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## Population analysis of ethnicity and first-phase insulin release

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### Abstract

**Objective**—A mechanistic, physiologically-based pharmacokinetic (PK/PD) model was developed to describe the biphasic insulin release and evaluate the racial effects on the glucose–insulin kinetics in response to intravenous glucose.

**Methods**—Fifteen African-American and 18 Caucasian children and adolescents between 8 and 18 years of age were enrolled in the study. Intravenous bolus of glucose (250 mg/kg) was administered and blood samples collected at frequent intervals for three hours following the glucose injection. A nonlinear mixed-effect population kinetic analysis with covariate structure was performed using Monolix.

**Results**—A significantly higher initial insulin secretion from a readily releasable pool, which is responsible for the first-phase insulin secretion, was detected in African-Americans compared to Caucasians ( $p < 0.05$ ).

**Conclusions**—The proposed kinetic model is able to describe the glucose-stimulated insulin response, account for the first-phase insulin release and identify a racially-based pharmacokinetic difference in insulin's biphasic secretion behavior. It is hypothesized that the first-phase insulin component may play an important role in the development of type 2 diabetes. The proposed mechanistic model provides a quantitative analysis of the biphasic insulin release that may be useful in the early detection of diabetes.

### Keywords

First-phase insulin; Beta-cell; Ethnicity; Intravenous glucose tolerance test; Insulin modeling; Pharmacokinetics; Pharmacodynamics

## 1. Introduction

The prevalence of obesity and type 2 diabetes among children is increasing in U.S., and this problem is greater in African-American children compared to Caucasian children [1]. A

number of recent reports have demonstrated that nondiabetic African-Americans have increased insulin resistance [2–5] which is a risk factor for developing type 2 diabetes [6,7]. Increased insulin secretion in African-Americans may compensate for or precede the insulin resistance [2,8–10]. In any event, it appears that there is an inherent difference in glucose and insulin metabolism between these two populations.

In our previous study [11] a nonparametric approach was used to analyze insulin dynamics by means of data from frequently sampled intravenous glucose tolerance tests (FSIVGTT). That study indicated that race, as a major covariate, only affects the insulin concentration in the first-phase for non-obese subjects. As first demonstrated by Cerasi and Luft *in vivo* [12], insulin secretion in response to glucose exhibits a biphasic pattern. Much effort has been devoted to understanding the underlying mechanisms of the biphasic release because of the apparent association between the onset of type 2 diabetes and the loss of the first-phase [12,13].

The objectives of this study are to kinetically evaluate the biphasic insulin release based on current knowledge of the beta-cell insulin physiology, and to apply population kinetic analysis principles to differentiate the kinetic parameters that contribute to the difference in insulin secretion between African-American and Caucasian. A wide variety of models have been used to study the glucose and insulin dynamics. The often applied minimal model [14] has been used for several decades due to its capability of estimating indices of insulin sensitivity. However, the minimal model does not represent the early insulin secretion, accurately. The early first-phase data points are commonly not included in the minimal model analysis. Thus, the minimal model is not suitable for analyzing the early phase release. Only few models have been proposed to describe insulin secretion based on the mechanism of a biphasic insulin secretion. Grodsky modeled multiple insulin pools in beta-cells [15]. They studied the phasic insulin secretion both *in vitro* and *in vivo* and suggested a two-compartment model to describe the biphasic secretion. However, the model only successfully simulated the first-phase secretion under certain levels of glucose stimulation. A more elaborate model with a biphasic mechanism of insulin secretion has been proposed by Gupta et al. [16]. However due to the greater complexity of that model the parameters that relate to first-or second-phase release are difficult to evaluate in a population kinetic framework.

In this work, the physiological mechanism of biphasic secretion is specifically considered in the PK/PD analysis, with a strong structural connection to current knowledge of the beta-cell insulin physiology. Compared to other models that consider a biphasic insulin models, the proposed model is simpler in structure and number of parameters and includes physiologically based parameters closely related to the important biphasic insulin secretion. Insulin concentrations obtained from FSIVGTT in the two racial groups were simultaneously analyzed in a population modeling framework using the Monolix software [17] according to the proposed mechanistic kinetic model. A specific covariate structure was used in the population analysis enabling the identification of distinct differences in physiologically relevant kinetic parameters between African-American and Caucasian youths.

## 2. Subjects and methods

### 2.1. Subjects

The database used here were the same as the one used in our previous noncompartmental analysis [11] except 5 subjects were excluded due to missing first- or second-phase. The FIVGTT analysis involved 15 healthy African-American (3 females and 12 males), age  $13.7 \pm 2.55$  (mean  $\pm$  SD), and 18 healthy Caucasian children (8 females and 10 males), age  $14.1 \pm 2.90$ . The BMI of African-Americans is  $25.6 \pm 4.63$ , which is significantly ( $p < 0.05$ ) higher comparing to that of  $21.5 \pm 4.13$  of Caucasians. The study was conducted in accordance with the guidelines in The Declaration of Helsinki and the protocol approved by the Ohio State University Office of Responsible Research.

### 2.2. Sampling procedure

Subjects received their routine diet for at least 3 days before the glucose tolerance test and then were admitted at 8 AM after 10 h fasting to the General Clinical Research Center, Ohio State University. On the morning of the test, a bolus glucose of 250 mg/kg was administered within 1 min through an intravenous catheter at time 0. Blood samples were collected at  $-10, 0, 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 27, 32, 42, 52, 62, 72, 82, 92, 102, 122, 142, 162$  and 182 min relative to the glucose administration at time 0.

### 2.3. Laboratory analysis

Plasma glucose was measured by the YSI model 2300 glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). The coefficient of variation (CV) of this method is  $<2\%$ . Plasma insulin was measured in the CORE laboratory of the GCRC using a double antibody radioimmunoassay (Coat-A-Count kit manufactured by Siemens Medical Solutions Diagnostics, Los Angeles, CA). The sensitivity of the insulin assay was  $2.5 \mu\text{U/ml}$ . The intra- and inter-assay CV were 6% and 10%, respectively.

### 2.4. PK/PD modeling

A physiologically based mathematical model was developed to describe plasma insulin concentration in response to an acute glucose challenge during FIVGTT. The model considers the physiologic mechanism of biphasic secretion of insulin at the beta-cell level. The model (Fig. 1) includes two insulin pools in the beta-cells, which store the biosynthesized insulin granules and account for the biphasic nature of insulin secretion. One pool, here denoted the reserved insulin pool ( $I_R$ ), governs the second-phase insulin secretion and the other denoted the readily releasable insulin pool ( $I_{RR}$ ) governs the first-phase insulin secretion.

Eq. (1) describes the second-phase insulin secretion from the  $I_R$  pool. It considers a rate of formation of  $I_R$  proportional to the plasma glucose concentration ( $C_G$ ) and a release of insulin empirically described by a Hill equation:

$$\frac{dI_R}{dt} = k_G C_G - \frac{E_{\max} C_G^\alpha}{C_{50}^\alpha + C_G^\alpha} I_R, \quad (1)$$

$$I_R(0) = I_{R_0}$$

with  $k_G$  denoting the first-order formation rate constant, and the  $E_{\max}$ ,  $C_{50}$  and  $\alpha$  the Hill equation parameters, while  $I_{R_0}$  is the estimated initial condition of the  $I_R$  pool.

The insulin in the readily releasable pool ( $I_{RR}$ ) has been “primed” (prepared for release) for ready release according to the biphasic secretion mechanism. The readily releasable insulin can in response to the glucose stimulation be released at a relatively fast rate compared to that in the  $I_R$  pool, to form the first-phase. The change in  $I_{RR}$  is given by:

$$\frac{dI_{RR}}{dt} = -k_{RR} I_{RR}, \quad (2)$$

$$I_{RR}(0) = I_{RR_0}$$

where  $k_{RR}$  is a first-order rate constant and  $I_{RR_0}$  is the estimated initial condition of the  $I_{RR}$  pool.

The insulin from the beta-cells ( $I_R$  and  $I_{RR}$  pools) needs to go through the liver prior to its entry into the circulation. A fraction of insulin described by the extraction ratio  $E$  is removed by the liver. The plasma is considered as a single compartment for insulin with first-order elimination kinetics. Accordingly, the rate of change in the plasma insulin concentration is described by:

$$\frac{dC_1}{dt} = \left( \frac{1-E}{V} \right) \frac{E_{\max} C_G^\alpha}{C_{50}^\alpha + C_G^\alpha} I_R - k_e \frac{I}{V} + \left( \frac{1-E}{V} \right) k_{RR} I_{RR} = P_e \frac{E_{\max} C_G^\alpha}{C_{50}^\alpha + C_G^\alpha} I_R - k_e C_1 + P_e k_{RR} I_{RR}, \quad (3)$$

$$C_1(0) = C_{1_0}$$

where  $C_1$  is the plasma insulin concentration,  $C_{1_0}$  is the initial plasma insulin concentration,  $k_e$  is a first-order elimination rate constant,  $V$  is the distribution volume of insulin in plasma and  $P_e$  has been introduced to represent the expression of  $(1-E)/V$  to avoid identifiability problems.

## 2.5. Population PK/PD modeling

A nonlinear mixed-effect model was used for the population analysis in which the insulin concentration profile is described by the above set of differential equations, and random effects account for variability between-and within-subjects. Let  $y_{ij}$  denotes the  $j$ -th observed plasma insulin concentration of subject  $i$  at time  $t_{ij}$  ( $i = 1, \dots, n, j = 1, \dots, k$ ), then the nonlinear mixed-effect model is written as:

$$y_{ij} = f(\phi_i, t_{ij}) + g(\phi_i, t_{ij}) \cdot \varepsilon_{ij}, \quad (4)$$

where  $f$  is the PK/PD model consisting of the solution to the differential Eqs. (1)–(3),  $g$  is the residual error model,  $\varepsilon_{ij}$  is the coefficient of the error model following a normal distribution with mean 0 and variance 1.

Various residual error models were investigated for the population analysis and the choice made based on the likelihood ratio test and plots of goodness-of-fit. As possibilities we consider the function  $g$  (Eq. (4)) to have the following constant, proportional and combined forms:

$$\text{Constant } g(\phi_i, t_{ij}) = a, \quad (5)$$

$$\text{Proportional } g(\phi_i, t_{ij}) = bf(\phi_i, t_{ij}), \quad (6)$$

$$\text{combined } g(\phi_i, t_{ij}) = a + bf(\phi_i, t_{ij}), \quad (7)$$

with  $\phi_i$  being a  $p$ -dimensional vector of individual parameters for subject  $i$  assumed to be log-normally distributed, ensuring non-negativity in the estimation. Race was evaluated as a categorical covariate to explain between-subject variability in the kinetic parameters. To describe racial effects,  $\phi_i$  was modeled with the following covariate structure:

$$\ln(\phi_i) = \mu + \beta C_i + \eta_i \text{ with } \eta_i \sim_{i.i.d} N(0, \Omega) \quad (8)$$

where  $c_i$  is a categorical vector corresponding to the ethnicity of subject  $i$  (African-American was used as reference class);  $\beta$  is a vector of coefficients of size  $p$ ;  $\mu$  is a vector of population means for parameters with length  $p$ ;  $\eta_i$  represents the random effect assumed to follow a normal distribution with mean of zero and  $\Omega$  is a diagonal variance matrix of the random effects. Significance of the racial effect on parameters was evaluated by the Wald test at a significance level of 0.05.

## 2.6. Data analysis

The data from all the subjects were simultaneously analyzed using the open-source free computer software Monolix, standalone version 3.1 developed by INRIA, France [17]. The population parameters were estimated based on the maximum likelihood estimation (MLE). A Stochastic Approximation Expectation Maximization (SAEM) algorithm coupled with Markov Chain Monte Carlo (MCMC) [18–21] procedure was used for MLE estimation of the parameters of the population model without the use of approximations in the estimation of the likelihood. We made use of Monolix because its algorithm showed much better convergence properties compared to the software NONMEM VI which failed to converge in our case. The Hastings–Metropolis algorithm was applied to compute conditional means and conditional standard deviations of the individual parameters. The glucose concentrations were represented by a linear spline interpolation.

### 3. Results

No racial difference in HOMA-IR was found between the African-American ( $2.60 \pm 1.92$ ) and Caucasian groups ( $2.04 \pm 1.87$ ) indicating similar insulin sensitivity. The residual error in the nonlinear mixed-effect population kinetic analysis was best described with a proportional model. Population estimates of parameters and between-subject variability (standard deviation) are presented in Table 1. A visual predictive check (VPC) with 1000 simulated dataset was performed. The simulated median and the 95% confidence interval are given in Fig. 2 together with the observed insulin concentrations. The VPC plot provides good evidence of the adequacy of the model.

In the present investigation race as a covariate was incorporated in the structure of population model (Eq. (8)). Inclusion or deletion of covariate was determined by a Wald test at a significance level of 0.05. We first applied the covariate of race to all parameters. It was then found that the initial value of the  $I_{RR}$  pool ( $I_{RR_0}$ ) is significantly associated ( $p < 0.05$ ) with race, and racial effect on the rate constant of  $k_{RR}$  is close to significant ( $p = 0.064$ ). If only the parameter  $k_{RR}$  or  $I_{RR_0}$  is considered in the covariate structure, then race shows a significant relationship. Accordingly, the derived parameter  $k_{RI} = k_{RR} \cdot I_{RR_0}$ , which provides a metric for the initial first-phase insulin secretion from beta-cell, was used in the population analysis. In this way race was found to have a significant effects on  $k_{RI}$ , resulting in a 63% decrease in  $k_{RI}$  for Caucasians compared with African-Americans. Fig. 3 shows the goodness-of-fit plots for this model. Apart from the high observed plasma insulin, the population predicted insulin concentrations and observations are clustered around the line of identity (Fig. 3A). Plotting the individual predictions against the observations for insulin (Fig. 3B) reduces the scattering around the line of identity as indicating that the population kinetic analysis is able to explain the variability in the insulin response. The scatter plot of population weighted residual against predicted concentrations of insulin (Fig. 3C) indicates that the residuals are randomly distributed. Thus, the population model appears to adequately describe the biphasic insulin dynamics.

### 4. Discussion

The current study combines a nonlinear mixed-effect population modeling approach and a physiologically based PK/PD model for analyzing insulin behavior in response to glucose stimulation. The structure of the PK/PD model contains expressions to describe the physiologic mechanism of insulin's biphasic secretion. The population PK/PD modeling with the covariate analysis approach enables identification of parameters specific to the difference found in the first-phase insulin secretion between African-American and Caucasian youths [11].

#### 4.1. PK/PD modeling rationale

The insulin release from the beta-cells involves the arrangement of insulin into secretory granules, trafficking of the insulin-containing granules to the plasma membranes and exocytosis from the beta-cell [22]. The release of insulin from the b-cells has been found to follow a biphasic time-course consisting of a rapid and transient first-phase followed by a slowly developing and sustained second-phase. The underlying mechanisms of the biphasic

pattern are still poorly understood in spite of the fact that biphasic release of insulin has been known for more than 40 years [12]. One explanation proposed by a number of studies [15,23–26] suggests that the biphasic release is due to the existence of at least two distinct pools of insulin granules in beta-cells. Based on their release competence or proximity to the plasma membrane it is proposed that the granules belonging to the readily releasable pool ( $I_{RR}$ ) can be rapidly discharged without any further modification and thus is responsible for the first-phase insulin secretion; the second-phase release is due to the insulin granules in the reserved pool ( $I_R$ ) which have to be translocated to the membrane and primed before they can be released.

The PK/PD model presented in this work subscribes to this current cellular biology understanding of the kinetic mechanism governing insulin biphasic secretion. Specifically, the  $I_{RR}$  and  $I_R$  pools of insulin are included in the proposed model (Fig. 1) to consider this mechanism. Upon glucose stimulation, the granules in the  $I_{RR}$  pool are secreted from beta-cells at a faster rate compared to the  $I_R$  pool, and thus form the first-phase release.

Glucose is the main physiological regulator of insulin production and secretion in the beta-cells [27–29]. Several studies [15,26,30] have reported that the magnitude of the peak of the first-phase is varying with the level of glucose, while the shape and the transient nature of the peak remain unchanged. Thus, the size of  $I_{RR}$  should be associated with the glucose level. However, we could not estimate the dependence of  $I_{RR}$  on the glucose dose since only one level of glucose stimulation was tested in our study subjects. For the effect of glucose on the second-phase, we assume the production rate of insulin in  $I_R$  to be proportional to the glucose concentration. The relationship between glucose and insulin release from the  $I_R$  pool was analyzed by using several PK/PD relationships, including the Hill function with or without sigmoidicity and a simple linear function. In order to determine a reasonable empirical structure for the population analysis as a first step, glucose and insulin data were fitted individually using WINFUNFIT which is a Windows version evolved from the FUNFIT program [31]. Our analysis suggests that the model using the Hill function with sigmoidicity best describes the insulin dynamics during FSIVGTT based on the Akaike information criteria [32] and graphical comparisons of fits.

#### 4.2. Model parameters

The extraction ratio parameter  $E$  influences the amount of insulin that reaches the systemic circulation. Approximately 50% ( $E = 0.5$ ) of insulin secreted from beta-cell is extracted by the liver, but the value of  $E$  may change with the glucose level [33]. Instead of estimating  $E$ , which is not possible based on the current data, our PK/PD analysis estimates the  $P_e$  parameter, which “encapsulates” the  $E$  and  $V$  parameters ( $P_e = (1 - E) / V$ ). The average value of  $V$  has been reported to be 10 l [34]. Thus, the value of  $P_e$  calculated according to these references is about 0.05, which is in agreement with the population estimate of  $P_e$  based on our model which is 0.0581 with a 95% confidence interval of 0.0436–0.0773 l<sup>-1</sup>. Thus, our results do not indicate a significant change in  $P_e$  in response to a glucose challenge.

The  $k_e$  has been reported from 0.1 to 0.3  $\text{min}^{-1}$  [35,36]. Our population  $k_e$  estimate of 0.194  $\text{min}^{-1}$  falls into this range, which corresponds to an insulin half-life time of 3.57 min with a 95% confidence interval of 1.33–6.93 min.

In the proposed model, the initial amount of  $I_R$  is treated as an estimated parameter and not calculated assuming an initial steady state condition. The rationale for this is the fact that the basal insulin level is not a stable parameter because the insulin concentration in blood changes in a pulsatile manner. At the fasting state, the contribution of the pulsatile insulin secretion has been found to be at least at least 75% of the total insulin secretion [37].

Higher insulin concentrations in the first-phase in the African-American subjects were detected compared to Caucasians [11] which may indicate beginning signs of progression towards insulin resistance and a pre-diabetic state in the African-American group. Increased insulin secretion in the African-Americans may also compensate for or precede the insulin resistance [2,8–10]. The increase in insulin level may be caused by an increase in insulin secretion from  $I_{RR}$  and/or  $I_R$ , or a decreased insulin removal *via* the extraction from the liver or elimination from plasma. Our covariate analysis suggests that the difference is due to the insulin secretion from the  $I_{RR}$  pool. The significant racial difference ( $p < 0.05$ ) in the initial insulin secretion ( $k_R I_R$ ) from the beta-cells resulted in the difference in the secretion profiles found in the first-phase. Secretion profiles from beta-cell (Fig. 4) during FSIVGTT can be reconstructed using population parameters and averaged glucose concentrations. No significant association was found between race and the insulin elimination, and race and insulin extraction by liver.

The present analysis is based on FSIVGTT experiments. Research is currently in progress to test the applicability of the model for analyzing OGTT data to possibly broaden the applicability of the analysis.

#### 4.3. Other possible factors

Because of the significant difference in the BMI parameter between the two racial groups the difference in degree of obesity cannot be ruled out as possible alternative explanation for the kinetic difference found between the two groups. A better balanced study is needed to investigate that possibility. Possible differences in other factors that have not been determined in this study such as physical activity that was found to be a possible contributor [38], or genetic factors may also need to be considered.

In summary, the proposed mechanistic PK/PD model is able to describe the biphasic release of insulin in response to a glucose challenge. The population analysis allowed an estimation of the variability of the parameters relating to the important biphasic release and enabled the effect of race on the parameters to be evaluated. A significant difference between races was identified in the derived parameter  $k_R \cdot I_R$  that relates to the first-phase insulin release. Our result is supported by our previous exploratory nonparametric analysis of the insulin–glucose kinetics where we found African-Americans to have a higher insulin concentration in the first-phase compared to Caucasians [11]. The proposed kinetic model in this work offers an opportunity to quantify the biphasic characteristic of insulin release and provides a

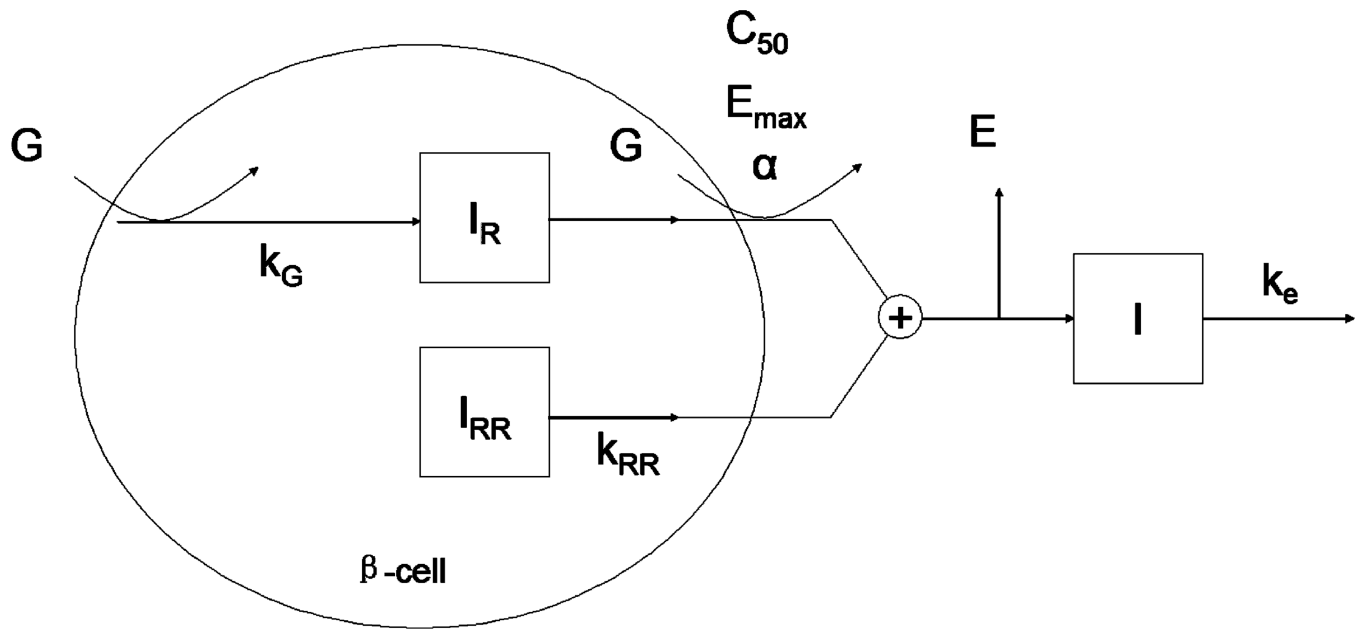


mechanistic and quantitative understanding of the first-phase release, which has been found to play an important role in the development of type 2 diabetes.

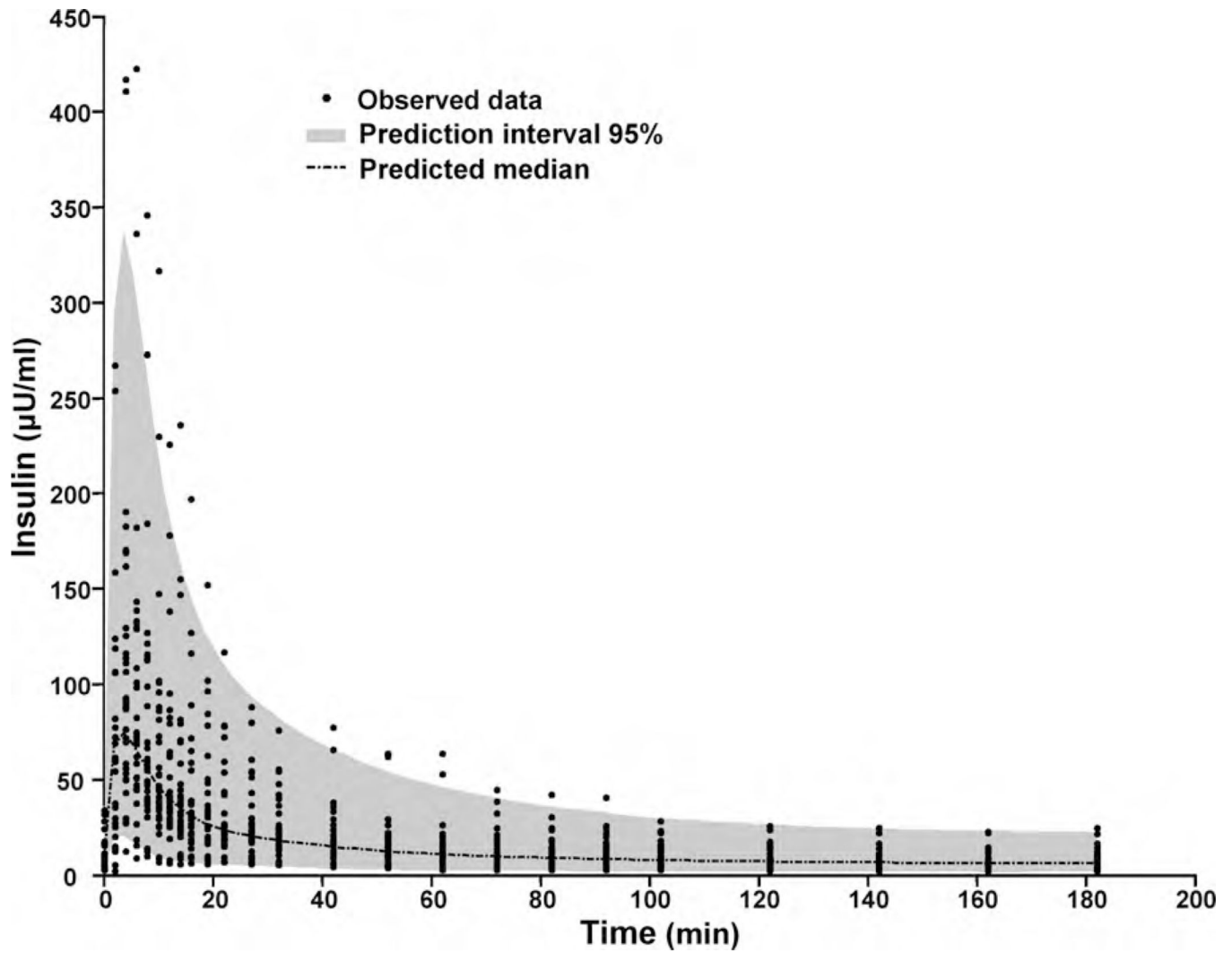
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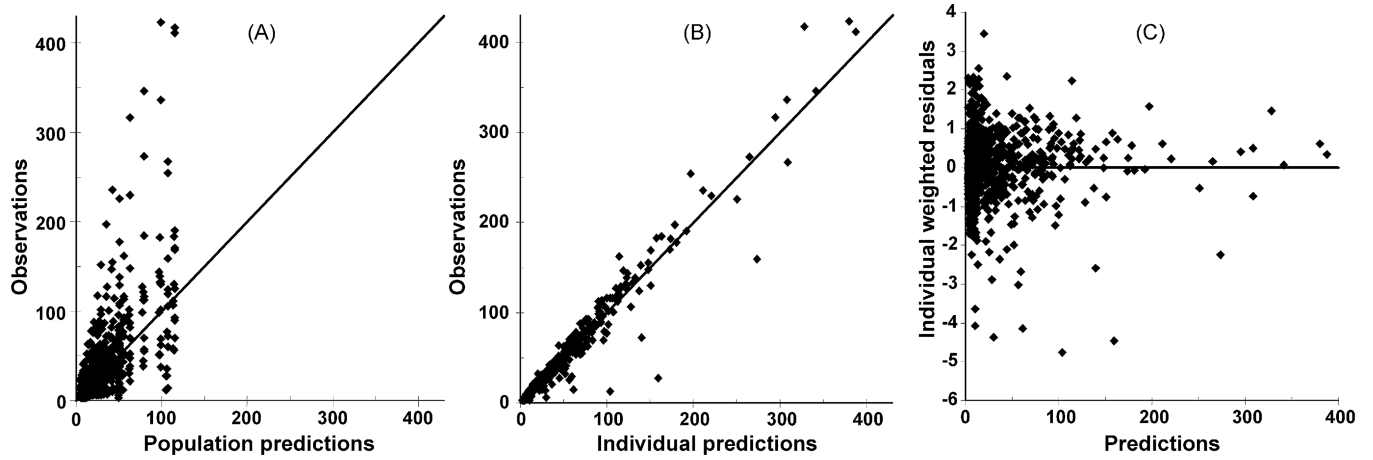
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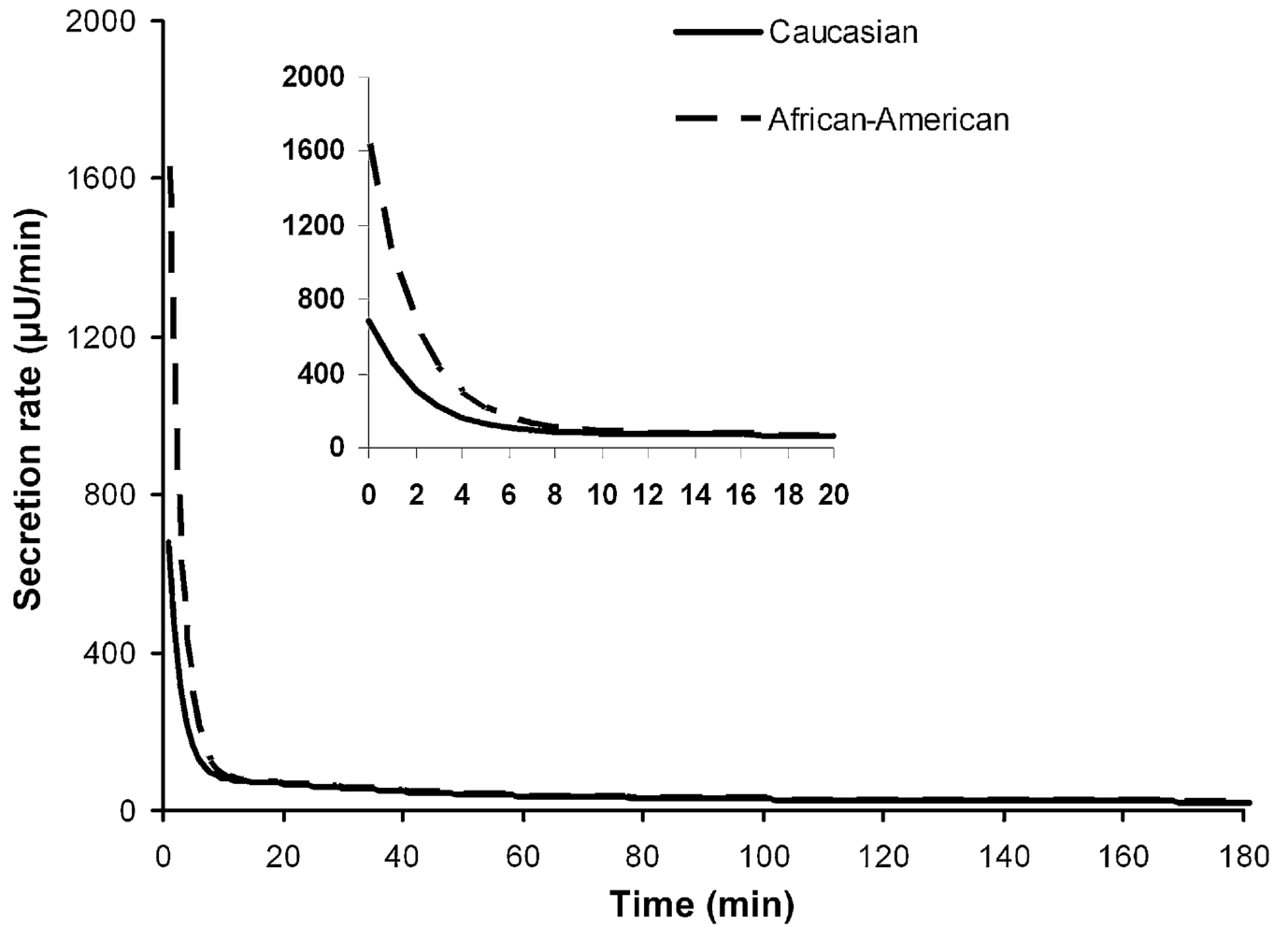
**Fig. 1.** Schematic of the proposed PK/PD model.  $G$  represents the glucose;  $I_R$ ,  $I_{RR}$  and  $I$  represent reserved insulin, readily releasable insulin and plasma insulin respectively.  $E$  denotes the extraction ratio of insulin by liver.  $k_G$ ,  $k_e$  and  $k_{RR}$  are rate constants;  $C_{50}$ ,  $E_{max}$  and  $\alpha$  are the parameters in the Hill equation.



**Fig. 2.** Visual predictive check of the population PK/PD model. Comparison of the observed concentrations with the median and 95% interval predicted for 1000 simulated datasets computed from the estimated population PK/PD model.



**Fig. 3.** Goodness-of-fit plots of population model. (A) Observed insulin concentrations vs. population predictions, (B) observed insulin concentrations vs. individual predictions, and (C) weighted residuals vs. population.



**Fig. 4.** Beta-cell secretion profile during FSIVGTT for African-American (dotted line) and Caucasian (solid line) according to population parameters and averaged glucose concentrations.

**Table 1**

Population estimates of the proposed PK/PD model using Monolix.

Parameters	Estimate	RSE (%) <sup>a</sup>
$k_G$ (min <sup>-1</sup> )	0.24	21
$E_{\max}$ (min <sup>-1</sup> )	0.0185	16
$C_{50}$ (mg/dl)	73.8	6
$P_e$ (L <sup>-1</sup> )	0.0581	25
$k_e$ (min <sup>-1</sup> )	0.194	12
$k_{RR}$ (min <sup>-1</sup> )	0.499	0.2
$\alpha$	4.05	13
$I_{R0}$ (μU)	4530	34
$C_{L0}$ (μU/ml)	8.7	12
$k_R \cdot I_R$ (μU/min) <sup>‡</sup>	1580 <sup>b</sup>	31
$\beta \cdot k_R I_R$	-0.922	27
$b$	0.185	3
Standard deviation of parameters		
$k_G$	0.296	
$E_{\max}$	0.189	
$C_{50}$	0.224	
$P_e$	0.146	
$k_e$	0.505	
$k_{RR}$	0.216	
$\alpha$	0.278	
$I_{R0}$	0.403	
$C_{L0}$	0.659	
$k_R \cdot I_R$ (African-American)	0.608	
$k_R \cdot I_R$ (Caucasian)	0.699	

<sup>a</sup>RSE, relative standard error.<sup>b</sup>The value is for African-American which is used as reference. $k_R \cdot I_R$  of Caucasian is calculated as:  $1580 \times \exp(-0.922) = 628$ .<sup>‡</sup> $p = 0.00016$ .