_{ghb} at the beginning of the experiment and to decrease toward zero as glycemia or insulinemia levels increase. Serum insulin, in a delayed fashion depending on its transport to the periphery and to the subsequent activation of cellular membrane glucose transporters, affects glucose clearance through (51) and the net hepatic glucose release rate through (52).

The kernel $\omega(s)$ represents the density of metabolic effect at time t for unit serum insulin concentration at time $t - s$ ($s \leq t$). It increases to a maximum at $s = 1/\alpha$, then decreases monotonically and asymptotically to zero. This represents the metabolic effect of insulin having to reach the tissues and activate intracellular enzymatic mechanisms (hence a delay in maximal action on glucose metabolism), and that natural breakdown of insulin induces progressive loss of effect of the hormone. A high α -value determines a concentrated kernel, corresponding to a fast-rising, fast-decaying effect of insulin on peripheral tissues.

The insulin-independent glucose tissue uptake process is modeled as a Hill function rapidly increasing to its (asymptotic) maximum value T_{xg} ; thus for glycemia values near 2 mM the insulin-independent glucose tissue uptake is already close to its maximum. This represents the aggregated apparent zero-order glucose utilization mechanism at rest (mainly the brain and heart), with the mathematical and physiological necessity that glucose uptake go to zero as glucose concentration in plasma approaches zero. The variation of insulin concentration is due to the external insulin infusion, to glucose dependent pancreatic insulin secretion and to the apparent first-order insulin removal from plasma.

Eq. (52) represents the rate of net hepatic glucose output, taking its maximal value T_{gh} at zero glucose and zero insulin and decaying monotonically with increases in both glucose and effective insulin plasma concentrations.

Steady-state conditions are used to decrease the number of free parameters to be estimated. At steady state, before the start of the clamp, we have

$$T_{xg} = \left(\frac{T_{ghb}}{V_g} - K_{xgl} I_b G_b \right) \frac{0.1 + G_b}{G_b}; \quad T_{iG} = \frac{K_{xi} I_b V_i}{G_b}. \quad (54)$$

Therefore the parameters T_{xg} and T_{iG} are completely determined by the values of the other parameters.

3.3.2. Stochastic models

As an alternative to the above deterministic model, a stochastic model was proposed in [63]. Assume that the underlying tissue glucose uptake process is not smooth, subject as it is to a variety of metabolic and hormonal influences, which change over time. In fact, tissue glucose uptake is determined not only by the varying concentrations of certain hormones (e.g. cortisol or growth hor-

mone) and by the rhythm of food intake, events which take place over periods of hours, but also by sudden changes in physical activity or emotional stresses induced by thought processes. Thus, the rate constant K_{xgl} is likely to exhibit irregular oscillations over time.

Let the parameter K_{xgl} vary randomly as $(K_{xgl} - \xi(t))$, where $\xi(\cdot)$ is a gaussian white-noise process. The system noise $\xi(t)dt$ can be written as $\sigma dW(t)$, where $\sigma > 0$ is the diffusion coefficient and W is a Wiener process. By incorporating the K_{xgl} variation into the deterministic model without delays, we obtain the following (Itô) stochastic differential equation

$$\begin{aligned} dG(t) &= \left[\frac{(T_{gx}(t - \tau_g) + T_{gh}(t))}{V_g} - T_{xg} \frac{G(t)}{0.1 + G(t)} - K_{xgl} G(t) I(t) \right] dt \\ &\quad + \sigma G(t) I(t) dW(t), \\ dI(t) &= \left[\frac{(T_{iG} G(t) + T_{ix}(t))}{V_i} - K_{xi} I(t) \right] dt, \end{aligned} \quad (55)$$

with

$$T_{gh}(t) = T_{gh}^* e^{-\lambda G(t) I(t)}; \quad T_{gh}(0) = T_{ghb} = T_{gh}^* e^{-\lambda G_b I_b} \quad (56)$$

and initial conditions $G(0) = G_b, I(0) = I_b$. All coordinates of this process stay positive.

Considerable simplification can be obtained in the stochastic model by only modeling the dynamics after insulin has reached its steady-state, as proposed in [62]. Then a one-dimensional model is sufficient, and insulin concentration is assumed constant and equal to the average insulinemia I^* . Consider the glucose dynamics described by

$$dG(t) = \left(\frac{T_{gx}(t - \tau_g) + T_{ghnet}}{V_g} - K_{xgl} I^* G(t) \right) dt + \sigma I^* G(t) dW_t, \quad (57)$$

where $G(t_0) = G_0$ is the recorded glycemia at time $t_0 = 40$ min for the given subject. The glucose infusion rate $T_{gx}(\cdot)$ is approximated by a smoothed step function, whose values $\lambda_1, \dots, \lambda_m$ change at times $0 = v_1 < v_2 < \dots < v_m$. It is defined as:

$$T_{gx}(t) = \sum_{v_j \leq t} \frac{(\lambda_j - \lambda_{j-1}) \cdot (t - v_j)^5}{v_j + (t - v_j)^5}, \quad t > 0, \quad \lambda_0 = 0, \quad j = 1, \dots, m, \quad (58)$$

where $T_{gx}(t) = 0$ for every $t \in [-\tau_g, 0], t = 0$ being the instant in which the insulin infusion starts (to be distinguished from the t_0 instant, which equals 40 min). The exponent of $(t - v_j)$ in (58) has been chosen to be the minimum integer such that the average of $\{|T_{gx}(v_j) - \lambda_j|\}_{j=1, \dots, m}$ is less than 3×10^{-3} .

The EHC procedure attempts to reach steady-state with a constant blood glucose concentration and infusion rate. Thus, it is reasonable to assume that the process is stationary towards the end of the experiment, which can be used to determine one parameter from the others. Thus, from (57) above, an estimate of T_{ghnet} is given by $T_{ghnet} = K_{xgl} I^* G^* V_g - T_{gx}^*$. $T_{gx}(\cdot)$ only depends on t and is bounded between 0 and 5 mmol/min/KgBW, approximately corresponding to the pump infusion of 50% glucose in water at a maximal rate of 100 ml in a subject of small size (50 kg). Thus, the model (57) and (58) fulfills the usual Lipschitz condition and linear growth bound, so that it has a unique t -continuous solution. Since $T_{gx}(\cdot)$ changes over time as an external forcing function, the distribution of $G(t)$ will depend on t and thus not be stationary.

When $T_{gx}(\cdot)$ is constant, the stationary distribution of $X_\infty := \lim_{t \rightarrow \infty} X_t$ is an Inverse Gamma distribution with shape parameter $1 + 2K_{xgl}/\sigma^2 I^*$ and scale parameter $2(T_{gx} + T_{ghnet})/V_g (\sigma I^*)^2$ (if X is Inverse Gamma, then $1/X$ is Gamma). The asymptotic mean glycemia is $G^* = (T_{ghnet} + T_{gx}^*)/(K_{xgl} I^* V_g)$, where T_{gx}^* is the

mean glucose infusion rate over the last hour of the experiment. This can be used as an alternative measure of insulin sensitivity.

4. Long-term diabetes

Models for metabolism in diabetes mellitus, in particular models for the glucose–insulin control system, typically span the (short) time along which perturbation experiments (IVGTT, OGTT or clamp) are performed and blood concentrations of metabolites are obtained. Longer time spans are used when assessing the response to antidiabetic therapy, during ‘classical’ clinical trials: in this case observation times can arrive to up to four years. It is evident that organizational and economic difficulties essentially prohibit the conduction of long-term experimental studies, where patients are followed for decades in the attempt to identify the mechanism of the development of diabetes. Still, a number of rational, physiology based hypotheses can be made regarding these mechanisms, extrapolating the existing limited-time experimental data. Among such hypotheses, of particular interest are those regarding the variation of the beta-cell mass in a given individual, its sensitivity to glucose toxicity (the assumed effect which high glycemia have in depressing beta-cell net replication rate), and its possible response to therapeutic interventions. The information available in this context is limited also by the fact that reliable non-destructive methods do not yet exist, which allow the quantification of the current size of the beta-cell mass in a given individual.

In this framework, mathematical modeling allows a coherent set of assumptions to be combined in a quantitative way. Even though parameter estimation is essentially impossible, given the timescale over which the development of diabetes mellitus takes place and in particular given the practical impossibility of observing the size of the beta-cell population, useful information can be obtained from a combination of qualitative behavior conclusions and of numerical simulations based upon the assessment of key model parameters from published results.

Not many models regarding the longtime development of diabetes have been published. The main contributions so far can be ascribed to the work of de Winter and colleagues [28], of Topp and colleagues [86], and of De Gaetano and colleagues [27].

The principles at the basis of short term and long term modeling may not be the same. The most common paradigm upon which short term metabolic modeling is built is that of mass action. According to this principle the system under investigation can be represented as a set of interconnected compartments and the flow of material from one another is regulated by simple mass balance considerations. While the validity of this approach seems unquestionable in the short term, extending a mass balance based model by simply reinterpreting time as representing “slow time” (i.e. months to years) instead of “fast time” (i.e. minutes to hours) seems wholly inappropriate. While, for instance, plasma glucose one minute after clearly depends in a mass balance way on plasma glucose one minute before, the same cannot be said of glucose one month after with respect to glucose one month before. In slow time the mass transfers accumulate and the variable values represent integrated influences. For this reason, the authors disagree with the model proposed by de Winter and associates, which does represent essentially an IVGTT model carried over to slow time.

The model here described in some details was published in 2008 [27] and represents an advancement over a previous model by Topp and colleagues, [86], with which it shares the basic philosophy.

In T2DM a progressive increase of glycemia is likely determined by progressively increasing insulin resistance at the level of both peripherals tissues and liver. In the face of this increasing insulin resistance, the pancreas is unable to increase its insulin output be-

yond a certain level. In fact, possibly due to glucose toxicity, replicating ability in the pancreatic beta-cells decreases and eventually beta-cell mass declines, with the eventual development of the frank clinical picture of diabetes. It is therefore essential that a mathematical model for the development of diabetes includes such key variables as beta-cell population, prevailing insulinemia and glycemia, and pancreatic beta-cell replication reserve.

The variation of beta-cell population, indicated as $B(t)$, follows a simple exponential growth:

$$\frac{dB}{dt} = \lambda B(t), \quad B(t_0) = B_0, \quad (59)$$

where the parameter λ is a function of prevailing glucose levels, through a third order Hill function, and depends on the current pancreatic reserve η :

$$\lambda(G) = \lambda_{\min} + \eta \frac{x^3}{1 + x^3}, \quad x(G) = x_0 \frac{G}{G_\lambda}. \quad (60)$$

In this way higher glucose levels stimulate beta-cell replication, in a way which is consistent with the controller action of the endocrine pancreas. In its turn, however, pancreatic reserve η follows a dynamics determined by a natural tendency to be restored, but affected negatively by high prevailing glucose concentrations through a glucose toxicity parameter $K_{\eta G}$.

$$\frac{d\eta}{dt} = -K_{\eta G}(G)\eta(t) + T_\eta, \quad \eta(t_0) = \eta_0. \quad (61)$$

Prevailing glucose and insulin levels are obtained by setting at equilibrium one of many possible models of fast glucose–insulin control:

$$G = \frac{\gamma}{\rho + I}, \quad I = h(G)I_{\max}B. \quad (62)$$

In fact, it can be shown that many such fast models, when taken at equilibrium, give rise to the same algebraic slow model. In this sense, the slow model proposed is a representative of an entire equivalence class of possible fast homeostatic models. The function h introduces the nonlinear, indeed saturable, dependency of insulin secretion upon glucose levels:

$$h(G) = \frac{\left(\frac{G}{G_h}\right)^{v_h}}{1 + \left(\frac{G}{G_h}\right)^{v_h}}. \quad (63)$$

The equations reported above express the main ideas underlying the model.

Given the practical impossibility of estimating parameter values from data sets, the parameter values with which the model has been made to run have been derived from a careful scrutiny of the existing literature, resulting in several instances what were perceived to be contradictions in the reported results. Just to make one example, reports of beta-cell apoptosis rates from cadaver preparations were felt to be excessive, and incompatible with other reported results on beta-cell replication rates in that if apoptosis were as high and replication as low as reported, then everybody would become type 1 diabetic in a very short time. In this case, the reported replication rate was assumed to represent both replication and apoptosis at equilibrium in the living human.

One of the main successes of the model has been its ability to predict what is commonly clinically observed, and for which a direct biologic explanation had been long searched for: the rapid development of diabetes after many years of essentially normal glycemia. The model predicts that prolonged very mild hyperglycemia, such as can be observed because of progressively increasing insulin resistance (due to accumulation of fat tissue, dietary habits and lack of physical activity) progressively undermines pancreatic

replication reserve. As long as pancreatic reserve is still sufficient to allow for progressively increasing ISRs, no major increase in blood glucose is observed. A point is reached, however, when pancreatic replication rates cannot sustain further increase in insulin secretion, at which point glycemia starts to rise, glucose toxicity is intensified and the entire compensation mechanism fails, with the apparent sudden development of frank diabetes. No specific determinants of the crisis need therefore to be looked for: the very nonlinear structure of the compensation mechanism determines an apparent normality for a long time in the face of actually worsening homeostatic derangement, followed by a rapidly accelerated decompensation once reserves have been exhausted.

5. Conclusions

Partly due to the high social impact of Diabetes (particularly of T2DM in industrialized societies, given its link with obesity), partly due to the potential simplicity in describing mathematically a two-variable system, the glucose–insulin homeostatic control has been one of the most intensely modeled biomedical problems. Modeling attempts to summarize the main features of the system, to improve the identification of the mechanisms involved, to make more reliable the prediction of the future condition of a given patient. Its applications are a more effective prevention or delay of the occurrence of frank diabetes mellitus, and the development of safer automatic insulin infusion devices for T1DM patients.

Given the vastity of the field and the large number of important results, which many authors contributed over the past four decades or so, it has been impossible to present all facets of the problem. In particular, many bottom-up, molecular mechanistic models have attempted to describe the biochemical chain of reactions giving rise to insulin secretion at the beta-cell level, and glucose uptake at the tissue level. These are not presented in this article.

Throughout the paper, a leading thread has been that while every model is an approximation to reality, these models interpret actual medical observations on the basis of shared or debated assumptions, offering to the critical reader a handle for further hypotheses and experimentation. In their counterpoint and in their chorality the voices of the many authors who worked in this field compose a very interesting chapter in the field of the application of mathematics to medical problems.

Acknowledgement

S. Ditlevsen is supported by the Danish Council for Independent Research Natural Sciences.

References

- [1] E. Ackerman, J.W. Rosevear, W.F. McGuckin, A mathematical model of the glucose tolerance test, *Phys. Med. Biol.* 9 (2) (1964) 203.
- [2] E. Ackerman, L.C. Gatewood, J.W. Rosevear, G.D. Molnar, Model studies of blood-glucose regulation, *Bull. Math. Biophys.* 27 (1965) S21.
- [3] G.E. Anderson, Y. Koglu, C. Papadopoulos, Fluctuations in postabsorptive blood glucose in relation to insulin release, *Metabolism* 16 (1967) 586.
- [4] A. Basu, C. Dalla Man, R. Basu, G. Toffolo, C. Cobelli, R.A. Rizza, Effects of type 2 diabetes on insulin secretion, insulin action, glucose effectiveness, and postprandial glucose metabolism, *Diabetes Care* 32 (2009) 866.
- [5] D.L. Bennett, S.A. Gourley, Asymptotic properties of a delay differential equation model for the interaction of glucose with plasma and interstitial insulin, *Appl. Math. Comput.* 151 (2004) 189.
- [6] R.N. Bergman, Y.Z. Ider, C.R. Bowden, C. Cobelli, Quantitative estimation of insulin sensitivity, *Am. J. Physiol.* 236 (1979) E667.
- [7] R.N. Bergman, in: R.N. Bergman, J.C. Lovejoy (Eds.), *The Minimal Model Approach and Determination of Glucose Tolerance*, LSU Press, 1997, pp. 3–50.
- [8] R.N. Bergman, The minimal model of glucose regulation: a biography, in: J. Novotny, M. Green, R. Boston (Eds.), *Mathematical Modeling in Nutrition and Health*, Kluwer Academic/Plenum, 2001.
- [9] R. Bertram, L. Satin, M. Zhang, P. Smolen, A. Sherman, Calcium and glycolysis mediate multiple bursting modes in pancreatic islets, *Biophys. J.* 87 (2004) 3074.
- [10] R. Bertram, A. Sherman, A calcium-based phantom bursting model for pancreatic islets, *Bull. Math. Biol.* 66 (2004) 1313.
- [11] A. Bertuzzi, S. Salinari, G. Mingrone, Insulin granule trafficking in β -cells: mathematical model of glucose-induced insulin secretion, *Am. J. Physiol. Endocrinol. Metab.* 293 (2007) E396.
- [12] V.W. Bolie, Coefficients of normal blood glucose regulation, *J. Appl. Physiol.* 16 (1961) 783.
- [13] E. Breda, M.K. Cavaghan, G. Toffolo, K.S. Polonsky, C. Cobelli, Oral glucose tolerance test minimal model indexes of β -cell function and insulin sensitivity, *Diabetes* 50 (2001) 150.
- [14] P.L. Brubaker, E.L. Ohayon, L.M. D'Alessandro, K.H. Norwich, A mathematical model of the oral glucose tolerance test illustrating the effects of the incretins, *Ann. Biomed. Eng.* 35 (2007) 1286.
- [15] A. Caumo, R.N. Bergman, C. Cobelli, Insulin sensitivity from meal tolerance tests in normal subjects: a minimal model index, *J. Clin. Endocrinol. Metab.* 85 (2000) 4396.
- [16] E. Cerasi, G. Fick, M. Rudemo, A mathematical model for the glucose induced insulin release in man, *Eur. J. Clin. Invest.* 4 (1974) 267.
- [17] C.-L. Chen, H.-W. Tsai, Modeling of physiological glucose–insulin system on normal and diabetic subjects, *Comput. Methods Programs Biomed.* 97 (2010) 130.
- [18] C.-L. Chen, H.-W. Tsai, S.-S. Wong, Modeling of physiological glucose–insulin dynamic system on diabetics, *J. Theor. Biol.* 265 (2010) 314.
- [19] H.F. Chou, E. Ipp, Pulsatile insulin secretion in isolated rat islets, *Diabetes* 39 (1990) 112.
- [20] C. Cobelli, G.M. Toffolo, C. Dalla Man, M. Campioni, P. Denti, A. Caumo, P. Butler, R. Rizza, Assessment of beta cell function in humans, simultaneously with insulin sensitivity and hepatic extraction, from intravenous and oral glucose test, *Am. J. Physiol. Endocrinol. Metab.* 293 (2007) E1.
- [21] C. Dalla Man, A. Caumo, C. Cobelli, The oral glucose minimal model: estimation of insulin sensitivity from a meal test, *IEEE Trans. Biomed. Eng.* 49 (2002) 419.
- [22] C. Dalla Man, A. Caumo, R. Basu, R. Rizza, G. Toffolo, C. Cobelli, Minimal model estimation of glucose absorption and insulin sensitivity from oral test: validation with a tracer method, *Am. J. Physiol. Endocrinol. Metab.* 287 (2004) E637.
- [23] C. Dalla Man, K.E. Yarasheski, A. Caumo, H. Robertson, G. Toffolo, K.S. Polonsky, C. Cobelli, Insulin sensitivity by oral glucose minimal models: validation against clamp, *Am. J. Physiol. Endocrinol. Metab.* 289 (2005) E954.
- [24] C. Dalla Man, F. Micheletto, A. Sathananthan, R.A. Rizza, A. Vella, C. Cobelli, A model of GLP-1 action on insulin secretion in nondiabetic subjects, *Am. J. Physiol. Endocrinol. Metab.* 298 (2010) E1115.
- [25] R.A. DeFronzo, J.D. Tobin, R. Andres, Glucose clamp technique: a method for quantifying insulin secretion and resistance, *Am. J. Physiol.* 237 (1979) E214.
- [26] A. De Gaetano, O. Arino, Mathematical modelling of the intravenous glucose tolerance test, *J. Math. Biol.* 40 (2000) 136.
- [27] A. De Gaetano, T. Hardy, B. Beck, E. Abu-Raddad, P. Palumbo, J. Bue-Valleskey, N. Prksen, Mathematical models of diabetes progression, *Am. J. Physiol. Endocrinol. Metab.* 295 (6) (2008) E1462.
- [28] W. de Winter, J. Dejongh, T. Post, B. Ploeger, R. Urquhart, I. Moules, D. Eckland, M. Danhof, A mechanism-based disease progression model for comparison of long-term effects of pioglitazone, metformin and gliclazide on disease processes underlying Type 2 Diabetes Mellitus, *J. Pharmacokinet. Pharmacodyn.* 33 (2006) 313.
- [29] G.T. Eddlestone, A. Goncalves, J.A. Bangham, Electrical coupling between cells in islets of Langerhans from Mouse, *J. Membr. Biol.* 77 (1984) 1.
- [30] L. Eliasson, F. Abdulkader, M. Braun, J. Galvanovskis, M.B. Hoppa, P. Rorsman, Novel aspects of the molecular mechanisms controlling insulin secretion, *J. Physiol.* 586 (2008) 3313.
- [31] K. Engelborghs, V. Lemaire, J. Bélair, D. Roose, Numerical bifurcation analysis of delay differential equations arising from physiological modeling, *J. Math. Biol.* 42 (2001) 361.
- [32] E. Ferrannini, O. Bjorkman, G.A. Reichard Jr., A. Pilo, M. Olsson, J. Wahren, R.A. DeFronzo, The disposal of an oral glucose load in healthy subjects: a quantitative study, *Diabetes* 34 (1985) 580.
- [33] E. Ferrannini, D.C. Simonson, L.D. Katz, G. Reichard Jr., S. Bevilacqua, E.J. Barrett, M. Olsson, R.A. DeFronzo, The disposal of an oral glucose load in patients with non-insulin-dependent diabetes, *Metabolism* 37 (1988) 79.
- [34] L.C. Gatewood, E. Ackerman, J.W. Rosevear, G.D. Molnar, Test of a mathematical model of the blood-glucose regulatory system, *Comput. Biomed. Res.* 2 (1968) 1.
- [35] P. Gilon, M.A. Ravier, J.C. Jonas, J.C. Henquin, Control mechanisms of the oscillations of insulin secretion in vitro and in vivo, *Diabetes* 51 (2002) S144.
- [36] C.J. Goodner, B.C. Walike, D.J. Koerker, J.W. Ensink, A.C. Brown, E.W. Chidekel, J. Palmer, L. Kalnasy, Insulin, glucagon and glucose exhibit synchronous sustained oscillations in fasting monkeys, *Science* 195 (1977) 177.
- [37] G.M. Grodsky, A threshold distribution hypothesis for packet storage of insulin and its mathematical modeling, *J. Clin. Invest.* 51 (1972) 2047.
- [38] J.C. Henquin, Triggering and amplifying pathways of regulation of insulin secretion by glucose, *Diabetes* 49 (2000) 1751.
- [39] J.C. Henquin, Regulation of insulin secretion by glucose: a matter of phase control and amplitude modulation, *Diabetologia* 52 (2009) 739.
- [40] J. Keener, J. Sneyd, *Mathematical Physiology*, Springer-Verlag, New York, 1998.
- [41] D.A. Lang, D.R. Matthews, J. Peto, R.C. Turner, Cyclic oscillations of basal plasma glucose and insulin concentrations in human beings, *N. Engl. J. Med.* 301 (1979) 1023.

- [42] J. Li, Y. Kuang, Analysis of IVGTT glucose–insulin interaction models with time delay, *Discrete Contin. Dyn. Syst. Ser. B* 1 (1) (2001) 103.
- [43] J. Li, Y. Kuang, Analysis of a model of the glucose–insulin regulatory system with two delays, *SIAM J. Appl. Math.* 67 (3) (2007) 757.
- [44] J. Li, Y. Kuang, C.C. Mason, Modeling the glucose–insulin regulatory system and ultradian insulin secretory oscillations with two explicit time delays, *J. Theor. Biol.* 242 (2006) 722.
- [45] J. Li, M. Wang, A. De Gaetano, P. Palumbo, S. Panunzi, The range of time delay and the global stability of the equilibrium for an IVGTT model, *Math. Biosci.* 235 (2012) 128.
- [46] A. Mari, S. Camastra, E. Toschi, A. Giancaterini, A. Gastaldelli, G. Mingrone, E. Ferranini, A model for glucose control of insulin secretion during 24 hours of free living 50 (2001) S164.
- [47] A. Mari, A. Tura, A. Gastaldelli, E. Ferrannini, Assessing insulin secretion by modeling in multiple-meal tests: role of potentiation, *Diabetes* 51 (2002) S221.
- [48] H.P. Meissner, Electrophysiological evidence for coupling between beta-cells of pancreatic islets, *Nature* 262 (1976) 502.
- [49] K. Millsaps, K. Pohlhausen, A mathematical model for glucose–insulin interaction, *Math. Biosci.* 23 (1975) 237.
- [50] A. Mukhopadhyay, A. De Gaetano, O. Arino, Modelling the intra-venous glucose tolerance test: a global study for a single-distributed-delay model, *Discrete Contin. Dyn. Syst. Ser. B* 4 (2) (2004) 407.
- [51] E. Muscelli, A. Mari, A. Natali, B.D. Astiarraga, S. Camastra, S. Frascerra, J.J. Holst, E. Ferrannini, Impact of incretin hormones on β -cell function in subjects with normal or impaired glucose tolerance, *Am. J. Physiol. Endocrinol. Metab.* 291 (2006) E1144.
- [52] E. Muscelli, A. Mari, A. Casolaro, S. Camastra, G. Seghieri, A. Gastaldelli, J.J. Holst, E. Ferrannini, Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients, *Diabetes* 57 (2008) 1340.
- [53] P. Palumbo, A. De Gaetano, An islet population model for the endocrine pancreas, *J. Math. Biol.* 61 (2) (2010) 171.
- [54] P. Palumbo, S. Panunzi, A. De Gaetano, Qualitative behavior of a family of delay-differential models of the glucose–insulin system, *Discrete Contin. Dyn. Syst. Ser. B* 7 (2) (2007) 399.
- [55] S. Panunzi, A. De Gaetano, G. Mingrone, Advantages of the single delay model for the assessment of insulin sensitivity from the intra-venous glucose tolerance test, *Theor. Biol. Med. Model.* 7 (9) (2010).
- [56] S. Panunzi, P. Palumbo, A. De Gaetano, A discrete single delay model for the intra-venous glucose tolerance test, *Theor. Biol. Med. Model.* 4 (2007).
- [57] M.G. Pedersen, R. Bertram, A. Sherman, Intra- and inter-islet synchronization of metabolically driven insulin secretion, *Biophys. J.* 89 (2005) 107.
- [58] M.G. Pedersen, A. Corradin, G.M. Toffolo, C. Cobelli, A subcellular model of glucose-stimulated pancreatic insulin secretion, *Phil. Trans. R. Soc. A* 366 (2008) 3525.
- [59] M.G. Pedersen, A. Sherman, Newcomer insulin secretory granules as a highly calcium-sensitive pool, *Proc. Natl. Acad. Sci. USA* 106 (2009) 7432.
- [60] M.G. Pedersen, G.M. Toffolo, C. Cobelli, Cellular modeling: insight into oral minimal models of insulin secretion, *Am. J. Physiol. Endocrinol. Metab.* 298 (2010) E597.
- [61] U. Picchini, A. De Gaetano, S. Panunzi, S. Ditlevsen, G. Mingrone, A mathematical model of the euglycemic hyperinsulinemic clamp, *Theor. Biol. Med. Model.* 2 (2005) 44.
- [62] U. Picchini, S. Ditlevsen, A. De Gaetano, Maximum likelihood estimation of a time-inhomogeneous stochastic differential model of glucose dynamics, *Math. Med. Biol.* 25 (2008) 141.
- [63] U. Picchini, S. Ditlevsen, A. De Gaetano, Modeling the euglycemic hyperinsulinemic clamp by stochastic differential equations, *J. Math. Biol.* 53 (5) (2006) 771.
- [64] K.S. Polonski, B.D. Given, E. Van Cauter, Twenty four hour profiles and pulsatile patterns of insulin secretion in healthy and obese subjects, *J. Clin. Invest.* 81 (1988) 442.
- [65] N. Pørksen, The in vivo regulation of pulsatile insulin secretion, *Diabetologia* 45 (2002) 3.
- [66] N. Pørksen, C. Juhl, M. Hollingdal, S.M. Pincus, J. Sturis, J.D. Veldhuis, O. Schmitz, Concordant induction of rapid in vivo pulsatile insulin secretion by recurrent punctuated glucose infusions, *Am. J. Physiol. Endocrinol. Metab.* 278 (2000) E162.
- [67] N. Pørksen, S. Munn, J. Steers, S. Vore, J. Veldhuis, P. Butler, Pulsatile insulin secretion accounts for 70% of total insulin secretion during fasting, *Am. J. Physiol.* 269 (1995) E478.
- [68] N. Pørksen, B. Nyholm, J.D. Veldhuis, P.C. Butler, O. Schmitz, In humans at least 75% of Insulin Secretion arises from punctuated insulin secretory bursts, *Am. J. Physiol. Endocrinol. Metab.* 273 (1997) E908.
- [69] M.A. Ravier, A.J. Sehlin, J.C. Henquin, Disorganization of cytoplasmic Ca^{2+} oscillations and pulsatile insulin secretion in islets from ob/ob mice, *Diabetologia* 45 (2002) 1154.
- [70] P. Rorsman, E. Renström, Insulin granule dynamics in pancreatic beta cells, *Diabetologia* 46 (2003) 1029.
- [71] R.M. Santos, L.M. Rosario, A. Nadal, J. Garcia-Sancho, B. Soria, M. Valdeolmillos, Widespread synchronous $[\text{Ca}^{2+}]_i$ oscillations due to bursting electrical activity in single pancreatic islets, *Pflügers Arch.* 418 (1991) 417.
- [72] J. Schirra, M. Katschinski, C. Weidmann, T. Schäfer, U. Wank, R. Arnold, B. Göke, Gastric emptying and release of incretin hormones after glucose ingestion in humans, *J. Clin. Invest.* 97 (1996) 92.
- [73] E.T. Shapiro, H. Tillil, K.S. Polonsky, V.S. Fang, A.H. Rubenstein, E. Van Cauter, Oscillations in insulin secretion during constant glucose infusion in normal man: relationship to changes in plasma glucose, *J. Clin. Endocrinol. Metab.* 67 (1988) 307.
- [74] T. Shibasaki, H. Takahashi, T. Miki, Y. Sunaga, K. Matsumura, M. Yamanaka, C. Zhang, A. Tamamoto, T. Satoh, J. Miyazaki, S. Seino, Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP, *Proc. Natl. Acad. Sci. USA* 104 (2007) 19333.
- [75] C. Simon, G. Brandenberger, M. Follenius, Ultradian oscillations of plasma glucose, insulin, and C-peptide in man during continuous enteral nutrition, *J. Clin. Endocrinol. Metab.* 64 (1987) 669.
- [76] C. Simon, M. Follenius, G. Brandenberger, Postprandial oscillations of plasma glucose, insulin and C-peptide in man, *Diabetologia* 30 (1987) 769.
- [77] P. Smolen, A model for glycolytic oscillations based on skeletal muscle phosphofructokinase kinetics, *J. Theor. Biol.* 174 (1995) 137.
- [78] J.I. Stagner, E. Samols, G.C. Weir, Sustained oscillations of insulin, glucagon and somatostatin from the isolated canine pancreas during exposure to a constant glucose concentration, *J. Clin. Invest.* 65 (1980) 939.
- [79] S.G. Straub, G.W.G. Sharp, Hypothesis: one rate-limiting step controls the magnitude of both phases of glucose-stimulated insulin secretion, *Am. J. Physiol. Cell Physiol.* 287 (2004) C565.
- [80] J. Sturis, K.S. Polonsky, E. Mosekilde, E. Van Cauter, Computer model for mechanisms underlying ultradian oscillations of insulin and glucose, *Am. J. Physiol.* 260 (1991) 439.
- [81] J. Sturis, W.L. Pugh, J. Tang, D.M. Ostrega, J.S. Polonsky, K.S. Polonsky, Alterations in pulsatile insulin secretion in the Zucker diabetic fatty rat, *Am. J. Physiol.* 267 (1994) E250.
- [82] J. Sturis, J.D. Blackman, E. Van Cauter, K.S. Polonski, Entrainment of pulsatile insulin secretion by oscillatory glucose infusion, *J. Clin. Invest.* 87 (1991) 439.
- [83] K. Thomaseth, A. Kautzky-Willer, B. Ludvik, R. Prager, G. Pacini, Integrated mathematical model to assess β -cell activity during the oral glucose test, *Am. J. Physiol. Endocrinol. Metab.* 270 (1996) E522.
- [84] G. Toffolo, R.N. Bergman, D.T. Finegood, C.R. Bowden, C. Cobelli, Quantitative estimation of beta cells sensitivity to glucose in the intact organism: a minimal model of insulin kinetics in dog, *Diabetes* 29 (1980) 979.
- [85] I.M. Tolić, E. Mosekilde, J. Sturis, Modeling the insulin–glucose feedback system: the significance of pulsatile insulin secretion, *J. Theor. Biol.* 207 (2000) 361.
- [86] B. Topp, K. Promislow, G. deVries, R.M. Miura, D.T. Finegood, A model of beta-cell mass, insulin, and glucose kinetics: pathways to diabetes, *J. Theor. Biol.* 206 (2000) 605.
- [87] E. Toschi, S. Camastra, A.M. Sironi, A. Masoni, A. Gastaldelli, A. Mari, E. Ferranini, A. Natali, Effect of acute hyperglycemia on insulin secretion in humans 51 (2002) S130.
- [88] K. Tsaneva-Atanasova, C.L. Zimlik, R. Bertram, A. Sherman, Diffusion of calcium and metabolites in pancreatic islets: killing oscillations with a pitchfork, *Biophys. J.* 90 (2006) 3434.
- [89] A. Tura, B. Ludvik, J.J. Nolan, A. Pacini, K. Thomaseth, Insulin and C-peptide secretion and kinetics in humans: direct and model-based measurements during OGTT, *Am. J. Physiol. Endocrinol. Metab.* 281 (2001) E966.
- [90] M. Valdeolmillos, A. Gomis, J.V. Sanchez-Andres, In vivo synchronous membrane potential oscillations in Mouse pancreatic β -cells: lack of co-ordination between islets, *J. Physiol.* 493 (1996) 9.
- [91] E. Van Cauter, D. Desir, C. Decoster, F. Féry, E.O. Balasse, Nocturnal decrease of glucose tolerance during constant glucose infusion, *J. Clin. Endocrinol. Metab.* 69 (1989) 604.
- [92] E. Van Cauter, F. Mestrez, J. Sturis, K.S. Polonsky, Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance, *Diabetes* 41 (1992) 368.
- [93] H. Wang, J. Li, Y. Kuang, Mathematical modelling and qualitative analysis of insulin therapies, *Math. Biosci.* 210 (2007) 17.
- [94] H. Wang, J. Li, Y. Kuang, Enhanced modelling of the glucose–insulin system and its application in insulin therapies, *J. Biol. Dynam.* 3 (1) (2009) 22.
- [95] H.-J. Woerle, M. Albrecht, R. Linke, S. Zschau, C. Neumann, M. Nicolaus, J. Gerich, B. Göke, J. Schirra, Importance of changes in gastric emptying for postprandial glucose fluxes in healthy humans, *Am. J. Physiol. Endocrinol. Metab.* 294 (2008) E103.
- [96] Y. Yang, K.D. Gillis, A highly Ca^{2+} -sensitive pool of granules is regulated by glucose and protein kinases in insulin-secreting INS-1 cells, *J. Gen. Physiol.* 124 (2004) 653.
- [97] Wu Zimei, C.-K. Chui, G.-S. Hong, S. Chang, Physiological analysis on oscillatory behavior of glucose–insulin regulation by model with delays, *J. Theor. Biol.* 280 (2011) 1.