Title: An integrated glucose-insulin model to describe oral glucose tolerance test data in healthy volunteers

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ABSTRACT

The extension of the previously developed integrated models for glucose and insulin (IGI)^{1,2} to include the oral glucose tolerance test (OGTT) in healthy volunteers could be valuable to better understand the differences between healthy individuals and type 2 diabetes mellitus (T2DM).

Data from and OGTT in 23 healthy volunteers was used. Analysis was based on the previously developed intravenous model with extensions for glucose absorption and incretin effect on insulin secretion. The need for additional structural components was evaluated. The model was evaluated by simulation and a bootstrap.

Multiple glucose and insulin concentration peaks were observed in most individuals as well as hypoglycemic episodes in the second half of the experiment. The OGTT data was successfully described by the extended basic model. An additional control mechanism of insulin on glucose production improved the description of the data. The model showed good predictive properties and parameters were estimated with good precision.

In conclusion, a previously presented integrated model has been extended to describe glucose and insulin concentrations in healthy volunteers following an OGTT. The characterization of the differences between the healthy and diabetic stages in the IGI model could potentially be used to extrapolate drug effect from healthy volunteers to T2DM.

INTRODUCTION

Provocation experiments are commonly used to study the regulation of glucose and insulin in healthy and diabetic individuals. Several mathematical models have been developed with the aim of describing glucose and insulin regulation in different situations, the most well known being the minimal model.³ A drawback of most previously presented models is that they use an open-loop approach, which significantly simplifies the model development but at the same time limits the possible use of these models for predictive purposes.^{3,4,5} Development of an integrated physiology based model for glucose and insulin was initiated by Silber *et al*¹ with a model describing glucose and insulin regulation during intravenous provocation experiment in both healthy volunteers and type 2 diabetes mellitus (T2DM) patients. The model was built using glucose, hot glucose and insulin plasma concentrations during different types of intravenous provocations including a clamp experiment and intravenous tolerance tests (IVGTT) with or without the addition of external insulin. The model was further extended by Jauslin *et al*² to describe an oral glucose provocation test (OGTT) in T2DM patients. In this integrated glucose-insulin (IGI) model, the glucose disposition is characterized by a two-compartment model with both insulin dependent and independent glucose clearance and with endogenous glucose production. The insulin disposition is characterized by a one-compartment model with linear elimination and separated 1st and 2nd phase secretion. Control effects were incorporated on glucose production, insulin secretion and insulin dependent glucose elimination. Differences between healthy volunteers and T2DM patients could be quantified. Patients were found to have a decreased glucose clearance (both pathways were affected), a lack of control of glucose production by glucose and an absence of first-phase insulin secretion. The main difference identified in T2DM patients during oral provocations compared to intravenous data was the identification of the incretin effect on insulin secretion. Additionally, the insulin dependent clearance was found to be increased in patients during the oral provocation compared to the intravenous situation.

The aim of the present paper was to extend on the previously published IGI model with oral glucose provocation data in healthy volunteers. Oral glucose provocations

are commonly used in early stages of clinical drug development starting in entry into human studies. A model for oral provocation experiments describing the glycemic regulation in the healthy state could be of value for the early assessment of anti-diabetic drug effects and for their extrapolation to the diabetic state.

MATERIAL AND METHODS

The data

The data included observations of glucose and insulin in 23 healthy male volunteers during an OGTT which was the placebo arm of a clinical study performed by Hoffmann-La Roche, Switzerland. The protocol was approved by an independent ethics committee. Written consent was obtained from all subjects included in the study. After an overnight fast the subjects were given a solution of 75 g of glucose to drink within 5 minutes. Blood samples for the determination of glucose and insulin concentrations were collected pre-dose and at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225 and 240 minutes after the glucose dose.

Variable glucose and insulin profiles, with multiple peaks of glucose and insulin concentrations, were observed in most individuals as well as hypoglycemic episodes during the second half of the experiment. This can be seen in figure 2 where plasma concentration data of glucose and insulin are shown in four representative individuals.

The structural model

The IGI model developed by Silber *et al*¹, with extensions by Jauslin *et al*.², was used as a starting point. The model equations stated in that publication underlie the present model unless otherwise specified. The equations of the model are included in appendix 1. The model was parameterized in clearance and volume and initially all parameters (i.e. parameters describing disposition of glucose and insulin as well as control mechanisms and variance parameters) were assumed to be identical to the values determined based on the intravenous provocations as presented by Silber *et al*. The validity of the assumption was later tested by estimation of separate disposition parameters for the OGTT. The validity of the included control mechanisms and the need for incorporation of additional control mechanisms were also evaluated. Different models for description of glucose absorption were tested including both semi-mechanistic and empirical models. Initially, a transit compartment model was tested which was used to describe the absorption phase in type 2 diabetic patients during the OGTT as presented by Jauslin *et al*.² However, this model could not describe the multiple glucose and insulin peaks which were seen in the healthy volunteer data and a more complex model describing the glucose absorption was

needed. The flexible input model is an empirical model in which the input rate is modeled as a series of zero-order inputs.⁶ The number of steps, the length and input rate of each step and the structure of the inter-individual variability in absorption need to be established.

It is a well established fact that oral glucose provocations results in a stronger insulin response compared to intravenous provocations.^{7, 8, 9} This effect is called the incretin effect and is mediated through the release of insulin stimulating hormones from the gut wall, mainly GLP-1, as a response to ingestion of glucose or other carbohydrates. In the model by Jauslin *et al* the incretin effect was included as a direct effect of glucose absorption rate on insulin secretion using an $E_{\max}$ function.² The incretin effect was included in the same way in the present model for healthy volunteers. Both linear and $E_{\max}$ functions were evaluated.

Inter-individual variability and residual error

Differences between individuals in model parameters were regarded as random and were modeled as eta-variables (η). The η -variables were assumed to be normally distributed with a mean of 0 and a variance of ω^2 , which was estimated by the model. The distribution of individual parameter values around the typical value was assumed to be log-normal. The need for inclusion of inter-individual variability was evaluated on all added parameters.

An additive model was used to describe the residual error on log-transformed data. Separate parameters were estimated for glucose and insulin of the OGTT as the residual error magnitude can be expected to be different between glucose and insulin as well as between the intravenous and oral provocation experiments. The additive error model on log-transformed data approximately corresponds to a proportional error model on non-transformed data.

Data analysis and model evaluation

Nonlinear mixed effects modeling using NONMEM VI with the first order estimation model (FOCE) was used for data analysis.¹⁰ Model selection was based on mechanistic considerations, plausibility of parameter estimates, the objective function value (OFV) and graphical assessment using the R-based program Xpose 4.¹¹ The

OFV, which is proportional to -2 times the logarithm of the likelihood of the data, is a measure of how well the model fits the data and a lower value indicates a better fit.¹⁰ The final model was evaluated using internal validation methods. A bootstrap was run to assess the uncertainty in parameter estimates and the predictive properties of the model was assessed by performing the visual predictive check (VPC).¹² For the bootstrap 50 bootstrap samples was run due to the long runtimes. For the VPC the median and the 90 % prediction interval (PI), based on 1000 simulations from the model, were compared to mean and the 90 % interval of the observed data.

RESULTS

Model development

The final model is illustrated graphically in figure 1 and the final parameter estimates are presented in table 1. Figure 2 shows the individual predictions of glucose and insulin together with the individual predicted absorption rate pattern in four representative individuals. The complex absorption profile of glucose with multiple peaks in most individuals was adequately described using an empirical flexible input model. The number of steps and the length of each step needed for adequate description of the absorption profile were determined by manual evaluation, and the use of 12 steps was found to be adequate. During the first two hours of the experiment 15-minute steps were used and during the last two hours a step length of 30 minutes was used. The fractional absorption rate was estimated during each of the 12 specified time intervals and is summarized in the parameter R1. The NONMEM code for the flexible input model is included in appendix 2. The glucose absorption rate (ABSG) was expressed according to equation 1. The bioavailability of glucose (BIO_G) was estimated to 72% which is in line with previous results in T2DM patients (81%) and with literature.^{2, 13, 14} The glucose absorption rate (ABSG) was expressed according to equation 1. The modified differential equation for the central glucose compartment including the glucose absorption rate is presented in equation 2.

$$ABSG(t) = R1(t) \cdot BIO_G \cdot DOSE \quad (1)$$

$$\frac{dG_C(t)}{dt} = ABSG(t) + G_{PROD}(t) + \frac{Q}{V_P} \cdot G_P(t) - \left(\frac{CL_G}{V_G} + \frac{CL_{GI}}{V_G} \cdot I_E(t) + \frac{Q}{V_G} \right) \cdot G_C(t), \quad (2)$$

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

The incretin effect (I_{ABSG}) could be described using a linear function of glucose absorption rate which was incorporated as a direct effect on insulin secretion (I_{SEC}). The first phase insulin secretion which was present in healthy volunteers during the intravenous provocations could not be identified during the OGTT and the insulin secretion was therefore described according to equations 3 and 4.

$$I_{SEC}(t) = I_{SEC,0} \cdot G_{CM2}(t) \cdot I_{ABSG}(t) \quad (3)$$

$$I_{ABSG}(t) = 1 + S_{INC} \cdot ABSG(t) \quad (4)$$

where $I_{SEC,0}$ is the baseline insulin secretion, G_{CM} is the glucose effect on second phase insulin secretion and S_{INC} is the slope describing the additional effect of glucose absorption rate on insulin secretion.

Based on the present data it was possible to identify an additional control effect of insulin plasma concentration on glucose production. This effect was found to be direct and was incorporated using a power function. The resulting equation for glucose production (G_{PROD}) is described by equations 5 and 6. The individual predicted glucose production-time profiles are shown in figure 3. Figure 4 shows the combined effect of glucose and insulin of glucose production during the experiment and also the effect of only glucose. From figure 4 it is evident that insulin has a dominating effect of the regulation of glucose production during the first half of the experiment and that glucose is important mainly during the last 2 hours. The two effects were incorporated in a multiplicative way, and therefore, if one goes towards zero this will also result in zero glucose production as can be seen in figure 3. Another parameterization was evaluated in which the insulin effect was incorporated as a proportional effect but that resulted in a significantly worse model fit.

$$G_{PROD}(t) = G_{PRDO,0} \cdot G_{CM1}(t) \cdot I_{CM}(t) \quad (5)$$

$$I_{CM}(t) = \left(\frac{I(t)}{V_I \cdot I_{SS}} \right)^{GPR1} \quad (6)$$

No parameters of the disposition model or of the control effects were found to be different between the intravenous and oral provocations. The residual error of glucose and insulin were found to be 8.6 % and 22.6 %, respectively, which are reasonable values for this type of data. The residual error of glucose with the OGTT was higher than with the intravenous provocations but for insulin it was somewhat smaller compared to the intravenous provocations.

Model evaluation

Basic diagnostic plots for assessment of the model fit (including population and individual predictions, conditional weighted residuals and individual residuals) showed that the model described the data adequately.

The bootstrap results (included in table 1) showed that the uncertainty in parameter estimates was generally small and in no fixed effects parameter was the uncertainty more than 37%. The uncertainty in the inter-individual parameters was larger

especially for the parameters included in the flexible input model. Of the 50 bootstrap samples 12 terminated with rounding errors or had parameter estimates near a boundary and were excluded from the calculations. The VPC showed that the model could adequately predict the data and the median curve of the simulated data closely resembled the observed data. The VPCs for glucose and insulin are shown in figure 5. Rather than plotting the median and limits of the prediction interval (5th and 95th percentiles) of the simulated data the 95% confidence intervals around these limits have been plotted as a shaded area. This simplifies the comparison between observed and simulated and the corresponding percentiles of the observed data should fall within the shaded areas. The figure shows that the PI interval of the insulin data closely resembled the observed data. The PI interval of the glucose data was wider than the observed data indicating that the variability in the simulated data was higher although the observed data fell within the confidence interval during the main part of the experiment. Inspection of individual simulated profiles further showed that the model was able to produce profiles similar to the observed data.

DISCUSSION

In this paper we have presented an extension to the previously presented IGI model to an oral glucose tolerance test in healthy volunteers. This additional development was necessary to finalize the model-based investigation of the difference in glucose homeostasis between the healthy and the diabetic states using the glucose provocations commonly used in clinical drug development.

An important result presented here was the ability of the model to separate the inhibitory effects of glucose and insulin on glucose production during a glucose provocation experiment. Both glucose and insulin are known to inhibit glucose production during hyperglycemia^{5, 15, 16} but we have not been able to separate the effects based on previously analyzed data of intravenous provocations. Insulin was predicted to have the dominating effect on glucose production during the first half of the experiment whereas glucose effect was predicted to be important during the second half when glucose production is increased to overcome the hypoglycemia which occurs in most individuals. The fast onset of the insulin effect is not surprising as the effect of insulin is mainly due to insulin present in the portal vein into which insulin is secreted. Figure 3 shows that the glucose production is more or less totally suppressed up to 2 hours following the ingestion of the glucose dose. The hypoglycemic episodes which were seen in most individuals during the last part of the experiment were probably due to the inhibition of glucose production which delays the return to baseline and this feature in the data was most likely of importance for the ability of the model to separate the effect of glucose and insulin on glucose production. When the additional control mechanism was not included in the model, or was included as a proportional effect, the hypoglycemic events were not well predicted. In the initial model development using the IVGTT data, only the effect of glucose on glucose production was found. Reasons for the inability of the model to identify both effects from intravenous data, which are normally expected to be more informative than oral, could be that much less hypoglycemic events were observed with the IVGTT experiments and also that the ones observed were mainly seen in a small group of individuals who received external insulin. It has previously been shown that externally administered insulin does not produce the same effects on glucose production as endogenous insulin, probably because the resulting portal vein

concentrations are much lower following external administration compared to endogenous secretion.¹⁷

The incretin hormones which are released from the gut as a response to ingestion of glucose or carbohydrates have multiple functions on glucose absorption, insulin secretion and insulin sensitivity.^{7, 8, 9} The main effect of incretin hormones are the stimulation of insulin secretion and this mechanism was also incorporated into the model as a direct effect of glucose absorption rate. In the model developed for T2DM patients an $E_{\max}$ model was identified and the maximum effect was two-fold. For the healthy volunteers a linear model was identified. In comparison to the T2DM patients the incretin effect was stronger in the healthy volunteers, which is in line with the expectation of a better insulin response and more efficient glucose control in healthy individuals compared to patients. The implementation of an $E_{\max}$ model in T2DM patients is more physiological than a linear model as many physiological systems tend to be saturable. However, when an $E_{\max}$ model was incorporated on the present data the parameter estimates were such that it indicated that only the linear part of the relationship was covered during the experiment and the model was therefore reduced to a linear model.

In contrast to the previously analyzed intravenous data in healthy volunteers¹ it was not possible to identify the first-phase secretion of insulin during the oral provocation experiment. The first phase insulin secretion has previously been shown to be important for the regulation of glucose metabolism and specifically to provide an initial inhibition of endogenous glucose production as a response to a glucose load.^{17, 18, 19} Therefore it is likely that the first-phase secretion is present also in this population although it cannot be separated from the incretin effect or the more slowly acting second-phase secretion.²⁰

The incretin hormones are known to inhibit gut emptying and glucose absorption which can explain the multiple peaks which were seen in the present data.⁸ Different semi-mechanistic models were evaluated for description of the glucose absorption, including the transit compartment model. An extension of the transit compartment model was tested which included a negative feedback loop on the transit rate through the chain to resemble the effect of incretin hormones on gut emptying. None of the

more mechanistic models were able to describe the complex absorption pattern which resulted in multiple peaks of plasma glucose concentrations. It is possible that the absorption rate, although influenced by the incretin hormones, is also determined by several other factors including random patterns, and that it is therefore difficult to find a mechanistic type model to describe the variable absorption rate based only on the effect of the incretin hormones. In the final model an empirical flexible input model was used and, as this model can describe almost any shape of absorption rate, it revealed a complex absorption pattern.

In the present study in healthy volunteers no disposition parameters were found to differ between the intravenous and oral provocation experiments. However, an additional regulatory mechanism on glucose production was needed for an accurate description of the glucose concentration data. In the previously developed model for T2DM patients insulin dependent clearance (CL_{GI}) was found to be approximately doubled during oral provocation experiments compared to intravenous.² In the patient population it was not possible to identify a regulatory mechanism on glucose production in either the intravenous or oral provocation experiment data and as a result the model predicts a constant production rate throughout the experiment. It is therefore possible that the higher CL_{GI} seen in the patient population during the oral provocation was needed to compensate for the constant production of glucose. In the present model this was instead handled by inhibition of glucose production both by glucose and insulin.

The analysis of healthy volunteer oral provocation data revealed additional complexity compared to the previously analyzed oral provocation data in T2DM patients. One reason for the additional complexity is that the control of glucose homeostasis in healthy volunteers is fully functional and therefore more difficult to characterize compared to the patients where the control is known to be deficient. Another explanation for the ability of the model to identify additional structures was that the healthy volunteer oral data was richer with up to 17 observations in each individual compared to only six observations in the patients.

The model which has been presented here has been shown to successfully describe the OGTT data in healthy volunteers. The VPC shows that it could predict the typical

curve and inspection of simulated individual profiles also displayed the expected pattern with multiple peaks. The variability in glucose was found to be somewhat larger in the simulated data compared to the observed data as can be seen in figure 5. The bootstrap showed that all fixed effects parameters were estimated with high precision. The variance parameters were estimated with larger uncertainty and part of the explanation is probably that for several of the intervals for which the absorption rate was estimated there was only one observation in each individual. This gives a large flexibility allowing for close description of the absorption rate pattern but the data probably does not contain information enough for precise parameter estimation.

In conclusion, we present an extension of a previously presented model to also include oral provocation experiments in healthy volunteers. With this additional development, the integrated glucose-insulin model can now be used to derive glucose homeostasis parameters from the glucose provocations that are most commonly used in early stage of clinical drug development, both in healthy volunteers and in T2DM. The mathematic representation of the known physiology and regulation of this system is also a possible asset for the identification of the effect of anti-diabetic drugs according to their mechanism of action. Finally, the characterization of the differences between the healthy and diabetic stages in the IGI model could potentially be used to extrapolate drug effect from healthy volunteers to T2DM patients.

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Table 1. Final parameter estimates

	Parameter¹	Typical value (RSE)²	IIV% (RSE)²
Glucose	V _G (L)	9.33	30
	V _P (L)	8.56	30
	CL _G (L/min)	0.0894	59
	CL _{GI} (L/min/(mU/L))	0.00829	53
	Q (L/min)	0.442	85
	k _{GE1} (/min)	0.0573	-
	k _{GE2} (/min)	0.0289	85
	GPRG (-)	-2.79	-
	BIO_G (-)	0.722 (6)	-
	S_{INC} (min/mg)	0.0818 (8)	21 (51)
Insulin	V _I (L)	6.09	41
	CL _I (L/min)	1.22	29
	k _{IS} (/min)	0.384	-
	k _{IE} (/min)	0.0213	58
	IPRG (-)	1.42	35
	GPRI (-)	-3.45 (7)	-
Residual	RES_{GPO} (%)	8.58 (6)	-
Error	RES_{IPO} (%)	22.6 (7)	-
Correlations	Corr _{VG-Q} (-)	-0.75	-
	Corr _{VG-VI} (-)	0.71	-
	Corr _{Q-VI} (-)	-0.35	-
Parameters of the flexible input model³	DEN1 (-)	1.48 (12)	-
	DEN2 (-)	1.2 (20)	44 (26)
	DEN3 (-)	0.914 (20)	-
	DEN4 (-)	0.634 (32)	61 (37)
	DEN5 (-)	0.874 (27)	48 (67)
	DEN6 (-)	0.973 (20)	-
	DEN7 (-)	0.675 (22)	-
	DEN8 (-)	0.906 (24)	48 (28)
	DEN9 (-)	0.512 (27)	-
	DEN10 (-)	0.261 (28)	-
	DEN11 (-)	0.134 (37)	-

¹ Estimated parameters are in bold, other parameters are fixed to the final estimates determined based on intravenous provocations by Silber et al

² RSE is relative standard error in %

³ The equations for the absorption model are presented in appendix 2

Figure 1. Schematic presentation of the integrated model including total glucose, insulin and regulation of glucose production, second-phase insulin secretion and glucose elimination. Broken arrows indicate control mechanisms with + or – sign indicating a stimulatory or inhibitory effect. Q , CL_G and CL_{GI} , clearance parameters of the glucose model; BIO_G , bioavailability of glucose; S_{INC} , incretin effect; CL_I , clearance of the insulin model; k_{GE1} , k_{GE2} and k_{IE} , rate constants for the effect compartments.

Figure 2. Individual profiles of glucose and insulin as well as the predicted glucose absorption rate in four representative individuals. The top to panels show glucose and insulin concentration time profiles, where the filled circles are the observed glucose and insulin concentrations and the black lines are the individual predicted profiles. The bottom panel shows the predicted glucose absorption rate.

Figure 3. Individual predicted glucose production profiles.

Figure 4. The solid lines show the total effect (glucose and insulin, black) and the insulin effect (grey) on glucose production. The horizontal line indicates 1, i.e. no effect on glucose production. If the effect is less than 1 this indicates that glucose production is suppressed. If the effect is greater than 1 this indicates that glucose production is stimulated. As can be seen from the figure, insulin is the dominating effect during the first two hours and glucose is important for stimulation of glucose production during the late part of the experiment.

Figure 5. Visual predictive check of glucose and insulin. The observed data are shown as black circles with the dashed black lines showing the median and the 90 % interval of the observed data. The 95 % confidence interval around the predicted median and the 5th and 95th percentiles of the simulated data are shown as shaded grey areas.

Appendix 1. Equations of the glucose-insulin model.

The model for glucose according to Silber et al (1) was described using the following equations, where differential equations 1.1-1.4 describe the kinetics of the different compartments and equations 1.5-1.7 describe the glucose production. Equations marked with * have been modified in the present publication.

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

$$G_C(0) = G_{SS} \cdot V_G \quad (1.1)^*$$

$$\frac{dG_P(t)}{dt} = \frac{Q}{V_G} \cdot G_C(t) - \frac{Q}{V_P} \cdot G_P(t), \quad G_P(0) = G_{SS} \cdot V_P \quad (1.2)$$

$$\frac{dG_{E1}(t)}{dt} = k_{GE1} \cdot \frac{G_C(t)}{V_G} - k_{GE1} \cdot G_{E1}(t), \quad G_{E1}(0) = G_{SS} \quad (1.3)$$

$$\frac{dG_{E2}(t)}{dt} = k_{GE2} \cdot \frac{G_C(t)}{V_G} - k_{GE2} \cdot G_{E2}(t), \quad G_{E2}(0) = G_{SS} \quad (1.4)$$

$$G_{PROD,0} = G_{SS} \cdot (CL_G + CL_{GI} \cdot I_{SS}) \quad (1.5)$$

$$G_{CM1}(t) = \left(\frac{G_{E1}(t)}{G_{SS}} \right)^{GPRG} \quad (1.6)$$

$$G_{PROD}(t) = G_{PROD,0} \cdot G_{CM1}(t) \quad (1.7)^*$$

The model for insulin was described using the following equations, where differential equations 1.8-1.10 describe the kinetics of the different compartments and equations 1.11-1.13 describe the insulin secretion.

$$\frac{dI(t)}{dt} = I_{SEC}(t) - \frac{CL_I}{V_I} \cdot I(t), \quad I(0) = I_{SS} \cdot V_I \quad (1.8)$$

$$\frac{dI_E(t)}{dt} = k_{IE} \cdot \frac{I(t)}{V_I} - k_{IE} \cdot I_E(t), \quad I_E(0) = I_{SS} \quad (1.9)$$

$$\frac{dI_{FPS}(t)}{dt} = -k_{IS} \cdot I_{FPS}(t), \quad I_{FPS}(0^+) = FPS \quad (1.10)$$

$$I_{SEC,0} = I_{SS} \cdot CL_I \quad (1.11)$$

$$G_{CM2}(t) = \left(\frac{G_{E2}(t)}{G_{SS}} \right)^{IPRG} \quad (1.12)$$

$$I_{SEC}(t) = I_{SEC,0} \cdot G_{CM2}(t) + k_{IS} \cdot I_{FPS}(t) \quad (1.13)*$$

Appendix 2. Equations for the flexible input model

The flexible absorption model was defined using 12 steps during which the glucose absorption rate was estimated for each of these steps. 15-minute intervals were used during the first 120 minutes (Q1-Q8) followed by 30 minute intervals during the last 120 minutes (Q9-Q12).

The NONMEM code used to implement the flexible input model is presented below.

```

DEN1 = THETA(1)
DEN2 = THETA(2)*EXP(ETA(2))
DEN3 = THETA(3)
DEN4 = THETA(4)*EXP(ETA(4))
DEN5 = THETA(5)*EXP(ETA(5))
DEN6 = THETA(6)
DEN7 = THETA(7)
DEN8 = THETA(8)*EXP(ETA(8))
DEN9 = THETA(9)
DEN10 = THETA(10)
DEN11 = THETA(11)

Q1=0
Q2=0
Q3=0
Q4=0
Q5=0
Q6=0
Q7=0
Q8=0
Q9=0
Q10=0
Q11=0
Q12=0

IF (TIME.LE.15) Q1=1
IF (TIME.GT.15.AND.TIME.LE.30) Q2=1
IF (TIME.GT.30.AND.TIME.LE.45) Q3=1
IF (TIME.GT.45.AND.TIME.LE.60) Q4=1
IF (TIME.GT.60.AND.TIME.LE.75) Q5=1
IF (TIME.GT.75.AND.TIME.LE.90) Q6=1
IF (TIME.GT.90.AND.TIME.LE.105) Q7=1
IF (TIME.GT.105.AND.TIME.LE.120) Q8=1
IF (TIME.GT.120.AND.TIME.LE.150) Q9=1
IF (TIME.GT.150.AND.TIME.LE.180) Q10=1
IF (TIME.GT.180.AND.TIME.LE.210) Q11=1
IF (TIME.GT.210.AND.TIME.LE.240) Q12=1

```

```

DEN=1+DEN1+DEN2+DEN3+DEN4+DEN5+DEN6+DEN7+DEN8+DEN9+DEN10+DEN11+DEN12

ABR1 = 1/DEN/15           ; absorption rate constant 0-15 min
ABR2 = DEN1/DEN/15        ; absorption rate constant 15-30 min
ABR3 = DEN2/DEN/15        ; absorption rate constant 30-45 min
ABR4 = DEN3/DEN/15        ; absorption rate constant 45-60 min
ABR5 = DEN4/DEN/15        ; absorption rate constant 60-75 min
ABR6 = DEN5/DEN/15        ; absorption rate constant 75-90 min
ABR7 = DEN6/DEN/15        ; absorption rate constant 90-105 min
ABR8 = DEN7/DEN/15        ; absorption rate constant 105-120 min
ABR9 = DEN8/DEN/30        ; absorption rate constant 120-150 min
ABR10 = DEN9/DEN/30       ; absorption rate constant 150-180 min
ABR11 = DEN10/DEN/30      ; absorption rate constant 180-210 min
ABR12 = DEN11/DEN/30      ; absorption rate constant 210-240 min

R1 = (Q1*ABR1+Q2*ABR2+Q3*ABR3+Q4*ABR4+Q5*ABR5+Q6*ABR6+Q7*ABR7+Q8*ABR8
+Q9*ABR9+Q10*ABR10+Q11*ABR11+Q12*ABR12) ;abs rate constant 0-240 min

ABSG = R1*BIOG*DOSE       ;rate of absorption 0-240 min

```