Assessing Glucagon Kinetics through Nonlinear Mixed Effect Modeling

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BACKGROUND AND AIM

Impaired glucagon suppression and defective insulin secretion contribute to the onset of diabetes. However, compared to insulin, glucagon is understudied, in part because of the absence of a kinetic model necessary to estimate its secretion. Recently, Laurenti and colleagues filled this gap and derived population parameters of glucagon kinetics using a standard-two-stage approach [1]. The best model describing glucagon kinetic data was a single-compartment one. However, a two-compartment model performed satisfactorily in a nonnegligible percentage of the analyzed subjects (39 out of 51).

The aim of this work was to assess whether a **two-compartment model for glucagon kinetics** can be identified in a population framework using **nonlinear mixed effects** (NLME) modeling.

DATABASE

❍ Subjects:

Fifty-three healthy subjects (17 M and 36 F, age = 54±13 y, weight = 81±15 kg).

❍ Protocol:

Volunteers underwent a constant infusion of **somatostatin** (60 ng/kg/min) to inhibit endogenous glucagon and insulin secretion, a variable infusion of **insulin** to replace normal plasma insulin levels and a constant infusion of **glucagon** (65 ng/kg/min), starting 2 hours after somatostatin infusion (Fig. 1, panel A). Plasma glucagon were measured using a two-site ELISA (Mercodia, Winston Salem, NC) in accordance with the manufacturer's instructions (Fig. 1, panel B). Demographic (age and sex) and anthropometric data (body weight, height, lean body mass, body mass index and body surface area) were also collected.

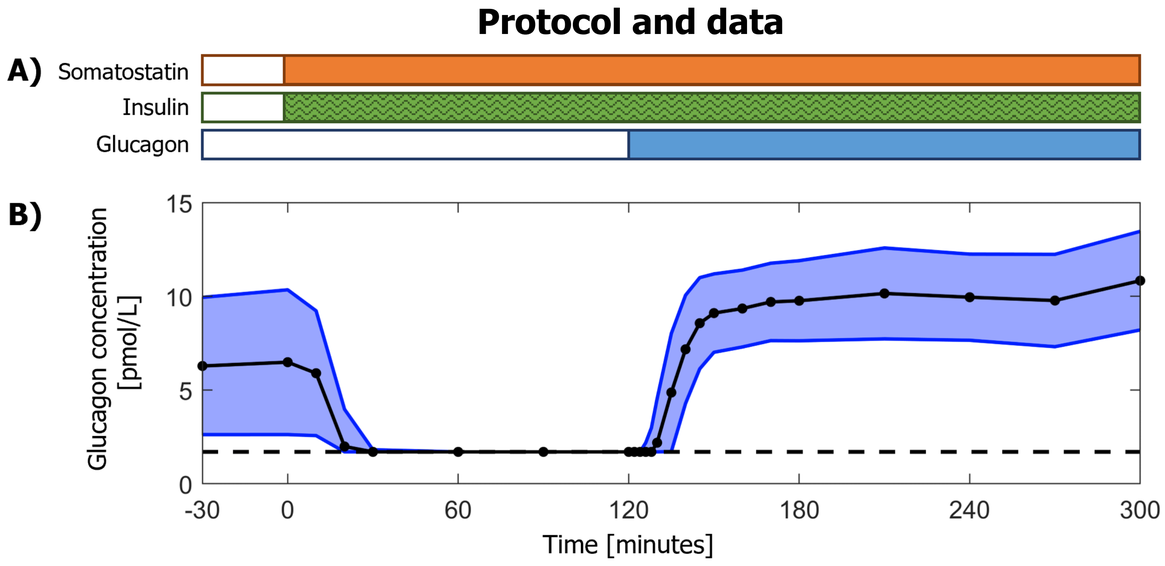

Fig. 1: Scheme of the experimental protocol and collected data. Panel A: filled bars represent the duration of the infusion of somatostatin (orange), insulin (green) and glucagon (blue). Panel B: mean (solid black line) ± standard deviation (blue area) of the plasma glucagon concentration. Dots indicates sampling times. The black dashed line indicates below limit of quantification.

METHODS

❍ Models:

The **NLME model for glucagon kinetics** consists of a structural model describing the individual kinetics of the hormone in plasma, and a stochastic model describing the variability of the structural model parameters among the subject population.

The **structural model** is a delayed two-compartment model. The model is reported in Fig. 2, where ir (pmol/min) is the infusion rate in input, $y(t)$ are the output measurements, CL (L/min) is the plasma clearance, Q (L/min) is the intercompartmental clearance, V_C and V_P (L) are the central and the peripheral volumes of distribution respectively, and t_0 (min) is a zero-order delay from the start of infusion that accounts for the time lag introduced by the glucagon pump.

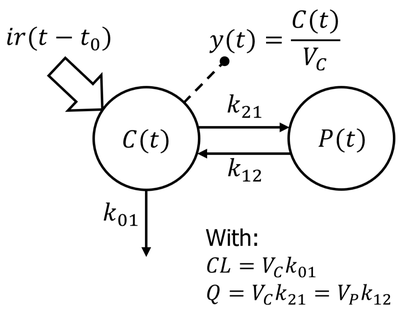

Fig. 2: Compartmental representation of the structural model.

The **stochastic model** assumes a log-normal distribution for all the kinetic parameters:

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

where $\psi_{i,j}$ is the i^{th} individual parameter of the j^{th} subject, θ_i is the i^{th} population parameter, the so-called fixed effects, and $\eta_{i,j}$ are Gaussian random variables, $\boldsymbol{\eta}\sim N(\mathbf{0},\boldsymbol{\Omega})$, the so-called random effects.

Finally, the model for the **measurement error** was assumed identical to [1]:

$$y(t)=\hat{y}(t)+[a+b\hat{y}(t)]\cdot\epsilon(t)$$

where $\epsilon(t)$ is a zero-mean and unit-variance Gaussian random variable, and the error parameters are fixed to a=0.07 pmol/L and b=0.049.

In this work, five different models were tested. They all share the **same structural and error model** described above, but **differ in the stochastic model**, in which different combinations of correlations between random effects were considered.

❍Identification strategy:

Models were identified using **Monolix** [2], which implements the stochastic approximation of expectation maximization in combination with a Markov Chain Monte Carlo method to estimate the maximum likelihood of the NLME model parameters. Prior information from [1] was employed for the estimation of the time delay, t_0. Only data from 120 min onward were used for model identification. The tested models were assessed in terms of **residual distribution** and **relative standard error** (RSE) of the estimates, and finally compared using a corrected **Bayesian information criterion** (BICc).

RESULTS

❍ Model comparison:

The results of model validation and comparison are reported in Tab. 1. Residual distribution was satisfactory for every tested model. Model 5 was rejected because one parameter was estimated with poor precision ($\rho_{Vc,Vp}$ with RSE=58.7%). Among the remaining models, **Model 2** was the one scoring the lowest BICc and, therefore, it was selected as the best model for describing glucagon kinetics.

#	Correlations between random effects	Number of model parameters	#RSE>50%	MEAN RSE [%]	BICc
1	-	10	0	15.37	3823.52
2	**$\rho_{CL,Vc}$**	**11**	**0**	**13.91**	**3814.01**
3	$\rho_{CL,Vp}$	11	0	13.82	3830.85
4	$\rho_{Vc,Vp}$	11	0	11.37	3840.39
5	$\rho_{CL,Vc}$; $\rho_{CL,Vp}$; $\rho_{Vc,Vp}$	13	1 ($\rho_{Vc,Vp}$)	17.82	3816.78

Tab. 1: Summary of the tested stochastic models. From left to right: model number; additional parameters ρ introducing a correlation between the random effects of model parameters; total number of estimated parameters; number of parameters estimated with RSE>50%; mean RSE; value of the BICc. The selected model is model number 2 (highlighted in bold).

❍The selected model:

The equations of the selected stochastic model are reported below:

$$\psi:\begin{cases}CL=CL^{pop}\exp(\eta_{CL})\\V_C=V_C^{pop}\exp(\eta_{V_C})\\Q=Q^{pop}\exp(\eta_Q)\\V_P=V_P^{pop}\exp(\eta_{V_P})\\t_0=t_0^{pop}\exp(\eta_{t_0})\end{cases}\quad\text{with:}\quad\Omega=\begin{bmatrix}\omega_{CL}^2&\omega_{CL}\omega_{V_C}\rho_{CL,V_C}&0&0&0\\\omega_{CL}\omega_{V_C}\rho_{CL,V_C}&\omega_{V_C}^2&0&0&0\\0&0&\omega_Q^2&0&0\\0&0&0&\omega_{V_P}^2&0\\0&0&0&0&\omega_{t_0}^2\end{bmatrix}$$

Parameter estimates are reported in Tab. 2 together with their precision. All population parameters were within physiological ranges. Finally, an overview of the model's ability to describe data can be seen in the **visual predictive check** (VPC, Fig. 3).

Fixed Effects	Value	RSE	SD of random effects	Value	RSE
CL^{pop}	1.25 L/min	3%	**ω_{CL}**	0.22	10%
Vc^{pop}	5.11 L	7%	**ω_{Vc}**	0.39	12%
Q^{pop}	0.49 L/min	10%	**ω_Q**	0.45	17%
Vp^{pop}	5.23 L	42%	**ω_{Vp}**	2.44	15%
t_0^{pop}	10.7min	7%	**ω_{t0}**	0.47	10%
Correlations	**Value**	**RSE**			
$\rho_{CL,Vc}$	0.68	20%			

Tab. 2: Parameter estimates, and their precision expressed as RSE.

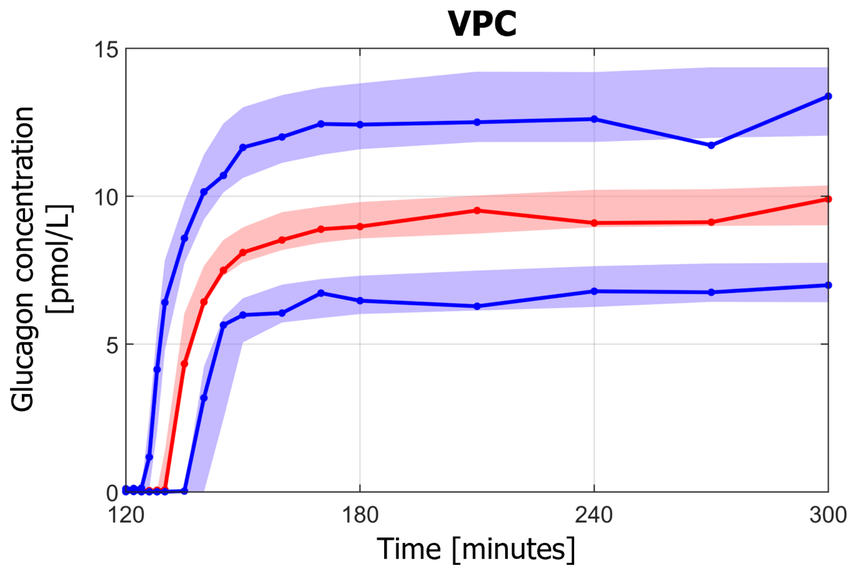

Fig. 3: VPC obtained with Model 2. Ninety-percent prediction intervals of the 10th (blue lower area), 50th (red central area), and 90th (blue upper area) percentiles are compared with the 10th (upper blue solid line), 50th (red central solid line), and 90th (lower blue solid line) empirical percentiles.

CONCLUSIONS

In this work, a **two-compartment model describing glucagon kinetics in plasma** has been developed in a NLME framework. The model assumes that kinetics parameters are log-normally distributed, and clearance and volume of distribution are correlated.

The model can be employed for the **estimation of glucagon secretion** via deconvolution as currently done for insulin, as well as it can be incorporated in **diabetes simulator platforms** to test therapies employing exogenous glucagon administration.

Future work includes adding a **covariate model** or **allometric scaling** to the presented NLME model.

REFERENCES

[1] Laurenti M. C., Vella A., Adams J. D., Schembri Wismayer J. D., Egan A. M., and Dalla Man C. "Assessment of Individual and standardized glucagon kinetics in healthy humans." *Am J of Physiol Endocrinol Metab.* 2021 Jan 1;320(1):E71-E77.

[2] Monolix, version 2020 R1, ©Lixoft, Antony, France. Documentation available at https://monolix.lixoft.com/.

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