Title: Optimization of the intravenous glucose tolerance test in T2DM patients using optimal experimental design

Authors: Hanna E. Silber*, Joakim Nyberg*, Andrew C. Hooker and Mats O. Karlsson

* Both authors have contributed equally

Address: Department of Pharmaceutical Biosciences, Uppsala University, Sweden.

Corresponding author:

Hanna Silber

Box 591

SE-751 24 Uppsala

Sweden

Phone: +46 18 471 44 37

Fax: +46 18 471 40 03

E-mail: hanna.silber@farmbio.uu.se

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Abstract

Intravenous glucose tolerance test (IVGTT) provocations are informative, but complex and laborious, for studying the glucose-insulin system. The objective of this study was to evaluate, through optimal design methodology, the possibilities of more informative and/or less laborious study design of the insulin modified IVGTT in type 2 diabetic patients.

A previously developed model for glucose and insulin regulation was implemented in the optimal design software PopED 2.0 (1, 2). The following aspects of the study design of the insulin modified IVGTT were evaluated; (1) glucose dose, (2) insulin infusion, (3) combination of (1) and (2), (4) sampling times, (5) exclusion of labeled glucose.

Constraints were incorporated to avoid prolonged hyper- and/or hypoglycemia and a reduced design was used to decrease run times. Design efficiency was calculated as a measure of the improvement with an optimal design compared to the basic design.

The results showed that the design of the insulin modified IVGTT could be substantially improved by the use of an optimized design compared to the standard design and that it was possible to use a reduced number of samples. Optimization of sample times gave the largest improvement followed by insulin dose. The results further showed that it was possible to reduce the total sample time with only a minor loss in efficiency. Simulations confirmed the predictions from PopED. The predicted uncertainty of parameter estimates (CV) was low in all tested cases, despite the reduction in the number of samples/subject. The best design had a predicted average CV of parameter estimates of 19.5%.

We conclude that improvement can be made to the design of the insulin modified IVGTT and that the most important design factor was the placement of sample times followed by the use of an optimal insulin dose. This paper illustrates how complex provocation experiments can be improved by sequential modeling and optimal design.

Introduction

Provocation experiments of different kinds are used to study the regulation of glucose and insulin in healthy individuals and in diseases such as diabetes. There is a wide range of provocations to choose from with respect to duration (from short-term experiments of a few hours to 24-hour profiles); route, rate and form of glucose (intravenous or oral, bolus or extended infusion, glucose solution or meals); insulin administration (short- or long-term infusion, short- or long-acting injections, glucose sensor-driven injections); and administration of other drugs and hormones, such as somatostatin and tolbutamide. Of these, the insulin modified intravenous glucose tolerance test (IVGTT) is one of the most frequently performed.

Intravenous glucose provocations are very informative for studying the glucose-insulin system and they are frequently used in drug development for assessment of potential new anti-diabetic treatment (3, 4). The intravenous provocations are, however, complex to perform as well as both time consuming and intensive in sampling. This makes them less appealing compared to the oral tests which are easier to perform but also less informative. When intravenous provocations are used in combination with efficient analysis tools such as non-linear mixed effect modeling it should be possible to simplify the design, while maintaining or increasing the information content of the experiment. Thereby, it would become more appealing to utilize the informativeness of the intravenous provocations as it would be possible to reduce the number of individuals included as well as the richness of the data needed and still gain enough information for estimation of model parameters.

The intravenous tolerance test was first introduced during the 1920s as a simple 1 hour test with four samples (5). It was later modified by increasing the study duration and frequency of sampling (6). The data generated with the frequently sampled IVGTT has been used for model based analysis with the minimal model which has become a standard tool for analysis of glucose data both for diagnosis and during drug development (7). The rich sampling common with the IVGTT was needed because the data was fitted on an individual basis and the minimal model used an open loop approach in which glucose is analyzed assuming that the insulin profile is known. With the minimal model, parameters

describing insulin sensitivity (S_I) and glucose efficiency (S_G) of glucose disappearance are estimated and can be used as a measure of diabetic state.

Several modifications of the IVGTT have been suggested including stimulation or suppression of insulin secretion and the addition of labeled glucose. The modifications generally aim at improving the power of estimation of parameters such as the S_I and S_G . The addition of a tolbutamide injection to the IVGTT was suggested and used in order to stimulate insulin secretion for better estimation of insulin sensitivity especially in diabetic patients (8, 9). However, tolbutamide injection requires pancreatic function for a sufficient insulin response which is commonly lacking in diabetic patients. As an alternative, the insulin modified IVGTT was introduced where a 5-minute insulin infusion is given at 20 minutes (10).

Optimal experimental design can be used to increase the efficiency of clinical trials by optimizing the sampling schedule and/or other design parameters, e.g. dose. When optimal experimental design is used together with population techniques for data analysis we expect the over all trial design of the IVGTT to be improved and made more efficient with respect to precision of parameter estimates. Optimal experimental design has been used previously in order to define a reduced sampling schedule for the IVGTT to be used in connection with the minimal model (11). A reduced sampling schedule including 14 samples was suggested by Cobelli et al. and was based on the minimal models for both glucose and insulin. This design was later evaluated using both an individual estimation method (standard two stage) and a population approach (iterative two stage) (12). When the individual estimation method was used the reduced sampling schedule was found to perform unsatisfactorily with respect to precision of parameter estimates. The population approach was found to improve the performance.

The objective of this study was to evaluate the possibilities of an improved study design of the insulin modified IVGTT in type 2 diabetic patients. Instead of the minimal model we use an integrated model for glucose and insulin regulation which has been developed previously using non-linear mixed effects modeling (1) and instead of optimizing the

design only with respect to sampling times, we optimize also for other design aspects, such as amounts and times of the glucose and insulin administrations.

Material and Methods

Optimal design

The optimal experimental design software PopED 2.0 was used for the optimization (2). PopED maximizes the Population Fisher Information Matrix (FIM) (13) with respect to the design variables X , that is

$$\arg \max_x (FIM(\Theta, X)) \quad (1)$$

where Θ are the population parameters. Maximizing the FIM will then, according to the Cramer-Rao inequality (Eq. 2), be an optimal lower bound for the precision of any unbiased maximum likelihood estimator.

$$COV(\Theta, X) \geq FIM(\Theta, X)^{-1} \quad (2)$$

There are several ways to maximize a matrix and the most common approach is to maximize the joint confidence volume of the parameters, which is equivalent to maximizing the determinant of the FIM. This criterion is called D-optimal design. With D-optimal design, optimization aims at minimizing the variances and covariances, i.e. uncertainty and correlation, of parameter estimates. In situations where some parameters are more interesting than others it is possible to focus the optimization on a subset of parameters, known as D_S -optimal design. In D_S -optimal design the determinant of the FIM for all parameters are divided by the determinant of FIM for the uninteresting parameters. By doing this, the correlation between the uninteresting part and the interesting part of the FIM will still be maintained, that is, the uninteresting parameters will contribute to the criterion only if they are correlated with the interesting parameters and hence will affect the precision of the interesting parameters. To derive the population FIM a linearization around the random effects is needed (13). In this work this linearization was done around the population mean of the random effects parameters as a first order linearization. Another approach is to linearize the model around the individual

values of the random effects parameters, e.g. the first order conditional method (FOCE) (14), but this approach is considerably slower computationally.

A population model for glucose and insulin regulation

A previously developed model for glucose and insulin regulation in healthy volunteers and T2DM patients (1) was implemented into PopED 2.0. For the aim of this project the model was reduced and only model structure applicable to the patient population was included. The model contains sub-models for glucose, insulin and hot glucose as well as interactions between glucose and insulin for regulation of insulin secretion and glucose uptake, see Figure 1. Typical concentration-time curves for glucose, insulin and hot glucose during the standard doses of the IVGTT are shown in Figure 2.

The different parts of the model are briefly described below and parameter values are given in Table 1.

Glucose sub-model.

Glucose was described by a 2-compartment disposition model with glucose elimination from the central compartment. Two elimination pathways were included, insulin dependent and insulin independent elimination. Glucose production from the liver was included as a constant rate production entering the central compartment. One effect compartment was included mediating the effect of plasma glucose on insulin secretion. The glucose sub-model is described by equations 3-6.

$$\frac{dG_C(t)}{dt} = G_{PROD} + \frac{Q}{V_P} \cdot G_P(t) - \left(\frac{CL_G}{V_G} + \frac{CL_{GI}}{V_G} \cdot I_E(t) + \frac{Q}{V_G} \right) \cdot G_C(t),$$

$$G_C(0) = G_{SS} \cdot V_G \quad (3)$$

$$\frac{dG_P(t)}{dt} = \frac{Q}{V_G} \cdot G_C(t) - \frac{Q}{V_P} \cdot G_P(t), \quad G_P(0) = G_{SS} \cdot V_P \quad (4)$$

$$\frac{dG_E(t)}{dt} = k_{GE} \cdot \frac{G_C(t)}{V_G} - k_{GE} \cdot G_E(t), \quad G_E(0) = G_{SS} \quad (5)$$

$$G_{PROD} = G_{SS} \cdot (CL_G + CL_{GI} \cdot I_{SS}) \quad (6)$$

Insulin sub-model.

Insulin was described by a 1-compartment disposition model with linear elimination and insulin secretion entering into plasma. One effect compartment was included mediating the effect of plasma insulin on insulin-dependent glucose uptake. The insulin sub-model is described by equations 7-11.

$$\frac{dI(t)}{dt} = I_{SEC}(t) - \frac{CL_I}{V_I} \cdot I(t), \quad I(0) = I_{SS} \cdot V_I \quad (7)$$

$$\frac{dI_E(t)}{dt} = k_{IE} \cdot \frac{I(t)}{V_I} - k_{IE} \cdot I_E(t), \quad I_E(0) = I_{SS} \quad (8)$$

$$I_{SEC,0} = I_{SS} \cdot CL_I \quad (9)$$

$$G_{CM}(t) = \left(\frac{G_E(t)}{G_{SS}} \right)^{IPRG} \quad (10)$$

$$I_{SEC}(t) = I_{SEC,0} \cdot G_{CM}(t) \quad (11)$$

Hot glucose sub-model

Hot glucose was assumed to be identical to regular glucose and the model was the same as for glucose with the exception of glucose production. The hot glucose sub-model is described by equations 12-13.

$$\frac{dH_C(t)}{dt} = \frac{Q}{V_P} \cdot H_P(t) - \left(\frac{CL_G}{V_G} + \frac{CL_{GI}}{V_G} \cdot I_E(t) + \frac{Q}{V_G} \right) \cdot H_C(t), \quad H_C(0) = 0 \quad (12)$$

$$\frac{dH_P(t)}{dt} = \frac{Q}{V_G} \cdot H_C(t) - \frac{Q}{V_P} \cdot H_P(t), \quad H_P(0) = 0 \quad (13)$$

Baseline model

In Silber *et al.* the model included the baselines of glucose and insulin as covariates (1). For the purpose of this project the baseline model was changed and an estimated log-normally distributed baseline was used instead. The difference in performance is small which has been shown previously (15) but the change reduces the dependence on observed baseline values and thereby makes the results of this publication more general. To make this change, the model was re-estimated in NONMEM in order to get the estimates for the baselines. The changed baseline model implementation gave only minor changes in other parameter estimates.

Inter-individual variability

Inter-individual variability was included using an exponential distribution of the individual values around the typical value according to the following equation.

$$P_i = P \cdot e^{\eta_i} \quad (14)$$

where P_i denotes the i^{th} individual's parameter value and is a function of P , the typical value of the parameter, and η_i , the individual random effect (a zero-mean random variable with the variance ω^2).

Residual error model

The model for the residual error was additive on log-transformed data and was separate for glucose, insulin and hot glucose. A multiplication factor was included for the early error during the first 2 minutes when the concentration changes are fast and therefore known to have a larger error.

Study design

Standard design

In the standard insulin modified IVGTT a bolus dose of glucose (0.3 g/kg bodyweight) is given at the start of the experiment followed by a 5-minute insulin infusion (50 mU/kg bodyweight) after 20 minutes. Hot glucose constitutes 10 % of the total glucose dose. Approximately 30 samples are taken during 240 minutes and analyzed for glucose, hot

glucose and insulin concentrations. In the standard rich design (SRD) that was used as a reference in this project 32 samples were taken at the following time points: 0, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 20, 22, 24, 26, 28, 30, 35, 40, 45, 50, 55, 60, 70, 80, 100, 120, 140, 160, 180, 210 and 240 minutes.

Because of the size and complexity of the glucose-insulin model it was necessary to reduce the number of samples in order to get the optimization to run with reasonable run times. The number of samples was reduced to 10 and placed at the following time points: 0, 2, 10, 15, 30, 45, 70, 100, 150 and 240 minutes. This standard design with 10 samples will be referred to as the standard sparse design (SSD) throughout this article. Glucose, insulin and hot glucose concentrations were measured at each sample time, however baseline measurements at time 0 were made only for glucose and insulin.

Optimization of design

The SSD as presented above was used as the basis for the optimization and one or more design aspects were optimized at a time while keeping the others fixed. The following aspects were evaluated alone or in combination: glucose dose, insulin dose, start time of insulin infusion, length (or stop time) of insulin infusion, sampling times, reduction of total sample time, and exclusion of hot glucose. In addition to optimizing using the SSD as a basis, optimization was also performed in a sequential manner in which the sample times were first optimized followed by a second step in which the optimized sample times were fixed and other design aspects were optimized. All tested scenarios are summarized in Table 2. To make sure that some information about the early residual error was found one of the samples had a maximum time of 2 minutes while the rest of the samples have an upper boundary of 240 minutes. The lowest possible sample time for all samples was 0.

A study size of 42 individuals was used. Bodyweight was fixed to the mean from the reference population, 89.4 kg.

D_S-optimal design was used and the parameters for residual error were treated as uninteresting parameters. Further; the correlations between individual η -values of V_G , Q and V_I were excluded from the optimization but they were still in the model, i.e. they were assumed fixed. A total of 24 parameters were included in the optimization.

Boundaries of allowed glucose concentrations

Boundaries were introduced in order to restrict the model space to physiologically plausible glucose concentrations. A lower boundary on glucose concentration introduces a limit to the size of the insulin dose and an upper boundary introduces a limit to the size of the glucose dose. The lower boundary was chosen so that 95 % of the individuals were above 58 mg/dl throughout the study. As there is no distinct definition of a hypoglycemic event a range of concentrations are used in clinical trials and a boundary of 58 mg/dL, as was used here, is within the range. The upper boundary was chosen more arbitrarily as short term hyperglycemia is not as critical as hypoglycemia and the limit was set so that the typical individual had a glucose concentration which was less than twice the baseline value 30 minutes after the start of the experiment. Optimizations and simulations were performed in PopED in order to evaluate the effect of using different values for the lower boundary.

PopED settings

Different optimization algorithms are available in PopED. Random search, stochastic gradient and line search were used sequentially for optimization. To speed up the optimization procedure a differential equation solver that stored the integrated solution over the whole design space in memory was used. However this method could only be used when optimizing over the sample times exclusively. The absolute and relative tolerance for the differential equation solver was set to a low value because of the stiff nature of the model. Further a complex differentiation method was used to ensure that a small enough step length for the calculation of the FIM could be used.

Evaluation of optimized design

Several measures of design improvement were used to measure the improvement of an optimized design compared to the standard design. The determinant of the Fisher Information Matrix (FIM) was used to calculate the efficiency according to equation 15.

$$Efficiency = \left(\frac{\begin{vmatrix} |FIM_{OPT_full}| & / & |FIM_{OPT_uninteresting}| \\ |FIM_{STANDARD_full}| & / & |FIM_{STANDARD_uninteresting}| \end{vmatrix}}{\begin{vmatrix} |FIM_{OPT_full}| & / & |FIM_{OPT_uninteresting}| \\ |FIM_{STANDARD_full}| & / & |FIM_{STANDARD_uninteresting}| \end{vmatrix}} \right)^{1/N_p} \quad (15)$$

Where $|FIM_{OPT_full}|$ is the full determinant of the FIM for the optimized design, $|FIM_{OPT_uninteresting}|$ is the determinant of the FIM for the uninteresting parameters given the optimal design, $|FIM_{STANDARD_full}|$ is the full determinant of the FIM for the standard design, $|FIM_{STANDARD_uninteresting}|$ is the determinant of the uninteresting parameters given the standard design and N_p is the number of interesting parameters in the model. Optimized designs were compared both to the SRD and the SSD. The efficiency corresponds roughly to the proportion increase or decrease of patients needed in the standard reference design to achieve the same parameter certainty as in the optimized design.

The predicted CVs (coefficient of variation) of the different parameters for the optimized design were compared to the predicted CVs for the basic designs. The predicted CVs provided from PopED are a lower boundary, thus when parameters are estimated from the model and future data, the true CVs can be expected to be higher.

Simulations were performed in NONMEM (16) to evaluate the performance of all of the optimized designs. For each design 100 simulations were performed and the model parameters were estimated from each of the 100 simulated data sets. The CVs of parameter estimates could be calculated from these 100 estimates and compared to the CVs predicted from PopED. Bias and imprecision were also calculated based on the simulated data. Data sets with 42 individuals corresponding to the reference population were created using the study design to be tested. The first order (FO) and the first order conditional estimation method (FOCE) were used for estimation. The same mean value for bodyweight was used in all individuals.

Results

The model was implemented into PopED 2.0 using the script version of the software. Simulations were performed in PopED to check that the model implementation was correct and that the simulations using the standard design were the same as when the model was used for simulation in NONMEM.

PopED was used for optimization and simulation using a range of insulin doses and boundaries to find a suitable lower boundary. The magnitude of the improvement in efficiency was strongly dependent on the level of the lower boundary of the glucose concentration. The potential improvement in the design was higher when the boundary was set to a lower value.

The results of the optimizations are presented in Table 2 where the optimized design parameters are presented as well as the efficiency calculated based on both the SRD and the SSD and the mean predicted CV%. For the optimization of single design parameters, the optimization of sample times gave the largest improvement in efficiency followed by optimization on insulin dose. Optimizing on glucose dose gave a smaller improvement and optimizing on the infusion start time or length of insulin infusion did not further improve the design to a significant degree.

The efficiency of the non-optimized SSD was only 67% of the SRD. The SSD was always improved when optimization was performed, and, in general the optimized sparse designs were less efficient compared to the SRD. However, in 3 optimizations the optimized design was more efficient than the SRD despite only including 1/3 of the samples. These information rich designs occurred when optimizing sample times, insulin dose at optimal sample times and both glucose and insulin dose simultaneously. This last optimization was the most efficient, indicating the non-linear correlation between design parameters (with linear correlation, optimization over the two most influential single design parameters, sample times and insulin dose, would give the largest increase).

When optimization of sample times was performed clustering of sample times was observed. This occurs because duplicate samples will improve the estimation of the residual error parameters through an increase in the signal-to-noise ratio. Sample times were also optimized with a restricted total sample time of 2 hours and the result showed a loss in efficiency of less than 1% compared to the SSD. It is obviously possible to shorten the sample period with only minor losses in efficiency if the sample times are placed optimally. Because the number of samples is still the same this shorter design has the advantage of being richer in a shorter time period.

The predicted precision in parameter estimates was good in all tested cases; even when the sparse design was used. The predicted average CV% when the design was optimized on sample times was 19.5 % compared to 26% with the SSD and 22.5% with the SRD. The precision was good in all tested scenarios, except for the case when hot glucose was excluded. This indicates that even though the efficiency was lower when a sparse design was used compared to the rich design, a sparse design may well give sufficient precision in parameter estimates.

Simulations were performed for all optimized designs and the simulated data were analyzed using both FO and FOCE. The resulting estimated CV was compared to the predicted CV of each parameter computed by PopED. The results showed that the estimated CV values were in general close to the predicted values, with the FOCE method resulting in slightly lower imprecision than the FO method. The estimated CVs were in most cases larger than the predicted values, which is expected as the predictions from PopED constitutes the lower boundary. The CVs for all parameters of the four most efficient designs are shown in figure 3. Bias and imprecision were small for most of the evaluated scenarios. FOCE performed better than FO and the mean absolute bias calculated per design was in most cases below 15 % for FOCE and below 36 % for FO. The mean imprecision calculated per design was in most cases below 88 % for FOCE and below 70 % for FO.

Discussion

In this study we have shown that it is possible to implement and optimize an experiment based on a complex model with multiple dependent variables. With the rapid increase in prevalence of T2DM and the need for understanding of the regulatory system of glucose and insulin and the development of efficient drugs for the treatment of T2DM it is important to make sure that studies are performed efficiently in order to cut costs but also to minimize the inconvenience for patients. In this study we have focused on optimizing the study design for estimation of parameters in the placebo model (i.e. system parameters) but the natural continuation is to look at design optimization for identification of the mechanism of action of potential drugs in development.

Provocation experiments are often used as screening methods for diagnosis of diseases, such as diabetes, in addition to their use in drug development for experimental purposes (4). In this situation, the efficiency of the method is essential. Previous attempts at improving the design of the IVGTT has mainly focused on sampling times (11, 12). However, with provocation experiments there are several other aspects which are of importance for the resulting effect on the system such as the dose and timing of intervention. This paper illustrates one example of how the optimization of these aspects can be done.

With sparse sampling, the most important single design aspect was found to be sample times. When optimal sample times were used the sparse design was shown to be equally efficient as the original rich design and we have thus shown that it is possible to reduce the number of samples from 32 to 10 without losing information as long as optimal sample times are used. The second most important design parameter was shown to be the insulin dose and the magnitude of the improvement of the design was strongly dependent on how the lower boundary of glucose concentration was chosen. A wider range of allowed glucose concentrations gives the possibility of more efficient designs but also increase the risk of the patients to experience hypoglycemic events.

When optimizing on multiple design variables at the same time, our results showed that optimization on insulin and glucose dose was more efficient than optimizing on the two most important single design aspects (sample times and insulin dose). This result indicates the complex correlation that can be seen between design parameters, as well as the benefit of optimizing on multiple design parameters.

Most of the sparse designs considered here were less efficient compared to the rich design which is normally used in clinic. However, it is worth noting is that even though the efficiency was lower with these sparse designs the predicted imprecision in parameter estimates was still quite low and often similar to what was predicted for the rich design. It should therefore be considered that a design with a low efficiency compared to the reference might still be sufficient for the purpose of which the design will be used.

Boundaries on glucose concentrations were incorporated in order to avoid unwanted glucose concentrations. The most important boundary was to limit the low glucose concentrations in order to avoid hypoglycemia. The boundary was implemented as a cut off and if a concentration was predicted to fall outside this boundary it was returned as an uninformative design. An alternative would have been to use a penalty function which would have avoided the very strong assumption that was used here. Because the FO method was used for optimization the boundary was implemented on the typical individual and a “trial and error” procedure was needed in order to find a suitable boundary for the population. If the FOCE method had been used for optimization the restriction could have been implemented on the individual profile instead of the typical individual thus avoiding the need for the “trial and error” process. However, this was not feasible because of the long runtimes that would have resulted from using the FOCE method with such a large model. It is however a solution that should be evaluated on a simpler problem.

When optimization is performed on several design parameters it is generally preferable to optimize on all simultaneously (17). Because of the complexity of this model simultaneous optimization was not always possible. Optimization of sample times was

especially time consuming and in the cases where sample times were optimized together with other design parameters this was done in a sequential manner by optimization of sample times followed by optimization of dose.

Ds-optimal design was used in this project which assumes that the point estimates of parameter are known to be the true values. The model was developed based on a combination of different provocation experiments but also on both healthy volunteer and T2DM patient populations (1). Most of the parameters were estimated based on all data and we can therefore expect some parameters to be estimated with less precision and to have a slightly different value when only patient data are included. In order to acknowledge the uncertainty in the parameter estimates ED optimal design where a distribution is assumed around the parameter estimate instead of assuming a known point estimate could have been an alternative in this situation (18-21). The ED-approach would be more robust but as the parameters were originally determined with reasonably low imprecision, Ds-optimality is a reasonable alternative. Furthermore, due to the complexity of the model ED-optimality was not considered because of long run times.

The standard design of the IVGTT is a very informative design thanks to its richness. However, the work presented in this article shows that there is room for improvement. The use of optimal design and population methods results in sparser designs and/or the need for fewer individuals in these studies. Uncertainty in parameter estimates was predicted to be low with these sparse designs and these predictions were also confirmed by simulations. In addition the sparse designs showed good accuracy in parameter estimates, especially when the FOCE method was used. The example presented here illustrates how complex provocation experiments can be improved by the use of population modeling and optimal design.

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Table I. Parameter values of the glucose insulin model in type 2 diabetic patients

	Parameter ^a	Typical value (CV%)	IIV% ^b (CV%)
Glucose	V _G (L/70 kg bw)	9.33 (6)	30 (18)
	V _P (L)	8.56 (5)	30 (28)
	CL _G (L/min)	0.0287 (15)	59 (35)
	CL _{GI} (L/min/(mU/L))	0.00297 (9)	53 (22)
	Q (L/min)	0.442 (16)	85 (27)
	k _{GE2} (/min)	0.0289 (14)	85 (43)
	G _{BASE} (mg/dL)	159	20
Insulin	V _I (L/70 kg bw)	6.09 (9)	41 (27)
	CL _I (L/min)	1.22 (5)	29 (24)
	k _{IE} (/min)	0.0213 (13)	58 (20)
	IPRG (-)	1.42 (12)	35 (46)
	I _{BASE} (mU/L)	11.5	49
Residual	RESG (%)	4.36 (4)	-
	RESI (%)	25.2 (6)	-
Error	RESH (%)	5.12 (8)	-
	RESE (-)	3.31 (21)	-

^a V_G, glucose central volume; V_P, glucose peripheral volume; CL_G, insulin independent glucose clearance; CL_{GI}, insulin dependent glucose clearance; Q, inter-compartmental glucose clearance; k_{GE2}, glucose effect compartment rate constant; G_{BASE}, glucose baseline; V_I, insulin volume; CL_I, insulin clearance; k_{IE}, insulin effect compartment rate constant; IPRG, glucose effect on insulin secretion; I_{BASE}, insulin baseline; RESG, glucose residual error; RESI, insulin residual error; RESH, hot glucose residual error; RESE, early error multiplication factor; IIV%, inter-individual variability as standard deviation in percent; CV%, relative standard error in percent.

^b Inter-individual variability given in % as standard deviation.

Table 2. Optimization scenarios

Optimization parameter	Optimization interval	Glucose dose (g/kg) ^a	Insulin dose (mU/kg)	Infusion start time	Infusion stop time	Sample times ^b	Efficiency (SSD) (%) ^c	Efficiency (SRD) (%) ^d	Predicted mean CV (%) ^e
Insulin dose	1-300 mU/kg	0.3	88.4	20	25	SSD	126.4	84.4	21.5
Start of insulin infusion	0-235 min	0.3	50	28.6	5	SSD	106.7	71.3	25.3
Stop of insulin infusion	25-240 min	0.3	50	20	34.8	SSD	101.9	68.1	25.8
Start, stop, dose of insulin infusion	1-300 mU/kg, 0.1-240 min, 0.1-240 min	0.3	83.9	28.9	31.5	SSD	134.1	89.6	21.1
Stop, dose of insulin infusion	1-300 mU/kg, 25-240 min	0.3	86.9	20	32.8	SSD	129.3	86.3	21.3
Glucose dose	0.1-3 mg/kg	0.81	50	20	25	SSD	114.6	76.5	28.0
Glucose dose, insulin dose	0.1-10 mg/kg, 1-1000 mU/kg	1.42	151	20	25	SSD	166.6	111.2	20.9
10 samples	0-240 min	0.3	50	20	25	0, 2.1, 9.6, 20, 23.2, 37.5, 56.7, 112, 240, 240	150.7	100.6	19.5
10 samples, 2 hours	0-120 min	0.3	50	20	25	0, 2.1, 2.1, 11.7, 23.7, 36, 55.2, 72, 120, 120	99.6	66.5	32.8
Exclude hot	-	0.3	50	20	25	SSD	72.9	48.7	78.8
Insulin dose with optimized sample times	1-300 mU/kg	0.3	95.2	20	25	0, 2.1, 9.6, 20, 23.2, 37.5, 56.7, 112, 240, 240	155.6	103.9	19.1

^a Bold numbers indicate optimized design parameter.

^b SSD indicates standard sparse design with the following sample times: 0, 2, 10, 15, 30, 45, 70, 100, 150, 240 minutes.

^c Efficiency calculated based on the Ds FIM and compared to the SSD.

^d Efficiency calculated based on the Ds FIM and compared to the SRD.

^e Predicted mean CV% for the SSD and the SRD were 26.1% and 22.5%.

Figure 1. Schematic presentation of the model for glucose and insulin regulation.

Figure 2. Simulated concentration-time profiles of glucose, insulin and hot glucose using the standard doses of glucose and insulin.

Figure 3. Estimated CV with FO (black) and FOCE (grey) versus predicted CV with PopED for the four best designs. Filled circles are typical parameters with FO, open circles are IIV parameters with FO, crosses are typical parameters with FOCE, triangles are IIV parameters with FOCE. As the predicted CVs constitute the lower boundary for the CV, the majority of the estimated CVs should lie to the left of the line of identity.