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Modeling Between-Subject Variability in Subcutaneous Absorption of a Long-Acting Insulin Glargine 100 U/mL by a Nonlinear Mixed Effects Approach*

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Abstract— **Subcutaneous insulin absorption is well-known to vary significantly both between and within subjects (BSV and WSV, respectively). This variability considerably obstacles the establishing of a reproducible and effective insulin therapy. Some models exist to describe the subcutaneous kinetics of both fast and long-acting insulin analogues; however, none of them account for the BSV. The aim of this study is to develop a nonlinear mixed effects model able to describe the BSV observed in the subcutaneous absorption of a long-acting insulin glargine 100 U/mL. Four stochastic models of the BSV were added to a previously validated model of subcutaneous absorption of insulin glargine 100 U/mL. These were assessed on a database of 47 subjects with type 1 diabetes. The best model was selected based on residual analysis, precision of the estimates and parsimony criteria. The selected model provided good fit of individual data, precise population parameter estimates and allowed quantifying the BSV of the insulin glargine 100 U/mL pharmacokinetics. Future model development will include the description of the WSV of long-acting insulin absorption.**

Clinical Relevance— **The proposed model will become an important component of *in silico* platforms for the development and testing of new basal insulin dosing strategies.**

I. Introduction

Long-acting insulin analogues are widely used to treat diabetes. They are commonly employed in multiple daily injection (MDI) therapy to cover the daily basal insulin need, especially in subjects with type 1 diabetes (T1D), but also in those with advanced stage of type 2 diabetes (T2D). However, despite the progress made in insulin preparation and titration, many patients are still experiencing dangerous fluctuations in their blood glucose levels, mainly due to the biological variability present in the absorption process. In fact, plasma insulin level after a subcutaneous (sc) administration is affected by several factors, such as concentration and volume of insulin bolus, injection site and depth, skin temperature and tissue blood flow [1]-[4]. These factors lead to a considerable between- and within-subject variability (BSV and WSV, respectively) that significantly hampers the achievement of the target glucose control.

The BSV is usually assessed with a post-hoc analysis from the individual parameters estimated with standard estimation methods [5]-[7]. However, this procedure does not account for the precision of individual estimates, thus possibly biasing the results [8]. A better approach to overcome this issue is the nonlinear mixed effects (NLME) modeling. In this framework, the parameters shaping the population variability (the so-called fixed effects) are directly assessed during the estimation process. Thereafter, the fixed effects are employed to support the estimation of individual parameters which are characterized by the deviation from the population value (random effects). In addition, the model predictive power can be improved by introducing some subjects' covariates to explain part of the population variability in a deterministic way [9].

Here, we aim to apply the NLME modeling technique to a previously validated compartmental model in order to describe the BSV present in the subcutaneous absorption of a long-acting insulin glargine 100 U/mL (Gla-100). Such model will become an important component of *in silico* platforms, like the UVa/Padova T1D simulator [6] and the Padova T2D simulator [10]. The incorporation of models that explicitly accounts for subject variability would allow running more realistic simulations, providing great benefits on the way to the approval of new insulin treatments.

II. Materials and Methods

A. Database

To build the model we used data of two independent studies. For both studies, a validated radio immunoassay (lower limit of quantification, LLOQ, measured by the lab equal to 5.02 μU/mL) was used to measure Gla-100 concentration. The measurements below the LLOQ were discarded. More detailed information about the databases is available in [7].

Study 1. Twenty-two T1D subjects (age = 42±10 years, body weight, BW = 78±10 kg, body height, BH = 176±7 cm) underwent a 36-h euglycemic clamp and received a single sc injection of 0.4 U/kg of insulin Gla-100, at around 9 am (t = 0) [11]. During the clamp, glucose was infused to keep blood glucose stable at around 100 mg/dl and avoid hypoglycemia. The blood samples were collected at t = 0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 28, 32 and 36 hours to measure glucose and insulin levels (Fig. 1, panel A).

Study 2. Twenty-five T1D subjects (age = 42±9 years, BW = 82±12 kg, BH = 178±8 cm) received a sc injection of 0.4 U/kg of insulin Gla-100, at around 8 pm (t = 0), for 8 days, and after the 8-day period they underwent a 36-h euglycemic clamp [12]. During the clamp, glucose was infused to keep blood glucose stable at around 100 mg/dl and avoid hypoglycemia. The blood samples were collected after

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dosing on day 8 at t = 0, 1, 4, 6, 8, 10, 12, 14, 16, 20, 24, 28, 32 and 36 hours to measure glucose and insulin levels (Fig. 1, panel B).

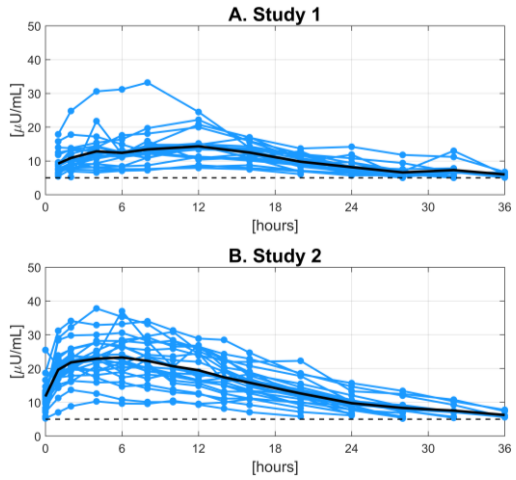

Fig. 1. Plasma insulin concentration [μU/mL] in study 1 (panel A) and 2 (panel B). Blue lines represent individual profiles, black lines the mean profile and dashed lines the LLOQ.

B. Nonlinear Mixed Effects Model

The proposed model consists of a structural model of Gla-100 sc absorption and kinetics combined with a stochastic model describing the BSV:

$$\boldsymbol{z}_j(t) = \boldsymbol{f}_j(\boldsymbol{x}_j, \boldsymbol{\psi}_j) + \boldsymbol{v}_j(t) \quad (1)$$

$$\boldsymbol{\psi}_j(t) = \boldsymbol{d}_j(\boldsymbol{c}_j, \boldsymbol{\theta}, \boldsymbol{\eta}_j) \quad (2)$$

$$\boldsymbol{\eta} \sim \mathcal{N}(\boldsymbol{0}, \boldsymbol{\Omega}) \quad (3)$$

where: $\boldsymbol{z}_j$ is the measurement vector for the jth subject, $\boldsymbol{x}_j$ is a vector incorporating variables such as time and injected dose, $\boldsymbol{f}_j$ is a vector function representing the structural model, $\boldsymbol{\psi}_j$ is the set of model parameters, $\boldsymbol{v}_j$ is the intra-individual error vector, $\boldsymbol{d}_j$ is a vector function describing the stochastic model of $\boldsymbol{\psi}_j$ using the covariates vector $\boldsymbol{c}_j$, the population parameters $\boldsymbol{\theta}$ (fixed effects) and the inter-individual variability $\boldsymbol{\eta}_j$ (random effects) assumed to be normally distributed with zero mean and covariance matrix $\boldsymbol{\Omega}$.

Structural Model. The model of sc absorption of Gla-100 was described by a linear two-compartment model recently proposed by Schiavon et al. [7]:

$$\begin{cases} \dot{I}_{q1}(t) = -k_{sp} \cdot I_{q1}(t) + F \cdot k \cdot u(t) \\ \dot{I}_{q2}(t) = -k_{sp} \cdot I_{q2}(t) + k_{sp} \cdot I_{q1}(t) + F \cdot (1-k) \cdot u(t) \\ Ra_I(t) = k_a \cdot I_{q2}(t) \end{cases} \quad (4)$$

where $I_{q1}(t)$ and $I_{q2}(t)$ represent insulin masses in the subcutis (precipitate and soluble state, respectively) and k [dimensionless] the precipitate fraction of insulin in the injected dose. From $I_{q1}(t)$, insulin dissolves with a constant rate k_{sp} [min^{-1}]. From $I_{q2}(t)$, insulin is absorbed into plasma, with a rate constant k_a [min^{-1}], thus, generating the flux $Ra_I(t)$. The model assumes a subject-specific bioavailability factor, represented by the parameter F [dimensionless]. The model described by (4) was then coupled to a linear single-compartment model to describe the whole-body insulin kinetic [13][14]:

$$\begin{cases} \dot{I}_p(t) = -k_e \cdot I_p(t) + Ra_I(t) \\ y(t) = \frac{I_p(t)}{V_I} \end{cases} \quad (5)$$

where k_e [min^{-1}] represents the fractional insulin clearance and V_I [L/kg] is the distribution volume.

Error Model. The measurement error is assumed to be independent, Gaussian, with zero mean and variance:

$$\sigma^2(t) = a^2 + b^2 y(t)^2 \quad (6)$$

as in [14], but with a and b unknown, estimated from the data.

Stochastic Models of BSV. Four models of BSV were tested and compared. They all shared the assumption that parameters k_{sp}, k_e and $\alpha\,(= k_{sp} - k_a)$ follow a log-normal distribution:

$$\psi_{i,j} = \theta_i \cdot \exp(\eta_{i,j}) \quad (7)$$

while F and k follow a logit-normal distribution:

$$\frac{\psi_{i,j}}{1-\psi_{i,j}} = \frac{\theta_i}{1-\theta_i} \cdot \exp(\eta_{i,j}) \quad (8)$$

In addition to these assumptions, Models 2, 3, and 4 also assume that some correlation ($\rho_{m,n}$) between the random effects of parameters m and n may take place. This is done by introducing the appropriate out-of-the-diagonal terms in $\boldsymbol{\Omega}$ and estimating them from the data. Furthermore, some subjects' covariates can be included in the stochastic model to explain the possible dependency of some parameter from macroscopic subjects' characteristics. In this case, the model of the parameter variability becomes:

$$\psi_{i,j} = \theta_i \cdot \exp\left(\beta_{i,k} \cdot (c_{j,k} - \bar{c}_k) + \eta_{i,j}\right) \quad (9)$$

where $c_{j,k}$ is the kth covariate and $\bar{c}_k$ its mean value. For each covariate added to the stochastic model, a coefficient $\beta_{i,k}$, i.e., between model parameter i and subject's covariate k, has to be estimated. Person correlation analysis among the estimated parameters was used to detect the most correlated covariate-parameter pairs and random effects.

C. Parameter Estimation

As reported in [7], the structural model is a priori non-uniquely identifiable since the rate parameters k_{sp} and k_a are interchangeable and only the ratio between F and V_I is identifiable. To overcome these issues, the parameter $\alpha = k_a - k_{sp}$ described by a log-normal distribution so that $\alpha > 0$ was introduced, and V_I was fixed to 0.135 L/kg [15]. In addition, as done in [7], a priori information on k_e was added to help a posteriori identifiability [15]. Therefore, the parameters that were estimated are: 5 fixed effects, ($\boldsymbol{\theta} = [F, k, \alpha, k_{sp}, k_e]$), 5 standard deviations of the random effects (ω_F, ω_k, ω_α, ω_{k_e}, $\omega_{k_{sp}}$), a number of coefficients $\beta_{i,k}$ and $\rho_{m,n}$ depending upon the selected model, and 2 parameters of the measurement error model (a and b).

For model identification and validation, we used the software Monolix (Monolix 2020R1, © Lixoft [16]) that implements the Stochastic Approximation of Expectation Maximization (SAEM) in combination with a Markov chain

TABLE I. MODEL COMPARISON

Model number	*Correlations*	*Covariates*	*Number of parameters*	*Precision*		*Residuals*		*BICc*
				Mean RSE	*RSE>50%*	*SW test*	*Runs test*	
1	—	—	12	13.8	0	96	100	2531
2	$\rho_{k,k_{sp}}$	—	13	19.4	0	94	100	2528
3	$\rho_{k,k_{sp}}$	$\beta_{k_e,\log(BMI)}$	14	22.0	1	94	100	2531
4	$\rho_{k,k_{sp}}$	$\beta_{F,\log(AGE)}$	14	21.8	2	94	100	2532

Summary of the results obtained with the tested BSV models, ordered by increasing complexity. From left: model number; correlations ρ between random effects; coefficients β allowing introducing a covariate in the model; number of unknown parameters; the precision of estimated parameters expressed as mean RSE and number of RSE > 50%; percentages of the subjects that passed the SW test and the Runs test; BICc.

Monte Carlo (MCMC) method to estimate the maximum likelihood of the NLME model parameters [17]. The Fisher information matrix was estimated with the Metropolis-Hasting's algorithm and the likelihood through an importance sampling method [16].

D. Model Validation and Comparison

Model results were assessed in terms of residual distribution, physiological plausibility of parameters, precision of the estimates and parsimony criteria. In particular, the goodness of the individual fits was checked by visual inspection of the data vs. individual predictions while the distribution of individual weighted residuals (IWRES) and normalized prediction distribution errors (NPDE, [18]) were compared to a standard Gaussian distribution. The Shapiro-Wilk (SW) test and the Runs test were used to assess normality and randomness of residuals, respectively. This analysis was performed in R (R version 4.0.3, © The R Foundation [19]) assuming a significance level p = 0.05. The uncertainty of the estimates was obtained from the inverse of the Fisher information matrix and summarized by the average relative standard error (RSE) and the number of parameters with RSE>50%. Finally, among the models providing satisfactory scores in all the previous metrics, model selection was performed using the Bayesian information criterion corrected for NLME model (BICc) [20].

III. RESULTS

A. Model Comparison

Results of model comparison are reported in Table 1. All the tested models provided satisfactory weighted residuals, with all the subjects passing the Runs and at least the 94% passing the SW. Similar average RSEs were obtained with the four models, with the lowest value achieved by Model 1. However, the lowest BICc was obtained with Model 2, which was therefore selected as best one. The observations versus predictions plot, IWRES and NPDE distributions obtained with this model are reported in Fig. 2.

B. The Selected Model

The selected model (Model 2) accounts for a correlation between the random effects of the parameters k and k_{sp} ($\rho_{k,ksp} = 0.474$) but does not include any covariates.

Model equations and covariance matrix of the random effects are:

$$\begin{cases} \frac{F}{1-F} = \frac{F^{pop}}{1-F^{pop}} \cdot \exp(\eta_F) \\ \frac{k}{1-k} = \frac{k^{pop}}{1-k^{pop}} \cdot \exp(\eta_k) \\ \alpha = \alpha^{pop} \cdot \exp(\eta_\alpha) \\ k_{sp} = k_{sp}^{pop} \cdot \exp(\eta_{ksp}) \\ k_e = k_e^{pop} \cdot \exp(\eta_{ke}) \end{cases} \quad (10)$$

$$\boldsymbol{\Omega} = \begin{bmatrix} \omega_F{}^2 & 0 & 0 & 0 & 0 \\ 0 & \omega_k{}^2 & 0 & \rho_{k,ksp}\omega_k\omega_{ksp} & 0 \\ 0 & 0 & \omega_\alpha{}^2 & 0 & 0 \\ 0 & \rho_{k,ksp}\omega_k\omega_{ksp} & 0 & \omega_{ksp}{}^2 & 0 \\ 0 & 0 & 0 & 0 & \omega_{ke}{}^2 \end{bmatrix} \quad (11)$$

The model parameter estimates are reported in Table 2 together with their precisions (as RSE in brackets). Kinetic parameters are consistent with those estimated in [7], and error parameters with those assumed in [14].

TABLE II. ESTIMATES OF THE POPULATION PARAMETERS

Fixed effects				
F [ ]	k [ ]	α [min^{-1}]	k_{sp} [min^{-1}]	k_e [min^{-1}]
0.88 (4)	0.823 (2)	0.00043 (24)	0.0013 (5)	0.107 (5)
Standard deviations of the random effects				
ω_F[ ]	ω_k[ ]	ω_α[ ]	ω_{ksp}[ ]	ω_{ke}[ ]
0.261 (44)	0.562 (15)	0.252 (46)	0.281 (13)	0.238 (12)
Correlation parameter			***Error parameters***	
$\rho_{k,ksp}$ [ ]			a [μU/mL]	b [ ]
0.474 (32)			0.754 (37)	0.0997 (15)

Estimates of the population parameters and their precision (as RSE in brackets). Top: fixed effects of insulin Gla-100 absorption and kinetics; middle: standard deviations of the random effects $\boldsymbol{\eta}$; bottom: correlation between the random effects of k and k_{sp}, and error model parameters.

IV. DISCUSSION

In this work, we proposed a NLME model of the sc absorption of insulin glargine 100 U/mL. It couples the structural model developed in [7] with a novel model of the BSV. The NLME model was able to capture both the typical trend and the variability of the absorption process, as well as, to provide an estimate of the deviation of each subject from the typical behavior through the random effects.

A significant ($p < 0.05$) positive correlation between η_k and η_{ksp} was detected, indicating that the higher the precipitate fraction of insulin after the injection (higher k), the higher the probability for an insulin molecule to dissolve

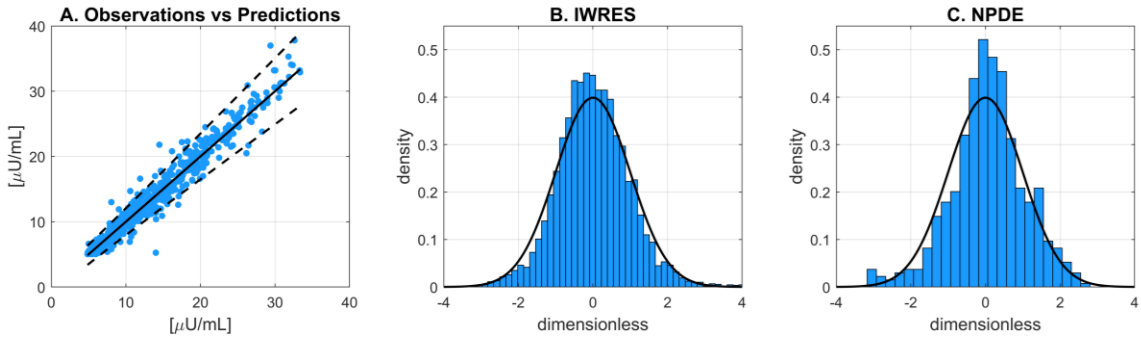

Fig. 2. Residual analysis plots for Model 2. Panel A: observations on the y-axes are compared with individual predictions on the x-axis; the line x=y (continuous black line) and the 90% prediction interval (dashed black lines) are also displayed. Panel B and C: empirical distributions (blue bars) of IWRES (panel A) and of NPDE (panel B) compared with a standard Gaussian (black lines).

(higher k_{sp}). Conversely, every attempt to introduce a covariate in the model failed, even if some weak relationships were identified, such as the dependency of the fractional clearance k_e on the body mass index (BMI) (Model 3) or of the bioavailability F on patient's age (Model 4). These findings seem reasonable from a physiological point of view, but perhaps we could not find a significant correlation due to the limited number of subjects, the specific experimental set-up, and the pre-processing of data, which are limitations of this work. Another limitation of this work is that the analysis was limited to insulin Gla-100 as representative of long-acting insulin compounds. Moreover, the available data did not allow assessing the WSV since blood samples were taken after a single insulin administration for each subject. Furthermore, the BSV estimated using data collected in a controlled setting may be an underestimation of the real variability observable in daily life conditions. Finally, only 3 possible independent (age, BW and BH) and 2 derived covariates (BMI and body surface area) were considered in the analysis, and only a log-transformation of them was tested together with their raw value. Additional studies are needed to validate the model in different experimental conditions and to assess the contribution of other possible covariates.

V. Conclusions

A NLME model describing the BSV of sc absorption of insulin Gla-100 has been presented. The model is able to accurately predict the insulin appearance in plasma from sc injection, and to provide an estimate of the BSV that affects this process. The incorporation of such model into simulation platforms of subjects with T1D or T2D (e.g., [6] and [10]) would allow running more realistic simulations of MDI therapy, providing great benefits on the development and testing of new treatment strategies.

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