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Mathematical Beta Cell Model for Insulin Secretion following IVGTT and OGTT

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Abstract—Evaluation of beta cell function is conducted by a variety of glucose tolerance tests and evaluated by a number of different models with less than perfect consistency among results obtained from different tests. We formulated a new approximation of the distributed threshold model for insulin secretion in order to approach a model for quantifying beta cell function, not only for one, but for several different experiments. Data was obtained from 40 subjects that had both an oral glucose tolerance test (OGTT) and an intravenous tolerance test (IVGTT) performed. Parameter estimates from the two experimental protocols demonstrate similarity, reproducibility, and indications of prognostic relevance. Useful first phase indexes comprise the steady state amount of ready releasable insulin A_0 and the rate of redistribution k_{rd}, where both yield a considerable correlation (both $r = 0.67$) between IVGTT and OGTT estimates. For the IVGTT, A_0 correlates well ($r = 0.96$) with the 10 min area under the curve of insulin above baseline, whereas k_{rd} represents a new and possibly more fundamental first phase index. For the useful second phase index γ, a correlation of 0.75 was found between IVGTT and OGTT estimates.

Keywords—Parameter estimation, Pancreatic beta cell, Mixed-effects, Physiological models.

ABBREVIATIONS

AUC	Area under the curve
BG	Baseline glucose
BOV	Between occasion variability
BSV	Between subject variability
CV	Coefficient of variation
FDR	First degree relatives to patients with diabetes
HGC	Hyperglycaemic clamp
IVGTT	Intravenous tolerance test
MTT	Meal tolerance test
OGTT	Oral glucose tolerance test
RRI	Ready releasable insulin
RRP	Ready releasable pool
SE	Standard error

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INTRODUCTION

Type 2 diabetes is a heterogeneous disorder characterized by a combination of impaired insulin secretion and insulin resistance,[7] in which either factor can be dominant. Of these interrelated subjects, the present work deals with the assessment of beta cell function, which relative to insulin resistance must be impaired for development of type 2 diabetes, and may even represent the primary factor predisposing individuals to type 2 diabetes.[9] Insulin secretion in response to an abrupt increase in plasma glucose is known to be biphasic with a rapid peak at 2–4 min (first-phase), decrease to nadir at 10–15 min, and then gradually increase within the next couple of hours (second-phase). Early insulin release after glucose ingestion is a key determining factor for the subsequent glucose concentration,[3] indicating that a reduced first-phase may be responsible for the development of impaired glucose tolerance.[6]

Evidently, diagnostic tests for the assessment of insulin secretion as well as insulin resistance have great value for epidemiological and clinical studies. The most common oral administration tests are the oral glucose tolerance test (OGTT) and the meal tolerance test (MTT), whereas the most common intravenous (IV) tests are the intravenous glucose tolerance test (IVGTT) and the hyperglycemic clamp (HGC). Several descriptive mathematical models and model based methods have been proposed to calculate indexes for characterization of beta cell function from the various tests.[2,17,27,28] Although useful for the analysis of a specific experiment type, the models can rarely be used across different tests, and similar indexes obtained from different experiments are not necessarily in agreement, leading to the conclusion that further work is needed for these indexes to be routinely used in clinical and epidemiological studies.[25]

Besides descriptive models for characterization, more comprehensive mathematical models[4,10,20] have been used to communicate and increase the understanding of the physiological mechanisms behind insulin secretion.[19] Of these, the distributed threshold hypothesis[10] has been used to argue and derive many of the descriptive models.[16,21] However, to our knowledge, descriptive insulin secretion models up until now all fail to incorporate the fundamental mechanisms that have enabled the distributed threshold hypothesis to describe insulin secretion in response to a long series of glucose challenges, and hereby increase understanding of the beta cell function. In the present work we formulate a model that includes threshold distribution, redistribution, and incretin effects, and investigate the applicability of this model to data from the IVGTT and the OGTT. At a longer perspective, the present work is a step towards a model for characterizing beta cell function, not only for one, but for many of the glucose tolerance tests; with parameters that are closer related to the physiology than those of more empirical models.

Model development and evaluation was performed on data from 40 individuals,[11,12] where each subject had both an IVGTT and an OGTT performed. Indexes obtained from the OGTT were compared to those from the IVGTT in order to demonstrate parameter reproducibility and similarity, giving credit to the applicability of indexes, e.g. in epidemiological studies. Indication of prognostic relevance was demonstrated by the extreme parameter values found for 4 subjects that subsequent to the study have developed type 2 diabetes, also other such parameters exist.[13]

RESEARCH DESIGN AND METHODS

A total of 40 healthy normoglycemic subjects had both an IVGTT and an OGTT performed; 20 subjects with no family history of diabetes and 20 first degree relatives (FDR) to patients with type 2 diabetes of which four subjects have developed type 2 diabetes themselves within 10 years of the initial investigation. The protocols were approved by the local Ethics Committee and informed written consents were obtained from all participants before testing both at the initial testing and at 10 years follow up. Clinical characteristics of the study populations are given in Table 1.

The OGTT was performed by ingestion of 75 g glucose in a liquid solution. Blood samples were obtained in the fasting state (three samples) and for a total of 3 h following the glucose load (15 samples). The IVGTT was performed, with an infusion of a 25% solution of glucose (300 mg of glucose per kg body weight (max 25 g)) being given over 1 min, immediately followed by a saline flush (50 ml). Time zero was taken as the start of the glucose bolus and samples were collected at −30, −20, −10, −1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 140, 160 and 180 min, for determination of plasma glucose and insulin. Plasma glucose concentration was measured at the bedside by the glucose oxidase method with a Glucose Analyzer (Beckman Instruments, Inc., Fullerton, Calif., USA). Blood samples for plasma insulin were immediately centrifuged at 4°C at the time of study and stored at −20°C until analysis and concentrations measured by a double antibody radioimmunoassay in duplicate (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden).

TABLE 1. Physical characteristics of the study subjects at initial examination.

	Relatives	Control subjects
N (F/M)	20 (8/12)	20 (8/12)
Age (yr)	29.4 ± 1.6	29.4 ± 1.7
HbA1c (%)	6.17 ± 0.13	6.12 ± 0.08
BMI (kg/m^2)	25.1 ± 1.0	25.1 ± 0.9
Weight (kg)	76.6 ± 3.3	78.8 ± 4.0
Fasting glucose (mM)	5.41 ± 0.08	5.16 ± 0.08
Fasting insulin (pmol/l)	45.6 ± 3.0	41.4 ± 3.0

Note. Values are mean ± SE. HbA1c: glycated haemoglobin (normal range: 5.4–7.4%); BMI: body mass index.

Model Description

Background

The distributed threshold hypothesis[10] successfully explains the dose dependent first phase insulin release following IV glucose administration with a pool of insulin stored in packets. According to this hypothesis, different packets have different thresholds, secreting insulin only when glucose concentration has exceeded this threshold. Changes in plasma glucose concentration will alter the distribution of insulin in the packets, while provision of new insulin and redistribution of insulin among packets ensures convergence toward steady state when glucose concentration is constant. In principle, each packet corresponds to a compartment, yielding a complicated numerical problem with a large number of coupled differential equations. The present simplification lump all active packets together and all passive packets together to a two-compartment system. A mathematical analysis of the differences and approximation between the present model and the distributed threshold model is given in Appendix A, whereas the following list summarizes the main differences:

1. Steady state provision was modeled with a sigmoid Emax function instead of the more complicated parametric function used in.[10]
2. Incretin effects during the OGTT were implemented for provision and packet activation.
3. The flow of insulin between passive and active packets is modeled to be unidirectional, i.e. all ready releasable insulin is released from an active packet before it is deactivated.
4. Redistribution was modeled to involve random activation rather than random change of threshold, see Appendix A.

5. All passive packets were approximated to contain the same amount of insulin.

The central approximation is that all passive packets contain the same amount of insulin. This will not be influential during simple glucose challenge tests, where glucose is single peaked. But for multiple glucose peaks, e.g. for glucose oscillations, the response of the threshold distribution hypothesis will be different from that of the present model.

Model Equations

As illustrated in Fig. 1, the model can be divided into four components, (1) provision of new insulin, (2) a pool of ready releasable insulin (RRI), i.e. insulin in passive packets available for quick release as a consequence of abruptly increasing glucose concentrations, (3) a pool of insulin in active packets, which is quickly being released, and (4) a plasma compartment that represents insulin pharmacokinetics.

The provision, P of new insulin is typically written as,

$$\frac{d}{dt}P = -\alpha(P - P(G,\infty))$$

$P(G,\ \infty)$ is the glucose dependent steady state provision, described below. Glucose (G) is implemented as the linear interpolation between measured concentrations of plasma glucose, except during the time interval between −1 and 1 min, where the baseline glucose (BG) value was used to ensure that the interpolated glucose does not rise prior to glucose administration at time zero.[21] When the system is not at steady state, e.g. due to rapid changes in glucose, $P(t)$ will be different from $P(G,\ \infty)$. Whereas changes in $P(G,\ \infty)$ are immediate, the changes in P will be delayed with time constant α, leading to smooth changes. Note that this delay is not the only contributing factor to the delay in the second phase insulin response, see Appendix B.

Oral ingestion of nutrients is known to enhance insulin secretion, the incretin effect, leading to higher insulin secretion during an OGTT than from an experiment with matched glucose concentrations obtained by IV infusion of glucose.[15] The incretin effect is mediated by insulinotropic intestinal hormones, as e.g. glucagon-like peptide-1, which enhances both first and second phase release, see e.g. the experiments by Fritsche *et al.*,[8] and consequently also the provision. We have

$$P(G,\infty) = \frac{E_{\max}G^h}{EC_{50}^h + G^h} + E_{OGTT}\frac{[G - BG]^+}{AUC_{G-BG}}$$

where the last term is the incretin effect, modeled with glucose above BG as a surrogate for incretin hormones, which are rarely measured in the OGTT. $[G - BG]^+$ is the maximum of zero and $(G - BG)$, and AUC_{G-BG} is the area under the curve (AUC) of $[G - BG]^+$. EC_{50} is calculated via the initialization given below. E_{OGTT} is the effect parameter for the incretin effect in an OGTT, which is zero for an IVGTT.

The two-compartment model for the passive and active pool, as derived in Appendix A, can be written:

$$\begin{aligned}
\tfrac{d}{dt}I_{passive} &= (1 - f(G))P - k_{\mathrm{rd}}I_{passive} - Ph_1\\
\tfrac{d}{dt}I_{active} &= f(G)P + k_{\mathrm{rd}}I_{passive} + Ph_1 - mI_{active}\\
SR &= mI_{active}\\
f(G) &= G^{h_2}/\left(G^{h_2} + FC_{50}^{h_2}\right)\\
FC_{50} &= BG\left(^{1-F_{BG}}/_{F_{BG}}\right)^{1/h_2}
\end{aligned}$$

where $I_{passive}$ is the total amount of insulin in packets with thresholds above G, and I_{active} is the amount in the active packets contributing to the total secretion rate SR. $f(G)$ is the threshold distribution function, i.e. the fraction of active packets at a certain glucose concentration. FC_{50} is the glucose concentration that activates 50% of the packets, F_{BG} is the fraction of packets activated at baseline, and h_2 is the hill coefficient. The phase 1 component, Ph_1, is the rate of insulin removal from the passive to the active packets caused by packet activation due to rising glucose concentration. The assumption that all passive packets contain the same amount of insulin allow us to calculate Ph_1 as the amount of insulin per passive packet $I_{passive}/N(1-f(G))$ times the rate of packet activation $N{\cdot}df(G)/dt$, where N is the total number of packets. Ph_1 is:

$$Ph_1 = \begin{cases} I_{passive}\frac{f'(G)\frac{d}{dt}G}{1-f(G)} & \text{for } \left(\frac{d}{dt}G > 0\right)\\ 0 & \text{for } \left(\frac{d}{dt}G \le 0\right)\end{cases}$$

Ph_1 is seen to be sensitive to glucose changes, giving us a first phase insulin release when glucose concentration increases abruptly. Incretin effects on the first phase release are explained by increased packet activation with oral glucose administration, which is implemented through two different values of h_2 in the OGTT and the IVGTT, h_{OGTT} and h_{IVGTT}.

Plasma insulin concentration is computed assuming first order elimination,

$$\frac{d}{dt}I_{plasma} = SR/V - k_I I_{plasma}$$

where I_{plasma} is the plasma concentration of insulin. V is the apparent volume of distribution for insulin, and k_I is the elimination rate constant. Since V and $E_{\max}$ (and E_{OGTT}) can be shown not to be simultaneous identifiable, V was fixed to the plasma volume (3 l), which is slightly higher than typical estimates of the central volume of distribution.[5]

Model Implementation

The model was implemented as a non-linear mixed-effects model in NONMEM V with FOCE.[1]

Parameters to be estimated: k_I, α, $E_{\max}$, h, E_{OGTT}, k_{rd}, m, F_{BG}, h_{IVGTT}, and h_{OGTT}, are described in Table 2. $E_{\max}$,

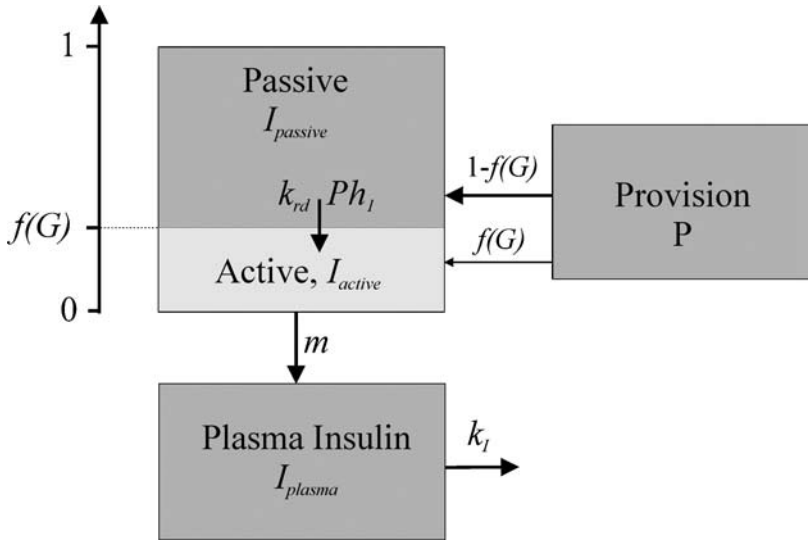

FIGURE 1. Model for observed insulin concentration in glucose tolerance tests. The model includes a one compartment model for insulin pharmacokinetics, two compartments to describe ready releasable insulin in the active and passive packets, and one differential equation to describe the relationship between glucose and the provision of new insulin. The provision is divided into the active and passive packets according to the fraction of active packets f(G), which is a function of the glucose concentration.

k_{rd}, and E_{OGTT} exhibit between subject variability (BSV) by the proportional model, $\theta \exp(\eta)$, and F_{BG} exhibit BSV via the logistic function, $F_{BG} = \exp(\theta + \eta)/(1 + \exp(\theta + \eta))$ to ensure values between zero and one, where θ is the fixed effect and η is a Gaussian random effect that varies between individuals. Data from the OGTT and the IVGTT were treated as if it was from different individuals, such that no false correlation is introduced in the parameter estimates from the two trials.

Observed (y) and predicted insulin plasma concentrations (I_{plasma}) were log-transformed for the residuals ε to be Normal distributed, i.e.

$$\log(y) = \log(I_{plasma}) + \varepsilon$$

TABLE 2. Description of parameters to be estimated.

k_I	The elimination rate of insulin
α	The rate constant for provision to reach steady state
$E_{\max}$	Maximum value of the incretin independent part of the steady state provision rate
h	Hill coefficient for the incretin independent part of the steady state provision rate
E_{OGTT}	Effect parameter for the incretin effect in an OGTT, which is zero for an IVGTT
k_{rd}	Redistribution rate constant from passive to active packets
m	The rate of insulin release from active packets
F_{BG}	The fraction of packets activated at the initial steady state glucose concentration
h_{IVGTT}	Hill coefficient for the threshold distribution function of the IVGTT
h_{OGTT}	Hill coefficient for the threshold distribution function of the OGTT

Initialization

The model is initiated in steady state, under the assumption that BG has produced a steady state provision corresponding to the baseline insulin concentration I_0, where BG/I_0 is calculated as the average of measured glucose/insulin concentrations prior to glucose administration. Initialization in steady state allow us to utilize the steady state equation to calculate EC_{50},

$$EC_{50} = BG \left(\frac{E_{\max}}{I_0 V k_I} - 1 \right)^{1/h}$$

Indexes

The amount of RRI in the ready releasable pool (RRP) is known to change according to the history of glucose concentration,[19] whereas the size of the pool at fasting, i.e. the initial amount of insulin in the passive compartment A_0 is suggested as a first phase index. In the presented model, A_0 is not estimated directly, but derived using the following equation,

$$A_0 = I_0 V k_I \frac{1 - F_{BG}}{k_{rd}}$$

It proved useful also to present a derived second phase index γ, which is the slope of the incretin independent part of the steady state provision with respect to glucose at BG, similar to indexes of other insulin secretion models.[27,28] γ is computed by,

$$\gamma = \frac{E_{\max} h BG^{h-1} EC_{50}^{h}}{\left(BG^{h} + EC_{50}^{h}\right)^{2}}$$

TABLE 3. Population Parameter Estimates.

Parameter	Unit	Estimate	SE of estimate	CV of parameter
k_I	1/min	0.161	0.03	—
α	1/min	0.111	0.045	—
$E_{\max}$	pM/min	103	15	49%
h	1	6.43	0.89	—
E_{OGTT}	nmol	8.86	1.78	55%
k_{rd}	1/min	0.00869	0.00124	58%
m	1/min	1.47	0.206	—
F_{BG}	%	27.4	11	90%
h_{IVGTT}	1	1.96	0.45	—
h_{OGTT}	1	5.14	1.76	—

Model Development

Different models were discriminated based on, robustness, likelihood function value, ability to capture individual insulin profiles, reproducibility, bias of parameter estimates compared to known physiological values, bias in the predictions made by the typical set of parameters, and whether the implementation seemed physiologically reasonable.

Based on the listed criteria for model selection, a number of incretin effects on the first phase in the OGTT were attempted and discarded. These attempts include: (1) no first phase incretin effect, (2) some packets were activated during the OGTT only, (3) the Ph_1 input to active packets was elevated compared to removal from passive packets, corresponding to recruitment of insulin from packets not contributing to steady state secretion, (4) glucose above baseline or transit compartment functions was used to elevate packets activation.

The described implementation of incretin effects for the second phase was chosen above the following list of discarded attempts. (1) transit compartments were used to model an incretin profile, (2) proportional rather than additive incretin effects, (3) incretin effects directly on the provision rather than on the steady state provision, (4) separate levels of $E_{\max}$ and/or hill coefficient h were used for the IVGTT and OGTT.

Besides investigations of different structural models, several combinations of between-subject variation were tested. BSV on the hill coefficients were judged to run unstable, whereas BSV on α and k_I worsened the correlation between IVGTT estimates and OGTT estimates, possibly because individual values of α and k_I cannot be estimated robustly from the OGTT.

RESULTS

The estimated typical parameter values for the study population are presented in Table 3, along with the standard error (SE) of the estimate and the coefficient of variation (CV) between subjects in the study.

Model Predictions

Two kinds of model predictions are calculated for this model, (1) individual predictions are based on the individually estimated parameter values, and (2) population predictions utilize the typical parameter values to compute a typical insulin concentration profile for a given glucose profile. The geometric mean of the individual- and the population-predictions are compared to data in Fig. 2, demonstrating ability of the model to capture differences between insulin response in the OGTT and the IVGTT.

Reproducibility and Similarity of Parameters

Parameter estimates obtained from the IVGTT are plotted against those obtained from the OGTT in Fig. 3, and the correlation coefficients between estimates are presented (for parameters plotted on a log-scale the correlation coefficient for the log-transformed parameters are presented). γ, A_0, k_{rd}, exhibit a clear correlation between experiments (correlation coefficient around 0.7), whereas FC_{50}, F_{BG}, and $E_{\max}$ demonstrate an intermediate correlation (correlation coefficient around 0.5). The high degree of correlation demonstrates parameter reproducibility, in the sense that subjects characterized with high values in one experiment are most likely associated with high values in the other experiment. All parameters except FC_{50} are close to the line of unity, demonstrating similarity of the parameter values, giving a hint to a similar physiological origin of parameters from the different experiments. The bias in FC_{50} is due to the implemented incretin effect, where a higher hill coefficient results in a higher fraction of packet activation when glucose starts to rise, resulting in a decrease in the level of glucose necessary to activate 50% of the packets.

Prognostic Indexes

The four subjects that subsequent to the study have developed type 2 diabetes are associated with a low A_0, a low F_{BG}, a high FC_{50}, and a high k_{rd}, especially k_{rd} for which all exhibited a high value. For the second phase, a low $E_{\max}$ seems to indicate diabetes for the OGTT, but not for the IVGTT, whereas γ and EC_{50} does not appear to be particularly predictive. As expected, also more descriptive indices, such as high BG and high AUC for glucose above BG, appear to implicate higher risk for development of type 2 diabetes. Of the four subjects in a pre-diabetic state, the one with lowest BG was found to have the lowest A_0 among the total study population, see Fig. 4, indicating that the first phase indexes (A_0, k_{rd}, and F_{BG}) carry separate prognostic information to that of BG.

DISCUSSION

We have suggested a new approximation of the distributed threshold hypothesis for parameter estimation that

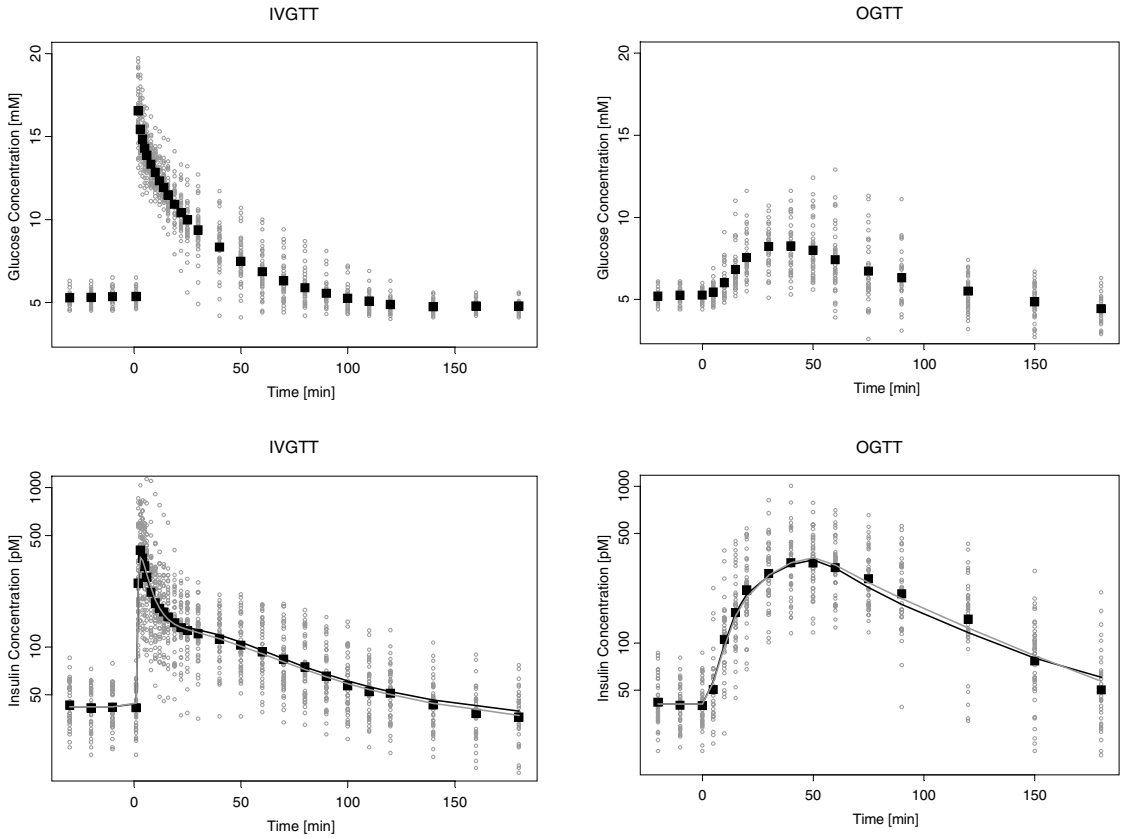

FIGURE 2. All observed glucose (top) and insulin (bottom) concentrations (small light colored circles), geometric mean of insulin and mean of glucose (large black squares), geometric mean of individual predictions (light colored line), and geometric mean of population predictions (black line), are presented for the IVGTT (left), and the OGTT (right).

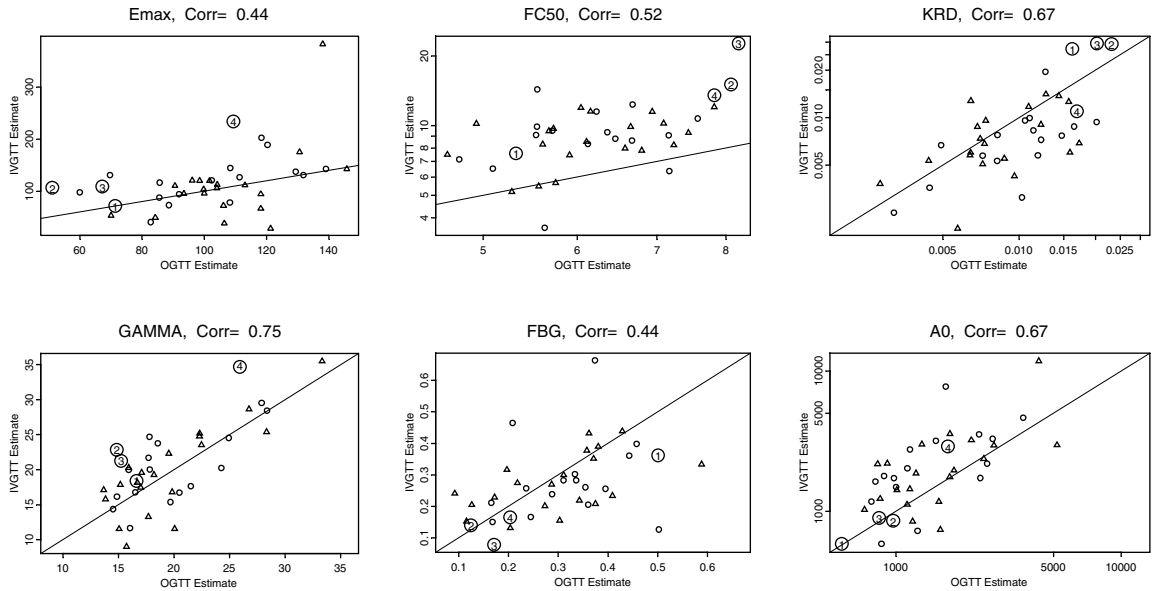

FIGURE 3. Individual parameter values obtained from the IVGTT are compared to those obtained from the OGTT. Triangles signify control subjects, circles signify first degree relatives of diabetics, and numbers 1–4 signify the four first degree relatives of diabetics that have developed type 2 diabetes within the 10 years since study completion. The correlation coefficient presented above each plot is calculated for the log transformed parameter for k_{rd}, FC_{50}, and A_0.

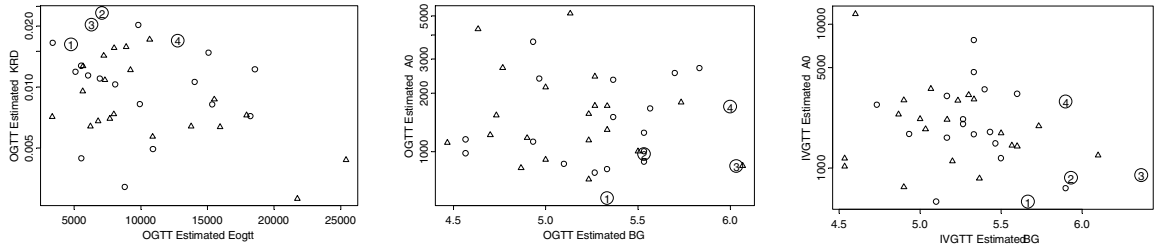

FIGURE 4. Individual parameter values are compared to illustrate separate prognostic relevance. Triangles signify control subjects, circles signify first degree relatives of diabetics, and the numbers 1–4 signify the four subjects that have developed type 2 diabetes within the 10 years since study completion. k_{rd} is compared to E_{OGTT} for the OGTT(left), A_0 is compared to BG for the OGTT(middle), and A_0 is compared to BG for the IVGTT(right).

conserves fundamental mechanisms of threshold distribution, active and passive packets, and redistribution among packets. Effects of incretin hormones were included on packet activation and provision of insulin, to allow quantification of beta cell function, both for the IVGTT and the OGTT. The parameter estimates exhibited similarity and reproducibility, and several parameters are promising candidates for an early diagnostic for development of type 2 diabetes. However, in spite of the usefulness, the reproducibility was less than perfect, which deserves some discussion.

Reproducibility

Three factors can be named to cause reduced correlation, as that observed between some of the parameter estimates in Fig. 3.

1. Between-occasion variability (BOV). For example, the less then perfect correlation of BG ($r = 0.70$) and I_0 ($r = 0.76$), data not shown, must be caused by BOV.
2. Suboptimal design, e.g. timing of measurements and chosen glucose administration. Compared to the IVGTT, the OGTT exhibit some technical design problems: (a) inevitably, incretin and plasma glucose effects occur over the same time period, making them difficult to separate. (b) Glucose rises slowly, making separation of first and second phase difficult. (c) Glucose elevation is lower, making estimation of $E_{\max}$ difficult.
3. Lacking physiological precision, in the sense that parameters in the OGTT and the IVGTT have separate physiological meanings. However, the similarity in parameter values obtained from the OGTT and IVGTT indicates that this last point is not a dominant issue.

Sampling Design

In analogy with other models,[2] the robustness of the individual parameter estimates in the OGTT were investigated for different sampling designs, and similar conclusions were obtained, i.e. the accuracy of the first phase parameter estimates drops significantly when fewer measurements are included around times 0–50 min. The original design includes sampling at (0, 5, 10, 15, 20, 30, 40, and 50 min). When sustaining the (0, 10, 20, and 30 min) samples, the first phase indexes (A_0 and k_{rd}) are robust, with high correlation to the original estimates ($r \sim 0.95$). This correlation is reduced to ($r \sim 0.86$), when using only the (0, 20, and 40 min) samples, and to ($r \sim 0.77$) when using only the (0 and 30 min) samples. Also the estimates of E_{OGTT} and $E_{\max}$ were sensitive to reduced sampling design.

Second Phase Indexes

γ and $E_{\max}$ were highly correlated for the IVGTT ($r = 0.85$), but less correlated in the OGTT ($r = 0.52$). Whereas $E_{\max}$ was estimated, the introduction of γ was motivated by (1) γ is theoretically similar to previous successful second phase indexes,[27,28] (2) a simulation study verified that γ is more robustly estimated than $E_{\max}$ in the OGTT, which is also seen as a higher correlation in Fig. 3. Both parameters were well estimated from the IVGTT, indicating design problems with the OGTT. (3) A clear relationship exists between parameter estimates of $E_{\max}$ and E_{OGTT}, but not between γ and E_{OGTT}.

Incretin Effect

For the OGTT, glucose above baseline exhibit similar dynamics as incretin hormones, see e.g. experiments by Rask *et al.*,[24] and was found the best available surrogate for their effect. However, the implementation leads to suspicion concerning the physiological origin (glucose or incretin hormones) of E_{OGTT}. Three observations indicate successful estimation of the incretin effects: (1) the similarity of $E_{\max}$ and γ estimates obtained in the IVGTT and the OGTT. (2) No correlation was found between E_{OGTT} and AUC_{G-GB}, which would be expected if glucose alone was causing what was estimated as incretin effects. (3) A simulation study confirmed that γ and E_{OGTT} can be estimated simultaneously from the OGTT, with a correlation

between simulated and estimated values of ($r = 0.9$) for γ and ($r = 0.95$) for E_{OGTT}.

First Phase Indexes

The AUC of insulin above I_0 during the first 10 min after glucose administration has been used as an index for first phase secretion.[26] This index correlated with A_0 ($r = 0.96$), k_{rd} ($r = -0.63$), and F_{BG} ($r = -0.30$), demonstrating a clear difference between the three indexes, where A_0 clearly represents the more traditional first phase index.

Insulin response to glucose in the OGTT has previously been successfully described by a static and a dynamic index ϕ_D,[2] where ϕ_D depend on the derivative of glucose, which is similar to Ph_1 in the present model. An insulin based calculation of ϕ_D demonstrated that ϕ_D and A_0 obtained from the OGTT correlates equally well to the 10 min AUC of insulin above baseline for the IVGTT ($r = 0.65$) and ($r = 0.67$). However, compared to previous models, the present model brings new and potentially important indexes to describe beta cell function.

Does Redistribution Represent a Fundamental Factor for Beta Cell Dysfunction?

Under the assumption that packets represent beta cells, as eluded to in other work,[23] redistribution would be the activity of passive cells, e.g. by random activation, so that fast redistribution will represent a high frequency of cells releasing their content. Note that the precise formulation of redistribution has changed slightly compared to the distributed threshold model, see Appendix A, and that redistribution has not yet been understood in a cell biological framework. In the whole body system, glucose is known to have potentiating effects on the first phase release,[10] and redistribution is a likely mechanism for the normalization of an elevated RRP, making k_{rd} a determining factor for the steady state RRP. Since provision of insulin to the RRP is necessary, steady state provision is another likely determining factor for the steady state RRP. For subjects 1–4 in Fig. 3, k_{rd} is large and A_0 is small, but k_{rd} is more pronounced than A_0, which could indicate that redistribution is the more fundamental factor for beta cell dysfunction. This makes sense, since A_0 depend upon baseline provision, which is known to increase during development of insulin resistance. Hence, A_0 may be a composite index, influenced in opposite directions by beta cell dysfunction and insulin resistance, whereas k_{rd} may be a more fundamental factor for beta cell dysfunction.

Provision

The provision of insulin to the RRP is a simple function of glucose that lump a number of intracellular processes together, such as the glucokinase, up regulation of proinsulin, and increased formation of new insulin granules. This clear approximation of reality means that the rate constant α and $E_{\max}$, may not be valid for other experiments on longer time horizons.

Threshold Distribution

In the light of the fact that some[22,23,29] believe heterogeneity in beta cells activation threshold to cause the biphasic insulin release, this heterogeneity should relate to the estimated threshold distribution. In fact, under the assumption that the potential size of the RRP in each beta cell is identical, the estimated distribution of thresholds and the measured distribution of activated beta cells should be identical. Some experiments have found that 53% of beta cells secreted detectable amounts of insulin at 5.6 mM glucose,[14] which is in the high end of the range of individual estimates of F_{BG} between 0.1 and 0.6. Two concerns for this comparison are that (1) redistribution from passive cells could also lead to insulin secretion, allowing some passive cells to be estimated as active, (2) oscillations of insulin secretion could interfere with the estimated activity frequency, which is not accounted for in the model.

It is worth noting that redistribution was estimated more robustly than F_{BG}, and also that the inclusion of BSV on redistribution was more important than for F_{BG}, to explain variations in the first phase response, possibly indicating a higher degree of uncertainty in the estimated values for F_{BG}.

Individual Secretion Profiles

In Fig. 5, individual and population predictions are compared to data for a few selected individuals. For subject 1 to 3, a small RRP was estimated for both experiments, corresponding to a lower than typical first phase for the IVGTT, and a lower than typical quick increase in plasma insulin concentration for the OGTT. Both subject 1 to 3 have developed type 2 diabetes post study, which is in agreement with the common understanding that a low first phase secretion is an early diagnostic marker for type 2 diabetes. Also subject 4 has developed type 2 diabetes, but for this individual the first phase response appears normal, and A_0 is among the highest 50%. Interestingly, this individual was associated with a relatively low F_{BG} (among the lowest 30%) and a relatively high k_{rd} (among the highest 30%), which should indicate a low first phase. The apparent slightly above normal first phase originates from the provision of new insulin at baseline glucose, for which the subject 4 had the largest value among the 40 subjects. Subject 5 and 6 exhibit an above average amount of RRI, and as expected none of these have developed diabetes. Subject 6 has an $E_{\max}$ around average in both experiments and E_{OGTT} was the second largest in the study, explaining why insulin concentrations during the later stage of the IVGTT are close to typical, while they are far above the population predictions in the OGTT.

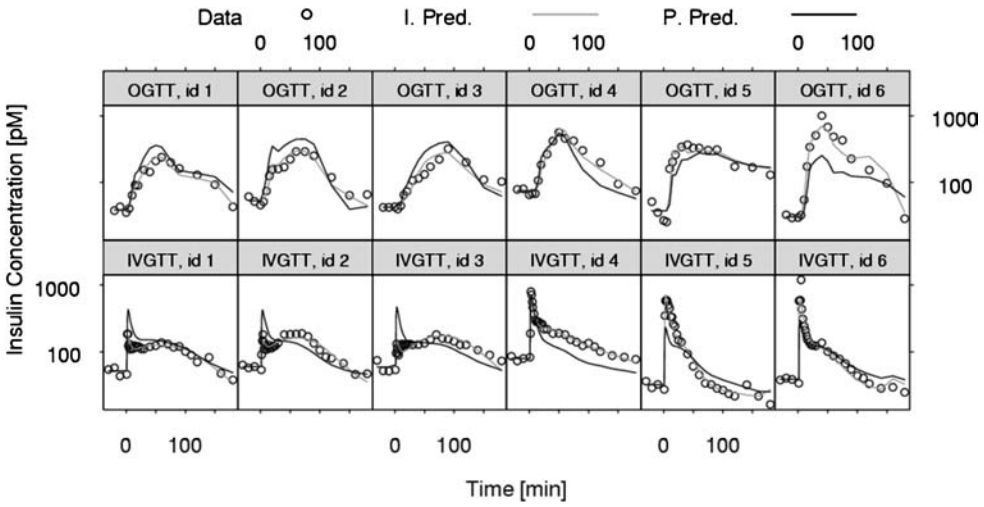

FIGURE 5. Observed insulin concentrations (circles), are presented together with individual predictions (light colored line) and population predictions (black line) for selected individuals. All 6 individuals were healthy at the time of the study, but individuals with id 1 to 4 have subsequently developed type 2 diabetes, whereas individuals with id 5 and 6 have remained healthy.

Using Insulin vs. C-peptide for Assessment of Beta Cell Function

Beta cells release an equimolar mixture of insulin and C-peptide, but C-peptide is cleared slower from plasma and does not suffer the same first pass effect of the liver, hence C-peptide has been used in many models and model based methods to asses beta cell function.[21] In the absence of C-peptide data, the present analysis was performed using insulin data, so the obtained secretion rate reflects a post hepatic secretion. One advantage of the higher elimination rate of insulin is that changes in secretion lead to more pronounced changes in plasma concentration, which we believe to produce high estimation accuracy.

Limitations and Potential Future Implementations

The presented method uses data from the IVGTT and the OGTT simultaneously to find all parameters. In order to use the model for estimation in a single experiment, it may be necessary to use Bayesian techniques or to fix some parameters. In our analysis, we modeled the OGTT alone by fixing some parameters to the values found by the simultaneous analysis, while estimating only parameters with BSV.

The present model is developed from oral and intravenous administration of glucose, using data within 3 h of glucose administration. If one wish to model longer experiments or use other combinations of nutrients, then the limitations of the model in its present formulation has been exceeded. Compared to more empirical beta cell models, one advantage of the present more mechanistic model is that it can more naturally be extended and adjusted to account for new situations. Fruitful model extensions and adjustments could possibly include: (1) a description of other experiments such as the HGC, to include new features at sustained high glucose challenges (2) model extensions to include simultaneous models of the MTT and the OGTT could possibly describe the incretin effects from various compositions and amounts of nutrients, and possibly link the incretin effect to measured incretin hormones, (3) model extensions that include a triple meal test could investigate whether the potentiating effects of insulin release[18] is caused by the RRP, or possibly another pool, (4) modeling of the modified IVGTT, to test whether the large tolbutamide driven insulin release can be related to the RRP included in the model, (5) extensions to include covariates for characterization of differences between patient populations, and (6) during development of drugs that target beta cells, description, understanding, and predictions of results in future trial designs could be aided by the presented model.

CONCLUSION

A new approximation of the distributed threshold hypothesis has been formulated, and it was verified that it can be used for parameter estimation of the IVGTT and the OGTT. With this initiative, we approach a population model for quantification of beta cell function that can be used for several of the different tolerance tests available. The present work focused on similarity, reproducibility, and prognostic relevance of individual parameters estimated from an IVGTT and an OGTT, for which validation further included comparison to other indexes, simulation studies, and prediction of individual profiles. First phase indexes comprise the steady state amount of ready releasable insulin, similar to traditional first phase indexes, and the rate of redistribution, which represents a new and possibly more fundamental

first phase index. The most useful second phase index γ is theoretically similar to the second phase index of other models, and it is precisely estimated for the IVGTT, and to some extend believed separable from the incretin effects of the OGTT. Lack of perfect correlation between parameters estimates from the two experiments is likely caused by between-occasion variability, and by the design of the OGTT, which yield more imprecise parameter estimates, but enables estimation also of the incretin effect.

APPENDIX A

In the present appendix we use the original threshold distribution hypothesis[10] to derive the equations for the active and passive amounts of insulin presented in the main text. This derivation involves three approximations/alterations to the original model that can be applied in arbitrary order, for instance as described below.

The fundamental hypothesis in the original distributed threshold model is that the readily releasable insulin is stored in small packets, where the different packets have different thresholds, secreting insulin into the plasma only when the glucose concentration has exceeded this threshold. The amount of readily releasable insulin in packets with threshold between θ and $\theta + d\theta$ is given by $\xi(\theta, t)d\theta$, so the threshold density distribution function can be used to model the total secretion into plasma. The original distributed threshold model,[10] can be written as,

$$\frac{d\xi(\theta,t)}{dt} = -m\xi(\theta,t)H(G-\theta) + f'(\theta)P(G,t) - k_{rd}\xi(\theta,t) + k_{rd}f'(\theta)\int_0^\infty \xi(\theta',t)d\theta'$$

$$SR = m\int_0^G \xi(\theta',t)d\theta'$$

The secretion of insulin into the plasma is realized through the first term, where $H(\cdot)$ is the Heaviside function. The second term is the provision of new insulin, and the last two terms are named redistribution terms corresponding to a random change of thresholds of the different packets. Note that $f'(\theta) = d/d\theta f(\theta)$, is the distribution density function. In the original analysis glucose started at zero, so that $f'(\theta)$ gives the initial insulin distribution density function $\xi(\theta,0)$. $I_{passive}$ and I_{active} can be written as,

$$I_{passive} = \int_G^\infty \xi(\theta',t)d\theta'; \quad I_{active} = \int_0^G \xi(\theta',t)d\theta'$$

The differential equations for $I_{passive}$ and I_{active} can be derived analytically,

$$\frac{d}{dt}I_{passive} = (1-f(G))P - k_{rd}(1-f(G))I_{passive} + k_{rd}f(G)I_{active} - \xi(G,t)\frac{dG}{dt}$$

$$\frac{d}{dt}I_{active} = -mI_{active} + f(G)P + k_{rd}(1-f(G))I_{passive} - k_{rd}f(G)I_{active} + \xi(G,t)\frac{dG}{dt}$$

The first suggested alteration to the distributed threshold model involves unidirectional flow of insulin from passive packets to active packets. Note that the beta cell action potential will either spike or not, where a spike will lead to exocytosis. It is not possible to stop the spike by a decrease in glucose and thereby stopping exocytosis of insulin that is currently being released, motivating a unidirectional flow. Whereas this change does simplify the model structure, the rate constant m is so large that it does not alter the results. We get,

$$\frac{d}{dt}I_{passive} = (1-f(G))P - k_{rd}(1-f(G))I_{passive} - \xi(G,t)\frac{dG}{dt}H\left(\frac{dG}{dt}\right)$$

$$\frac{d}{dt}I_{active} = -mI_{active} + f(G)P + k_{rd}(1-f(G))I_{passive} + \xi(G,t)\frac{dG}{dt}H\left(\frac{dG}{dt}\right)$$

In the distributed threshold model, redistribution can be understood as a random change in the sensitivity to glucose, where passive packets may change threshold but still be passive. This formulation is slightly changed. In the present model, redistribution involves random activation of packets, in the sense that a passive packet may by a random mechanism release all insulin in its ready releasable pool, and then return to the passive state. By this mechanism, redistribution will not decrease to zero when all packets are passive. The equations become,

$$\frac{d}{dt}I_{passive} = (1-f(G))P - k_{rd}I_{passive} - \xi(G,t)\frac{dG}{dt}H\left(\frac{dG}{dt}\right)$$

$$\frac{d}{dt}I_{active} = -mI_{active} + f(G)P + k_{rd}I_{passive} + \xi(G,t)\frac{dG}{dt}H\left(\frac{dG}{dt}\right)$$

The two alternative models for redistribution were compared during model development, and the chosen formulation produced superior correlations between OGTT and IVGTT parameter estimates, superior objective function value, and was numerically more robust when changing initial estimates in the estimation.

The central approximation in the present approach is that all passive packets contain the same amount of insulin, so that $\xi(G,t) = I_{passive}f(G)/(1-f(G))$. This approximation will not be influential during simple glucose challenge tests, where glucose is single peaked. But if a second and identical peak is seen immediately after the first peak, the

threshold distribution hypothesis will predict the second peak to give no first phase, because the passive packets involved are empty, whereas the present approximation will predict a nonzero first phase, since all passive packets contain the same amount of insulin. This difference may be of particular relevance for predictions of insulin response to rapid oscillations in glucose. Following this approximation we get the equations for the amount of insulin in the passive and active packets that were presented in the main text,

$$\frac{d}{dt}I_{passive} = (1 - f(G))P - k_{rd}I_{passive} - \frac{I_{passive}f(G)}{1 - f(G)}\frac{dG}{dt}H\left(\frac{dG}{dt}\right)$$

$$\frac{d}{dt}I_{active} = -mI_{active} + f(G)P + k_{rd}I_{passive} + \frac{I_{passive}f(G)}{1 - f(G)}\frac{dG}{dt}H\left(\frac{dG}{dt}\right)$$

APPENDIX B

The present appendix provides the exact solution to the amount of insulin in the passive and active packets following a step increase in glucose from G_1 to G_2, at time $t = 0$. Starting at the steady state solution:

$$P(0^-) = P(G_1, \infty)$$

$$I_{passive}(0^-) = \frac{P(G_1,\infty)(1-f(G_1))}{k_{rd}}$$

$$I_{active}(0^-) = \frac{P(G_1,\infty)}{m}$$

For a step increase in glucose, the Ph_1 contribution can be computed via a Dirac delta-function,

$$Ph_1 = \frac{P(G_1, \infty)(f(G_2) - f(G_1))}{k_{rd}}\delta(t)$$

The amount of insulin in the passive packets can be computed as

$$I_{passive}(t) = \frac{(1 - f(G_2))P(G_1, \infty)}{k_{rd}} + D(t); \quad t > 0$$

$$D(t) = (1 - f(G_2))(P(G_2, \infty) - P(G_1, \infty)) \times \frac{\alpha\left(1 - e^{-k_{rd}t}\right) - k_{rd}(1 - e^{-\alpha t})}{k_{rd}(\alpha - k_{rd})}$$

where the first term reflects the immediate removal of insulin due to packet activation, corresponding to the first phase release. The second term $D(t)$ represents the elevation in the amount of insulin in the passive packets coming from an elevated provision, i.e. the second phase contribution to the passive packets. α and k_{rd} constitute the two timescales for the second phase contribution. Since α is more than a factor 10 larger than k_{rd}, the main contribution of the delayed increase of the second phase comes from k_{rd}.

The amount of insulin in the active packets can be computed as,

$$I_{active} = \frac{P(G_1, \infty)}{m} + \frac{P(G_1, \infty)(f(G_2) - f(G_1))}{k_{rd}}e^{-mt} + D_2(t); \quad t > 0$$

$$D_2(t) = e^{-mt}\int_0^t e^{mx}\left(f(G_2)(P(G_2, \infty) - P(G_1, \infty)) \times (1 - e^{-\alpha x}) + k_{rd}D(x)\right)dx$$

where the first term in I_{active} corresponds to the initial amount, the second term is the first phase contribution, and the third term D_2 gives the second phase contribution. Since m leads to a rather fast decay compared to the remaining time scales, it is reasonable to approximate $D_2(t)$ as,

$$D_2(t) \approx \frac{(P(G_2, \infty) - P(G_1, \infty))}{m}\left((f(G_2)(1 - e^{-\alpha t}) + (1 - fG_2))\frac{\alpha\left(1 - e^{-k_{rd}t}\right) - k_{rd}(1 - e^{-\alpha t})}{k_{rd}(\alpha - k_{rd})}\right)$$

One part originates from the immediate increase in provision and another part originates from the steady increase in passive packets.

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