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Population modelling approach for the study of insulin clearance in previous gestational diabetes

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Abstract

Gestational diabetes is a high blood glucose condition that develops during pregnancy and although it usually disappears after giving birth, women who experienced gestational diabetes are more prone to develop type 2 diabetes later in their life. Insulin clearance, a physiological process representing the removal of insulin from the blood in the entire organism, is one of the main processes underlying the development of type 2 diabetes, together with insulin resistance and altered insulin secretion. Due to the role of insulin clearance in the development of type 2 diabetes, it is important to investigate this process also in women who experienced a history of gestational diabetes. Population modelling is a tool that allows to find correlations between heterogenic characteristics of subjects and to study the metabolism of certain molecules within the body. The aim of the present thesis was to exploit a population modelling approach for the study of insulin clearance in previous gestational diabetes. To this purpose, a mathematical model able to segregate hepatic and extrahepatic insulin clearance, previously proposed by Polidori et al., has been considered. The involved population consisted of 114 women with an history of gestational diabetes (pGDM) and a group of 41 healthy women as controls (CNT) who underwent an insulin modified intravenous glucose tolerance test. Data were processed with Monolix, a software providing simple solution for non-linear mixed effects modeling for pharmacometrics. To exploit the information about the heterogeneous characteristics among the population certain covariates, among those suggested by the software, were included into the model. Analyses were performed either on the complete dataset (OVP, overall population) and including the group (CNT, pGDM) as a categorical variable or considering the CNT and pGDM datasets separately. Population estimates of extrahepatic insulin clearance for pGDM women resulted almost three times smaller with respect to that of CNT group (0,32 L/min vs 0,91 L/min). Instead, concerning the hepatic insulin clearance population estimates, the pGDM population showed a higher value with respect to CNT (29,7% vs 44,7%). Individual estimates for FE_L resulted significantly different for pGDM vs CNT, whereas CL_P were found different only when considering the separate datasets. In conclusion, the proposed population modelling approach showed its capability to provide population parameter estimates related to hepatic and extrahepatic insulin clearance. Hepatic insulin clearance may be affected by the presence of a history of gestational diabetes, whereas extrahepatic insulin clearance requires further investigation in wider populations.

Introduction

Gestational diabetes is a high blood glucose condition that develops during pregnancy. Although it usually disappears after giving birth, women who experienced gestational diabetes are more prone to develop type 2 diabetes later in their life. Type 2 diabetes is another type of diabetes mellitus; the three main processes underlying its development are tissue resistance to the action of insulin (i.e., insulin resistance), altered insulin secretion by the pancreas and altered insulin clearance.

Insulin clearance is a physiological process representing the removal of insulin from the blood in the entire organism. It occurs in the liver, but also in other organs such as kidneys and skeletal muscles, thus it is possible to distinguish hepatic and extrahepatic insulin clearance, respectively. Due to the role of insulin clearance in the development of type 2 diabetes, it is important to investigate this process also in women who experienced a history of gestational diabetes.

Insulin clearance is a phenomenon that can be directly measured only through invasive procedures that cannot be performed in human subject. In this context, mathematical modelling approaches can be used to quantitatively assess insulin clearance from easily measured data, coming from venous blood samples. However, quantification of insulin clearance from mathematical modelling procedures may be affected by differences of subjects characteristics (age, sex, weight, etc.), called covariates.

Population modeling is a tool to identify and describe relationships between a subject's physiologic characteristics and observed drug exposure or response. Population models usually have fixed effect as well as random-effect parameters and are therefore called “mixed-effect” models. Fixed effects are population parameters assumed to be the same each time data is collected, and random effects are random variables associated with each sample (individual) from a population.

The aim of the present thesis is to exploit a population modelling approach for the study of insulin clearance in previous gestational diabetes. To this purpose, a previously proposed mathematical model able to segregate hepatic and extrahepatic insulin clearance has been considered.

1. Outline of physiology

1.1. Glucose homeostasis

The process of maintaining plasma glucose concentration at steady-state levels, in the narrow range 70-110 mg/dl, is called “glucose homeostasis”. Food consumption is the most important source of glucose. As shown in Fig. 1, to utilize the available glucose, the β-cells of the pancreas produce insulin, a hormone that stimulates glucose uptake at a cellular level, enhancing in this way glucose metabolism. Moreover, the glucose in excess is converted into glycogen in liver and skeletal muscles. Insulin secretion occurs in response to elevation of glucose concentration, but also to positive rate of change of glucose concentration. Once it absolved its function, insulin goes through a receptor-mediated uptake process, followed by its degradation, accomplishing “insulin clearance”. Instead, when glucose level becomes too low, pancreatic α-cells release glucagon, a hormone which provokes the catabolic division of glycogen into glucose, which is released into the blood in order to restore the glucose level [1].

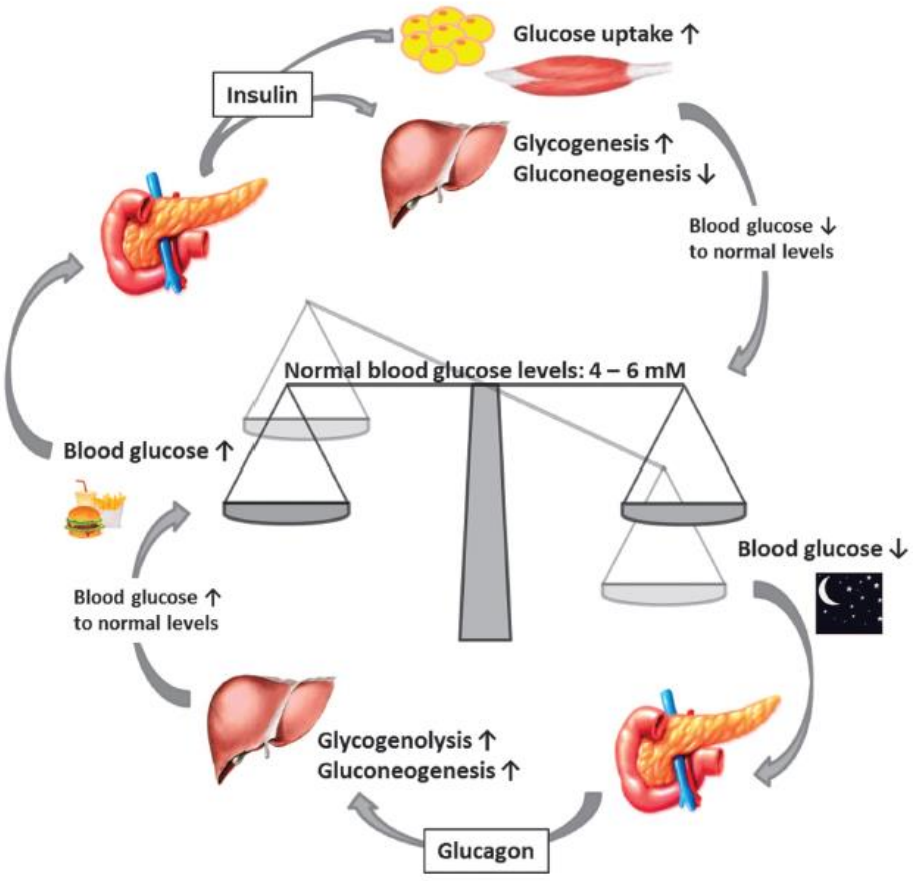

Fig. 1

Glucose homeostasis system [1].

As well as insulin action is essential to avoid hyperglycemia, glucagon action is aimed to prevent hypoglycemia, in both healthy and diabetic subjects. Incretin hormones, like glucose-dependent Insulinotropic Polypeptide (GIP) and Glucagonlike-peptide-1 (GLP-1), are secreted by the gastrointestinal tract in response to nutrient ingestion and are responsible for the "incretin effect". It consists in an increase of the glucose-dependent insulin secretion ("insulin potentiation") during an oral glucose tolerance test (OGTT), compared to an isoglycemic intravenous glucose infusion (I-IVG). In fact, it is not possible to observe the incretin effect in an intravenous glucose tolerance test, since there is no glucose bolus which passes through the gastrointestinal tract and triggers the incretin hormones production. GIP is secreted by k-cells and GLP-1 is released by L-cells, which are both cells of the small intestine, activating in response to nutrient ingestion. Another hormone involved in glucose homeostasis is the amylin. It is secreted together with insulin by β-cells in response to increasing food compounds (such as glucose) levels and its functions are inhibition of glucagon secretion, reduction of endogenous glucose production during the postprandial period and favoring glycogen synthesis. Production of amylin is impaired in diabetes patient's bodies. The use of synthetic pramlintide, which is an amylin analogue, showed to improve glycemic control in diabetic subjects [2].

1.2. Diabetes Mellitus

Impaired glucose metabolism can manifest in three forms of diabetes mellitus: type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) and gestational diabetes mellitus. In T1DM subjects, insulin is not secreted at all since all the β-cells in the pancreas are destroyed by an antibody autoimmune response [1]. Finally, it can happen that a pregnant woman is diagnosed with gestational diabetes, impairment that usually ends with the birth of the baby. This pathologic condition can often go unnoticed, in fact, it is important to often check the glycemic level. Women who pass thought gestational diabetes have an increased probability to develop T2DM in old age. Impairments in the insulin sensitivity and β-cells function are present in women with a history of gestational diabetes. Insulin sensitivity is defined as the capability of insulin to stimulate glucose uptake, while β-cells function refers to the ability of β-cells to produce adequate amounts of insulin [3]. Insulin clearance is another physiological process which regulates glucose tolerance, and it occurs in the liver, but also in other organs such as kidneys and skeletal muscles [4]. Since it is not possible to heal T1DM and T2DM, patients need regular insulin supplements and/or medications, based on a necessary and frequent glycemic control. Healthy and balanced diet, as well as physical activity are also recommended to diabetic subjects [5]. Formulation of diagnosis of diabetes can be accomplished through the observation of blood levels of glycated hemoglobin (Hba1c) and/or the fasting plasma

glucose (FPG). For Hba1c levels higher than 6,5%, the diagnosis of diabetes is very likely. Concerning FPG, levels comprised between 5,6 and 6,9 mmol/L indicates an impaired fasting glucose, while levels greater than 7,0 mmol/L indicate provisional diagnosis of diabetes, and further investigations could confirm the diagnosis or not [6]. Some pharmacological therapies will be presented in the next chapter, in relation to the included studies.

2. Review on population modelling in the field of glucose

2.1. Population modelling

The control of glucose in both diabetic and healthy people is an active field of research and in-silico models are widely used for it. Among all the approaches which are usually employed, in this study we focus on the nonlinear mixed-effect approach, through which it is possible to build a population model and to perform a population analysis. In this field, a population model allows to find correlations between heterogenic features of people (age, sex, weight, etc.), called covariates, and the metabolism of a certain molecule or drug in human body. A nonlinear mixed-effect model incorporates both fixed and random effects. Fixed effects are population parameters assumed to be the same each time data is collected, and random effects are random variables associated with each sample (individual) from a population. This approach allows to exploit input data to quantitatively evaluate the influence of the covariates on the variables (parameters). Population models are often pharmacokinetic (PK) or pharmacodynamic (PD) models, or the two mixed together (PK/PD models). The PK part is referred to how the drug is processed within the body, while PD modelling is based on a quantitative integration of several factors: pharmacokinetics, pharmacological systems, pathological and physiological processes. In this way it's possible to understand the intensity and time-course of drug effects in the human body and it's then possible to identify the optimal therapeutic dosing regimen for a specific patient.

2.2. Bibliographic Review

The research in the literature has been performed with the screening process described in Fig. 2.

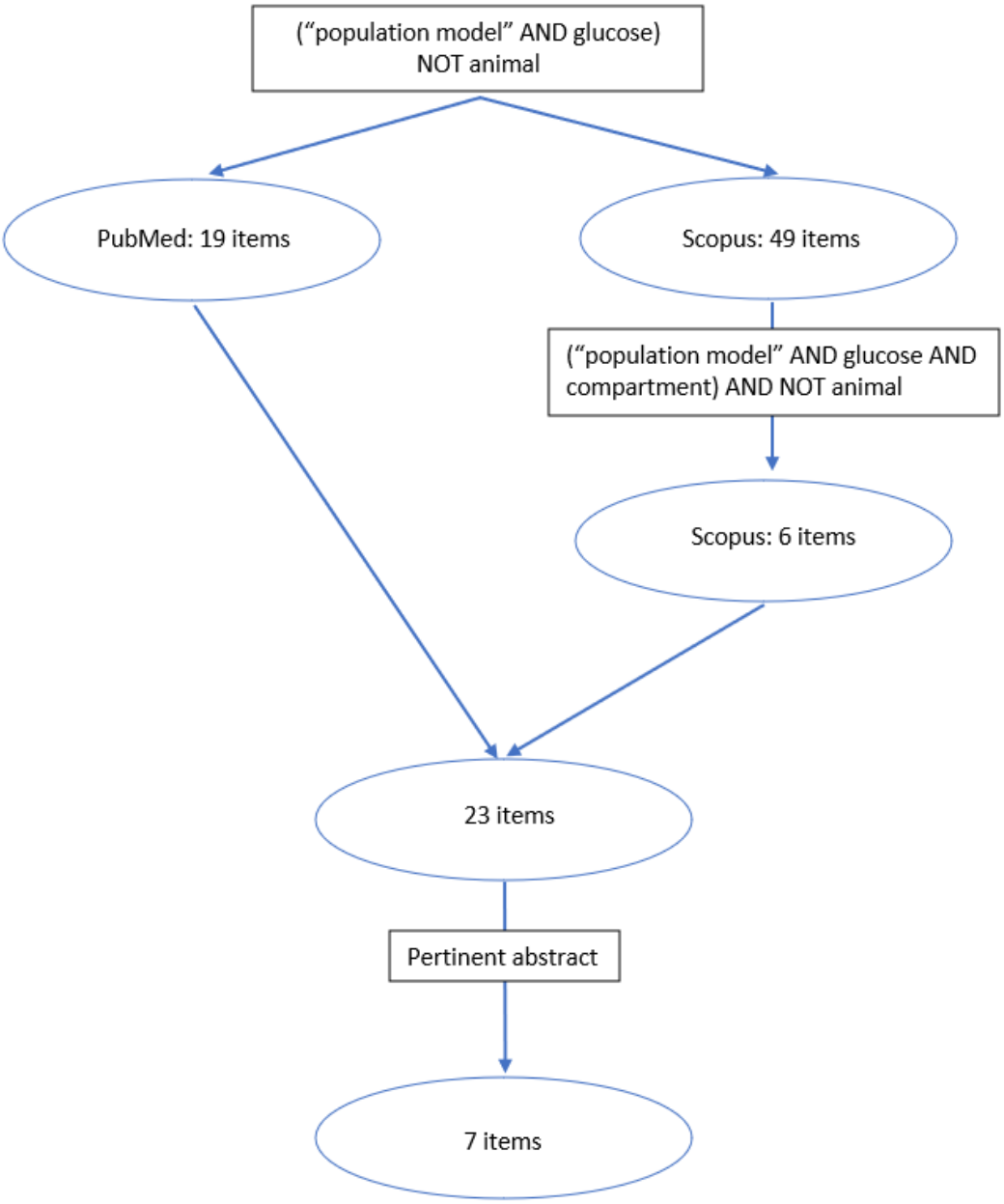

Fig. 2.

Systematic Review procedure flowchart: selection of the papers of interest.

Searching with the string *"population model" AND glucose) NOT animal* Pubmed showed 19 results, while Scopus showed 49 results. Since this latter gave too many results, the research has been repeated with the string (*"population model" AND glucose AND compartment) NOT animal,* obtaining 6 results. The same string in Pubmed would give just 1 result, so we preferred to keep the previous string for it. Among these 23 papers, reading the abstracts, 7 of them have been deemed appropriate

for inclusion. A further research was done adding “AND covariate” in the string, in order to include papers with a covariate analysis, but nothing pertinent was found. The included studies will be presented in chronological order.

The study by R. Hovorka and coauthors [7] is titled “Pancreatic β-Cell Responsiveness during Meal Tolerance Test: Model Assessment in Normal Subjects and Subjects with Newly Diagnosed Noninsulin-Dependent Diabetes Mellitus” and was published on *Diabetologia journal* in 1993. Its aim was to quantify pancreatic beta cell responsiveness through the estimation of two indexes: the postprandial sensitivity M_1, representing the ability of stimulating β-cells of postprandial glucose, and the basal sensitivity M_0, representing the ability of stimulating β-cells of fasting glucose. The measurements of plasma glucose and C peptide occurred in relation to a meal tolerance test (MTT), or oral glucose tolerance test (OGTT), in 16 healthy subjects and in 16 analogue newly diagnosed noninsulin-dependent diabetes mellitus (NIDDM) patients. The standardized meal consisted in 75 g of carbohydrates, 500 Cal. The model proposed in this study is a model of C peptide secretion and kinetics and it is composed by 2 compartments: the central one and the peripheral one, as shown in Fig. 3.

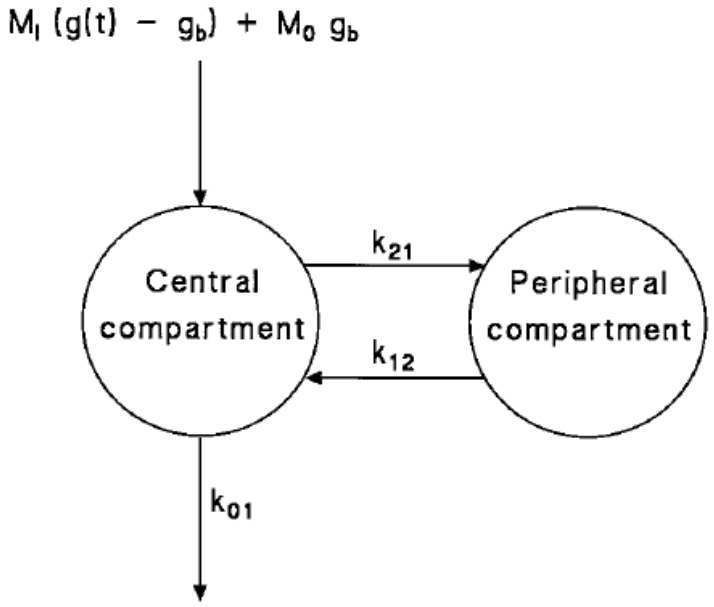

Fig. 3.

Model of C peptide secretion and kinetics during an OGTT [7].

C peptide secretion is a linear function of plasma glucose concentration (g(t)), and M_1 and M_0 are the parameters of the linear relation. Their estimation highlighted the difference in sensitivity of β-cells between newly diagnosed NIDDM and control subjects. Fig. 4 shows the estimation of M1 and M0, while Fig. 5 shows plasma concentrations of glucose, C peptide and insulin along the time.

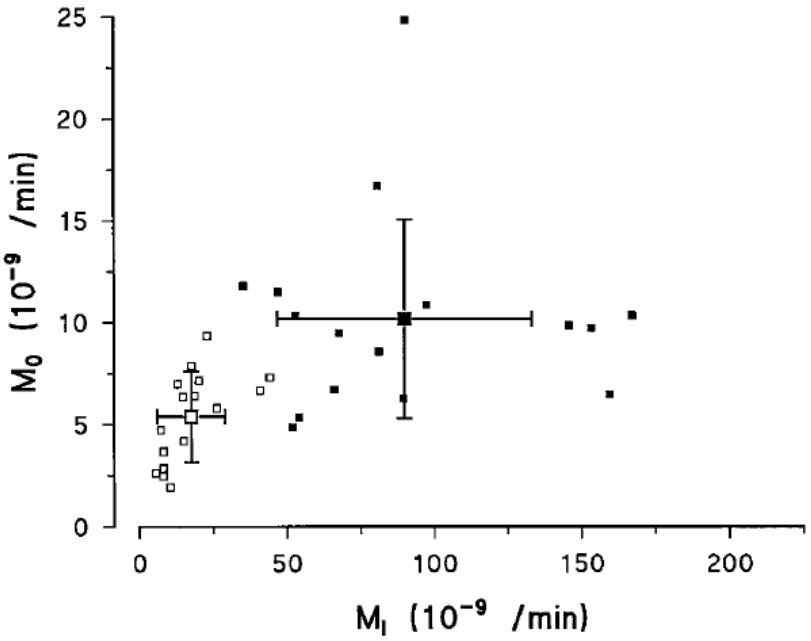

Fig. 4.

Estimates of postprandial sensitivity (M_1) plotted against estimates of basal sensitivity (M_0) in normal subjects (solid squares) and subjects with newly diagnosed NIDDM (open squares). The mean standard deviation (SD) has been plotted for each group [7].

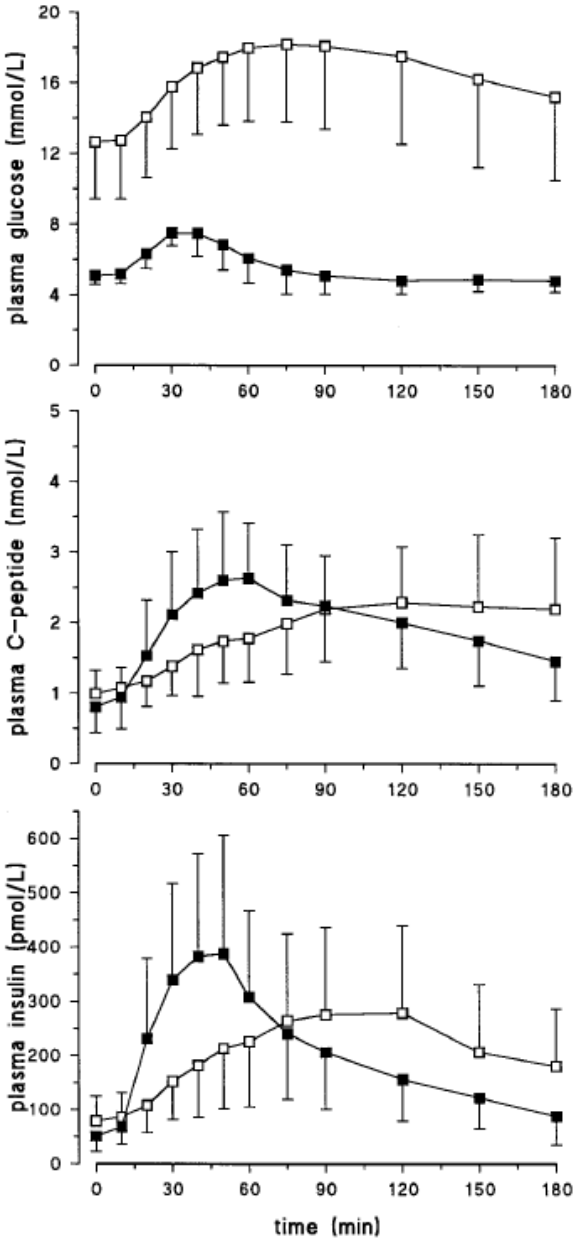

Fig. 5.

Top panel, middle panel and bottom panel represent plasma glucose, plasma insulin and plasma C peptide respectively during an OGTT in normal subjects (solid squares) and 16 body mass index (BMI)-matched subjects with newly diagnosed NIDDM (open squares) [7].

From the last graphics, it is possible to have a measure of the impaired glucose control in newly diagnosed NIDDM patients. For example, it is possible to see how the diabetic subject's plasma glucose level fails to go back to the fasting level (Fig. 5). The authors also observed that the intersubject variability influenced the measures, in particular the ones relative to control subjects. Moreover, an individual estimation of k_{01} would have given a better accuracy of the indexes M_1 and M_0, due to the heterogeneous differences among the subjects. Finally, M1 is a composite index,

representing the overall pancreatic responsiveness after an OGTT at that moment. This means that it does not allow the separation of defects in the net glucose effect and the incretin effect. In conclusion, Hovorka and colleagues built a model capable of assessing the β-cells responsiveness during an OGTT, successfully highlighting the overall differences in the metabolism between metabolically healthy people and newly diagnosed NIDDM subjects [7].

The study by J. B. Moller and coauthors [8] is titled "Mechanism-based population modelling for assessment of L-cell function based on total GLP-1 response following an oral glucose tolerance test" and was published in 2011. Its aim was to build a mechanism-based population model which describes the time course of blood GLP-1 concentration, and to assess capability of GLP-1 secretion from L-cells in each subject through indices. A mixed group of healthy volunteers and T2DM patients took part to an OGTT (standard 75 g dose of glucose). Once the dose is ingested and the gastrointestinal tract is stimulated, the production of GLP-1 in the L-cells starts. The half-life time of GLP-1 is very short for both T2DM subjects and control subjects, lasting about 2-3 min. This means that the decreased GLP-1 response in T2DM patients is due to lower post-prandial secretion. The model is composed by 2 sub-models: a glucose-insulin model (model (A)) and a GLP-1 secretion model (model (B)), as it is possible to see in Fig. 6.

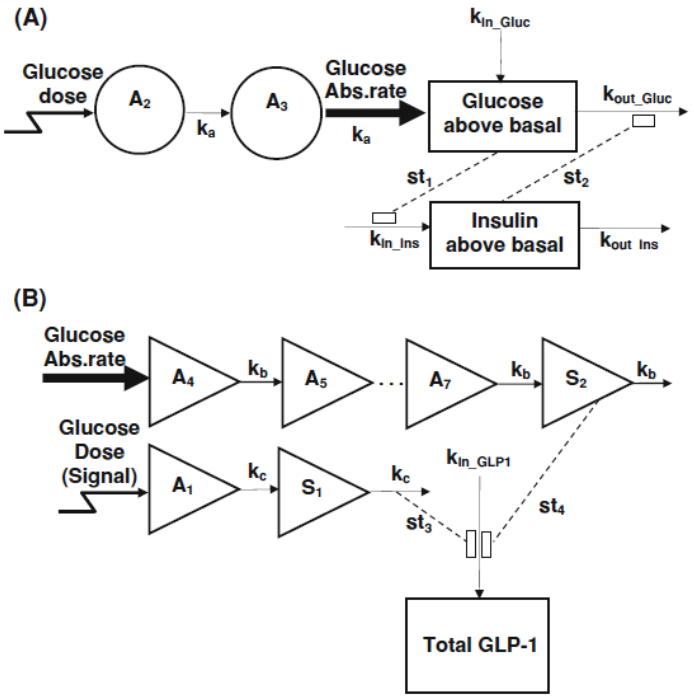

Fig. 6.

(A) Glucose-insulin model; (B) GLP-1 secretion model [8]

The Glucose-insulin model was needed for the estimation of the glucose absorption rate constant, which is k_a (instead, k_a*A_3 is the glucose absorption rate). The GLP-1 secretion model is an indirect response model with a zero-order input and first-order loss. The zero-order input is composed by two mechanisms with different times of onset, one faster and one slower. The fast one is the mechanism triggered by the glucose dose signal and it provokes a peak in GLP-1 concentration around 40 minutes after the ingestion. The slower mechanism, instead, is the glucose absorption. The delay was implemented through the transit compartments and k_b, a transit rate constant representing the delay between glucose absorption rate and stimulation of late phase of GLP-1 secretion. Instead, k_c is the neural signal rate constant, and it is related to the stimulus for GLP-1 secretion from the gastrointestinal tract. Moreover, the elimination of GLP-1 is represented like a first-order process. The choose to model separately model (A) and model (B) was made in order to avoid biasing the estimation of glucose absorption towards the prediction of GLP-1 concentration. The implementation was performed in the software NONMEM VI. The results of this study are strictly linked with the estimation of k_a, k_b and k_c. Looking at Fig. 7, it is possible to observe two peaks (darker plots) of the two stimuli for GLP-1 secretion. In fact, S_1 and S_2 are transit compartments mostly influenced by kc and kb respectively. The peaks relative to them are at around 25 min and 100 min respectively, and this seems to be consistent with the GLP-1 profiles usually observed after a meal. The faster stimulus, relative to k_c, is probably due to the activation of a proximal-distal neuroendocrine loop, due to the nutrients passing in the duodenum. In conclusion the model proposed in this study is not free of defects, mostly linked to the accuracy of the employed tools. However, the authors chose not to perform the covariate analysis and to leave it to future studies, to evaluate the influence of different demographic factors on the GLP-1 secretion.

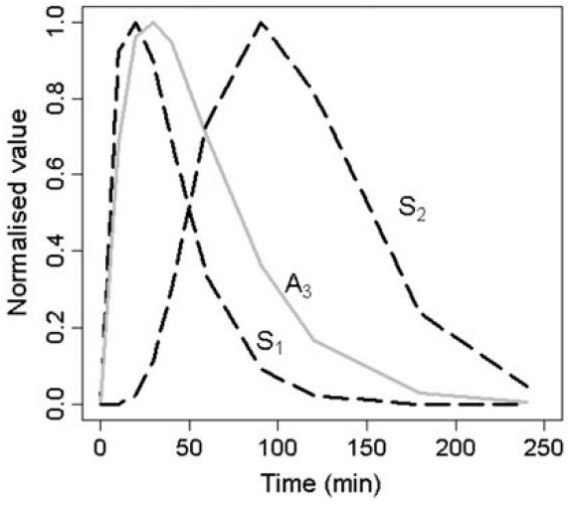

Fig. 7.

Normalized mean of simulations of compartments S_1, A_3 and S_2 versus time [8].

The study by J. Fang and coauthors [9] is titled “Study reanalysis using a mechanism-based pharmacokinetic/pharmacodynamic model of pramlintide in subjects with type 1 diabetes” and was published on *AAPS Journal* in 2013. Pramlintide is an amylinomimetic which showed to be helpful in improving glycemic control in diabetic patients. The data were taken from a previous study, in which 25 male T1DM patients participated. Plasma glucose and drug concentration were measured after ingestion or two hours intravenous infusion (at three different dose levels), or placebo, in the postprandial period. The proposed PK/PD model is shown in Fig. 8 and it consists of a pharmacokinetics part, a two-compartmental model (central and peripheral compartments) with zero order infusion and first order elimination.

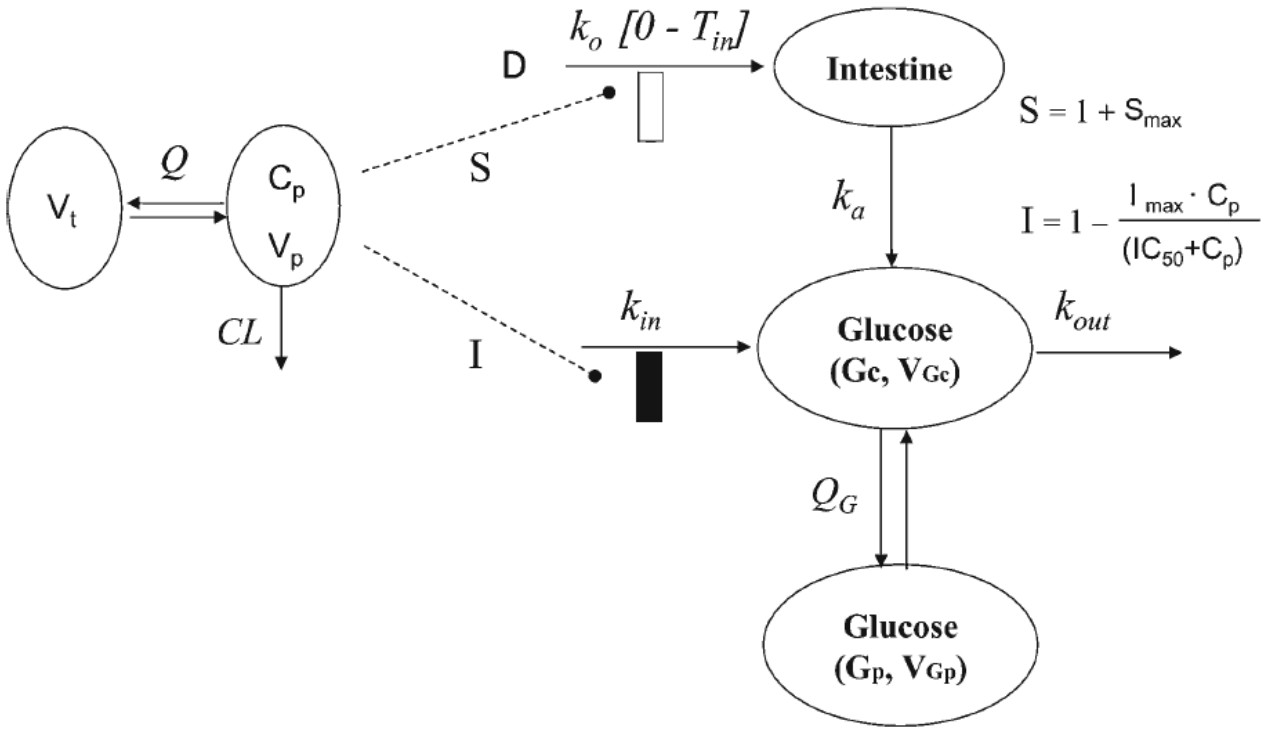

Fig. 8.

Proposed PK/PD model. On the left there is the two-compartmental model relative to the pharmacokinetics of pramlintide, and the dotted lines represent its effect on the pharmacodynamic. The open bar represents the stimulation (S), while the closed bar represents the inhibitory effect [9].

Since glucose homeostasis is a turnover process, the model is structured like an indirect response model. K_0 represents the sum of net entry glucose from the meal, k_{in} represents the endogenous glucose from the liver, and k_{out} represents the removal of glucose by uptake and utilization. Instead, k_a is the first-order rate constant representing the absorption of glucose from the intestine. The

suppression of postprandial glucagon was represented with an inhibitory sigmoidal function. The influence of pramlintide on the process of gastric emptying is one of the most focused aspects in Fang and coauthors' study and their model was able to quantify the delayed gastric emptying time through the variable S (pramlintide prolongation). Such delay seems not to be dependent on the dosage, since there is no significant increase of it increasing the dosing regimens. Moreover, Pramlintide provoked a significant decrease (at least 40%) in the glucose area under the curve net (AUC_{net}) for all the employed dosages except for the lowest one (30 μg), as it is possible to see in Fig. 9.

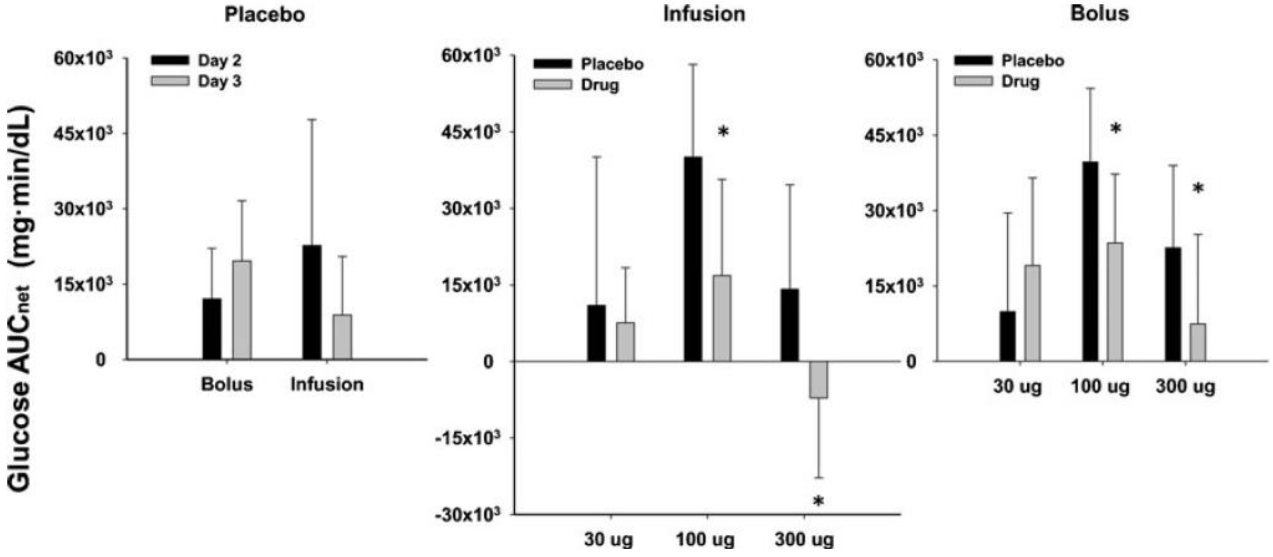

Fig. 9.

Glucose AUC_{net} for all the dosing regimens and placebo. Data are shown as means ± SD. The asterisks indicate the most significant differences between pramlintide and placebo [9].

Several software was employed in this study. NONMEM Version VII level 2.0 was used for the nonlinear mixed-effect models, S-Plus was used for the Diagnostic graphs and Phoenix WNL 6.1 for the non-compartmental PK analysis. Fang et al. successfully developed a model describing the fundamental effects of pramlintide on postprandial glucose regulation in T1DM patients [9].

The study by H. Li and coauthors [10] is titled "Target-mediated pharmacokinetic/pharmacodynamic model based meta-analysis and dosing regimen optimization of a long-acting release formulation of exenatide in patients with type 2 diabetes mellitus" and it was published on *Journal of Pharmacological Science* in 2015. In this study a PK/PD population model of exenatide ER was built. The aim of the simulations is to find the optimal dosing regimen to maximize the exposure to the

drug in T2DM patients. In fact, exenatide ER ("extended release") is gradually released along the time, thanks to the employment of a Poly-lactic-co-glycolic acid (PLGA) matrix excipient which, after the injection, gradually releases the drug in the subcutaneous tissue. In the nonlinear mixed-effect PK/PD model, built on Monolix, GLP-1R is taken into account too, as it is possible to see in Fig. 10, while FPG and HbA1c are observed to evaluate the efficacy of the dosing regimens.

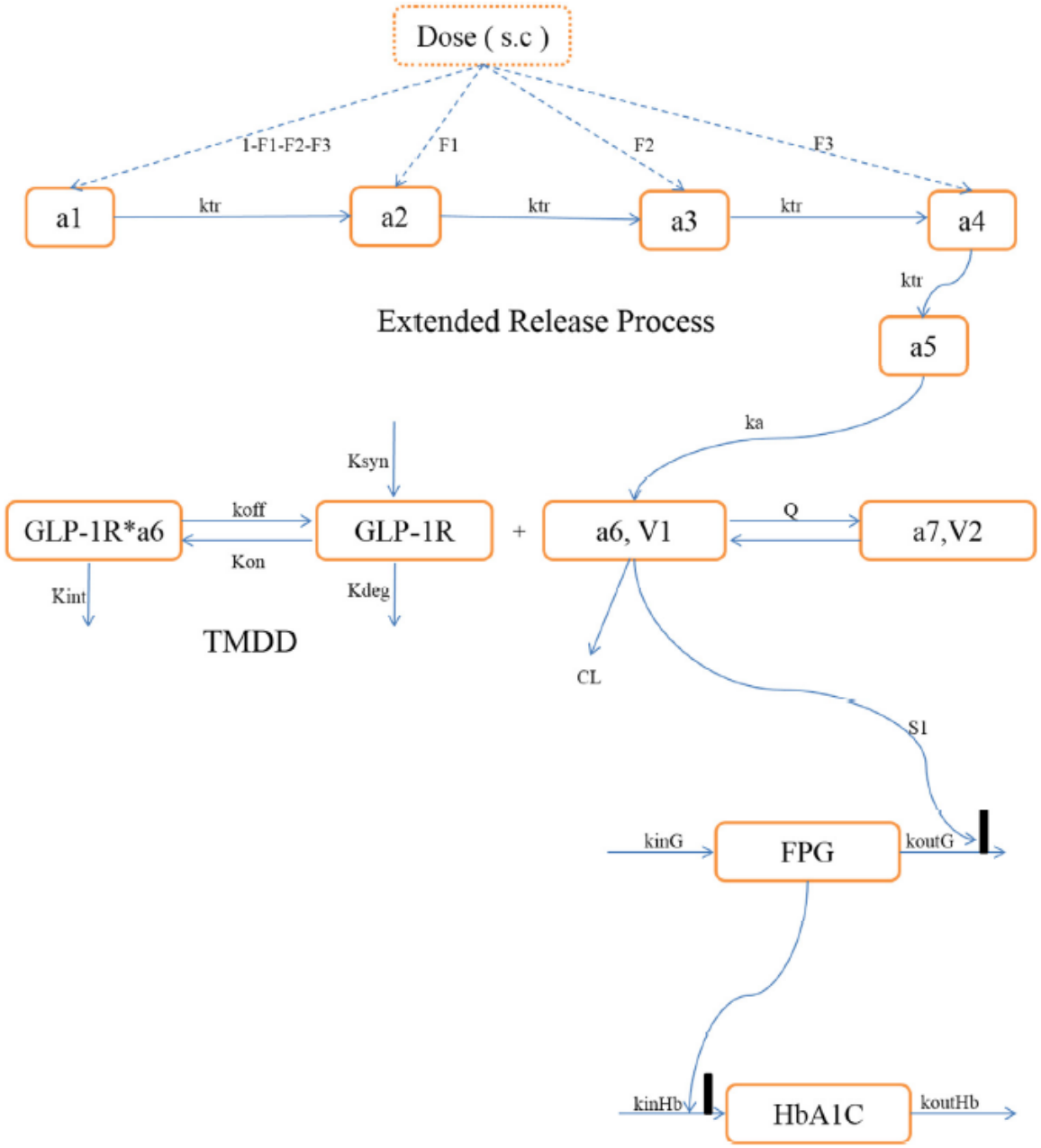

Fig. 10.

Nonlinear mixed-effect PK/PD model of exenatide ER for T2DM patients [10].

The proposed model can be divided into two parts: the PD part, composed by the FPG and HbA1c compartments, and the PK part, composed by the upper rest of the model. The PK model is composed by 4 transit compartments (a1-a4) before the absorption compartment (a5), to represent the gradual subcutaneous distribution carried on by the PLGA excipient. V1 and V2 are equal to central and peripheral compartments, respectively, while a6 and a7 are the amount of exenatide in the same compartments. CL and Q represent the elimination and inter-compartment clearances. Instead, a8 and a9 represent the amount of GLP-1 receptors and GLP-1R*a6 complex, respectively. Solid lines with the arrows represent the transit (ktr), the absorption (ka) and the elimination (CL). The PK/PD data was taken from 6 different publications, reported in the paper [10]. Fig. 11 shows how the population model fits well the data of one of the studies taken into account, in which exenatide ER dosages of 0.8 and 2.0 mg were considered. The model demonstrated its capability to evaluate the efficacy of an exenatide ER-based therapy with several dosages and timings, assessing the changes in FPG and HbA1c levels. No covariate analysis was performed in this study.

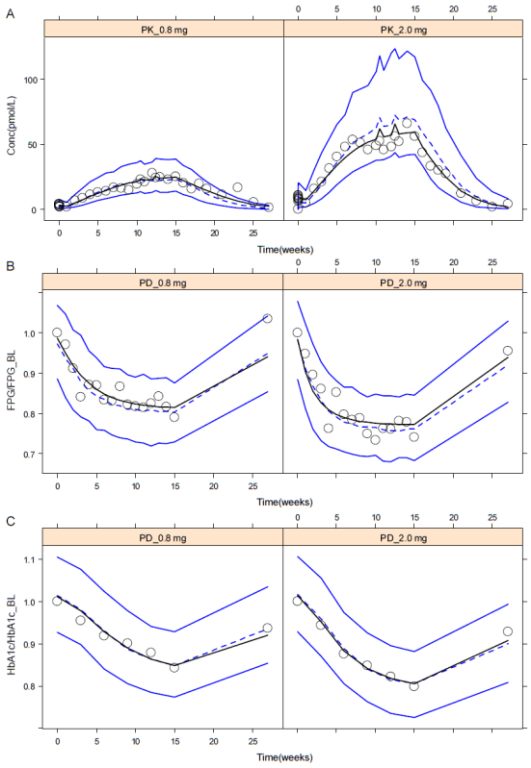

Fig. 11.

Model prediction distribution. Mean plasma exenatide concentrations (A), mean change in FPG levels (ratios of FPG/FPG_BL) (B) and mean change in HbA1c levels (ratios of HbA1c/HbA1c_BL) (C) considering multiple injections of exenatide ER. Panels on the left are relative to 0.8 mg dosages, while panels on the right are relative to 2.0 mg dosages. Median model prediction is represented by blue dashed lines, the 95% confidence interval of the prediction is represented by the two blue solid lines, individual prediction values are represented by black solid lines, and observed values are represented by open circles [10].

The study by N. Bouazza and coauthors [11] is titled “Evaluation of the pharmacokinetics of glibenclamide tablet given, off label, orally to children suffering from neonatal syndromic hyperglycemia” and it was published on the *European Journal of Clinical Pharmacology* in 2016. The pharmacokinetics of glibenclamide (Gb) in 18 children with neonatal syndromic hyperglycemia was studied. Gb is a sulfonylurea and it have been recently substituted to insulin for treatment of hyperglycemia in T2DM patients. In this study, the young patients have mutations of the genes relative to Kir6.2 and SURI, the two types of subunits of ATP-sensitive potassium channels in the pancreas beta cells. This genetic impairment is very rare. Gb has the capability to hyperpolarize the plasmatic membrane of the beta cells which prevents the normal secretion of insulin in response to blood glucose and it is completely metabolizable for the liver, so this drug is not toxic for the patients. Blood samples were frequently collected in the 12 h after the intake of the Gb dosage. The model employed are: a one-compartment model for the description of the data, a proportional error model for the residual variability and an exponential error model for the inter-subject variability. The covariates employed in this study are age, size, body mass index and genetic polymorphism. Concerning the covariate analysis, the covariate selection criteria to be incorporated in the model are physiological plausibility of the effect, production of a minimum threshold decrease in the objective function value and production of a reduction in the variability of the pharmacokinetic parameter, assessed by the associated inter-subject variability. The most significant covariate has been discovered to be the weight, since this parameter is responsible of variability of Gb clearance in children. In fact, Gb clearance increases with body weight linearly. Concentration time courses and relation between drug concentration and efficacy were described in the results. How it is possible to observe the venous plasma glucose concentration plotted against time in Fig. 12, a higher dosage of Gb allows to keep glycemia under control [11].

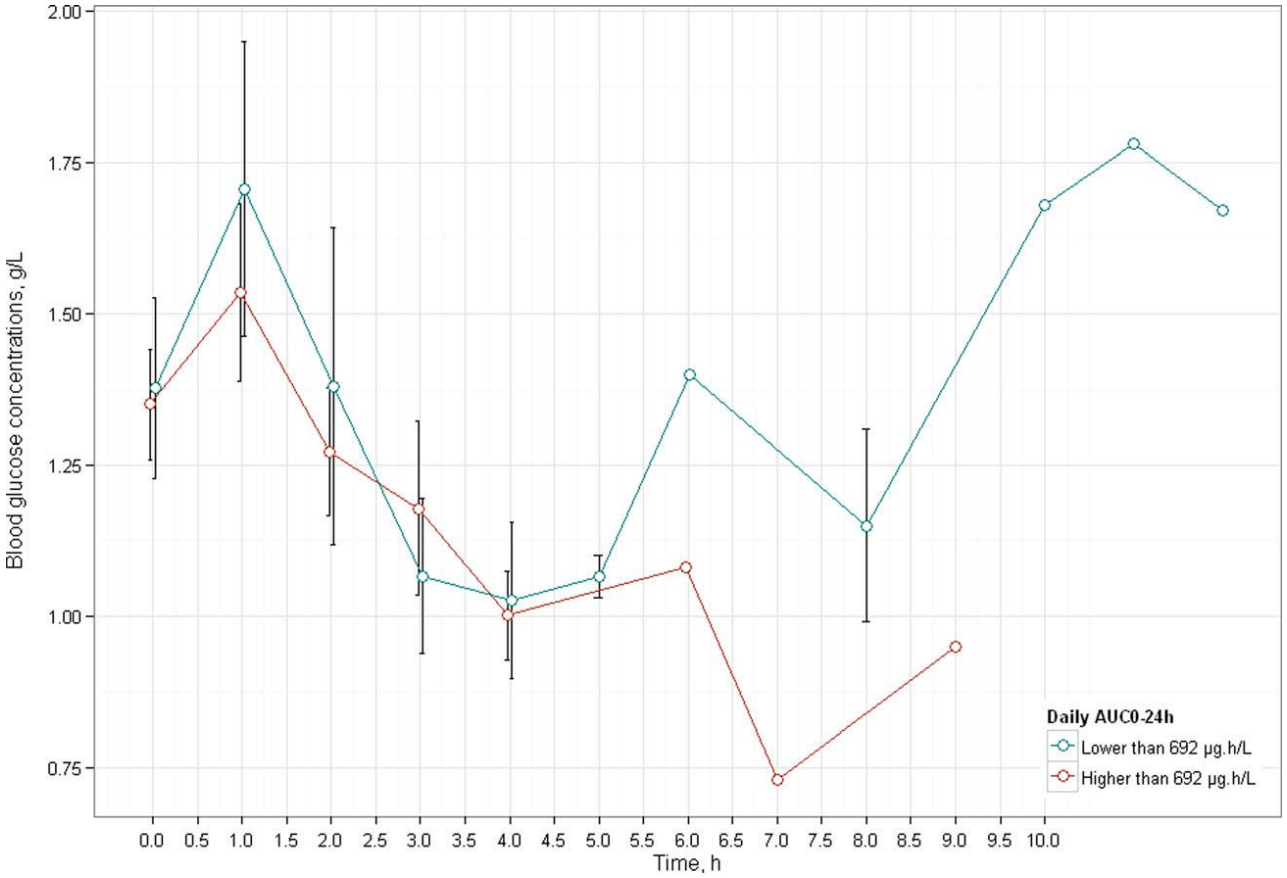

Fig. 12.

Venous plasma glucose measurements as a function of time after drug intake for different drug dosages (in the right lower corner) [11].

The study by S. Choy and coauthors [5] is titled "Weight-HbA1c-insulin-glucose model for describing disease progression of type 2 diabetes" and it was published on *CPT: Pharmacometrics and Systems Pharmacology* in 2016. A model describing changes in fasting serum insulin (FSI), FPG and HbA1c in obese patients with newly diagnosed T2DM was presented. At the beginning of the study, the patients had the 61% and 25% of the normal beta cells function and insulin sensitivity respectively, but they have been managed with therapy, diet and exercise for 67 weeks with a consequent reduction of the body weight and a relevant improvement of insulin sensitivity (to 30,1% of the normal). The proposed model is a weight-HbA1-insulin-glucose semi-mechanistic model, in which FPG, FSI and HbA1c are exploited as biomarkers of diabetes. We can see it in Fig. 13. EF_W represents the combined effect of diet and exercise, placebo, and an upward counter-effect dependent on time acting on the input of weight. EF_S stands for "effect on insulin sensitivity". It depends on changes in weight (ΔWGT) and influences the insulin sensitivity (IS). EF_B indicates the treatment effect on beta cell function, and it depends on an increase component and a decrease component. B,

representing the natural loss on beta cells functionality, together with the natural feedback from FPG, determines the production rate of FSI. Instead, FPG compartment is driven by both IS and FSI. Finally, FPG and postprandial glucose (PPG) determine the production of HbA1c, designed with three transit compartments [5].

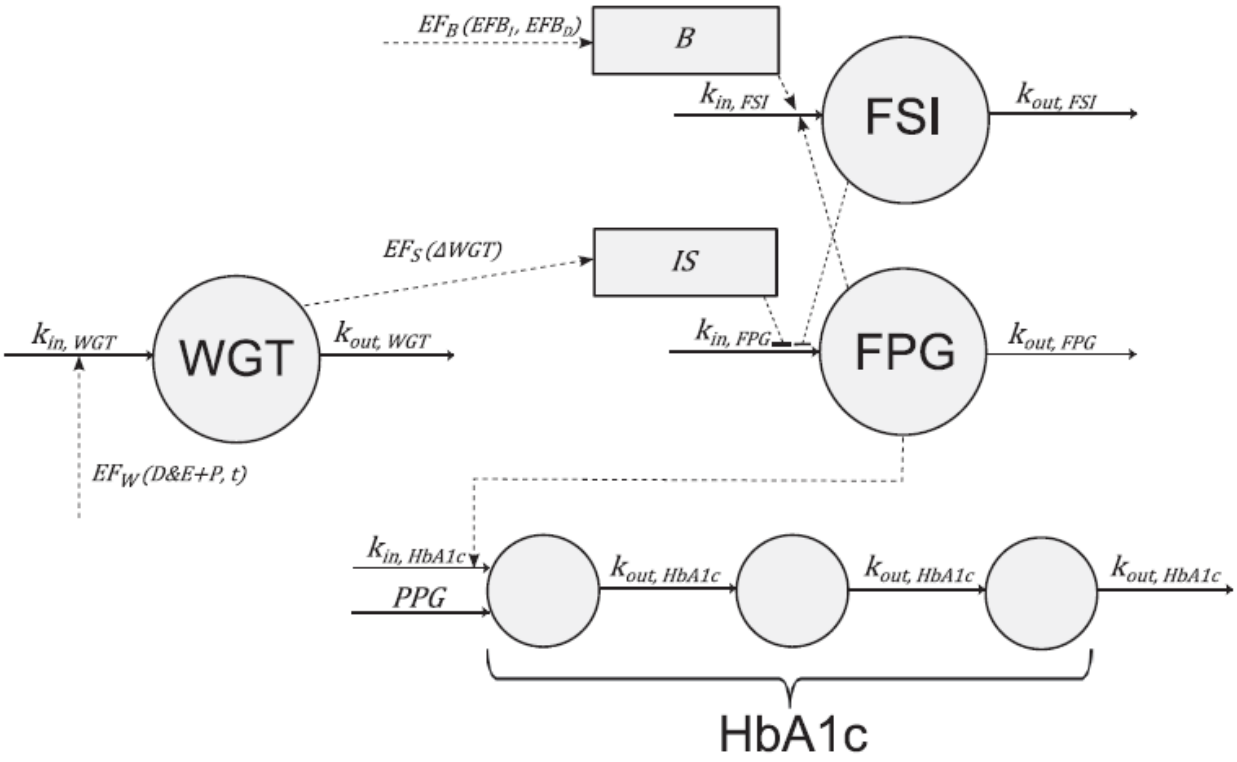

Fig. 13.

The weight-HbA1-insulin-glucose model proposed by Choy and coauthors [5].

Employing this model, it has been possible to estimate the influence of weight loss on IS, and to evaluate the overall functioning of the homeostatic system and the diabetes biomarkers. The relationship between weight loss and insulin sensitivity is shown in Fig. 14. [5].

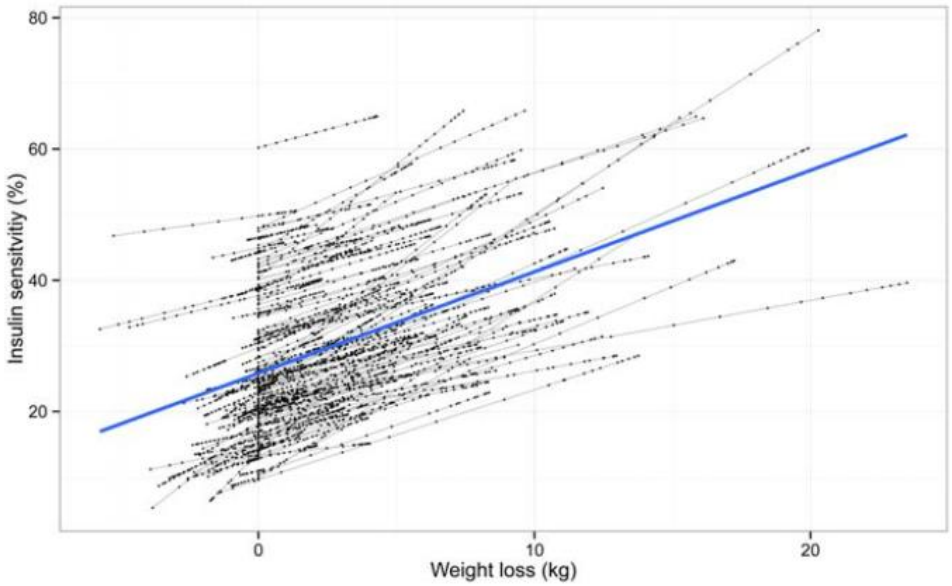

Fig. 14.

The relationship between weight loss and insulin sensitivity. Each gray line represents an individual patient, while the black dots are the estimation data from the model. The blue line represents the linear regression of all points, then, in the weight-HbA1-insulin-glucose model, weight loss and insulin sensitivity are linearly proportional. For each kilogram lost, an obese T2DM patient is expected to regain about 1.5% insulin sensitivity [5].

The study by A. Rostami-Hodjegan and coauthors [12] is titled "Population-based modeling to demonstrate extrapancreatic effects of tolbutamide" and it was published on the *Journal of Applied Physiology* in 2020. The authors built a model to investigate the PK and PD of tolbutamide and the eventual influence of covariates on the process in healthy subjects. Tolbutamide is part of the sulfonylurea, in fact, it can be used to help T2DM treatment stimulating the secretion of insulin by the beta cells.

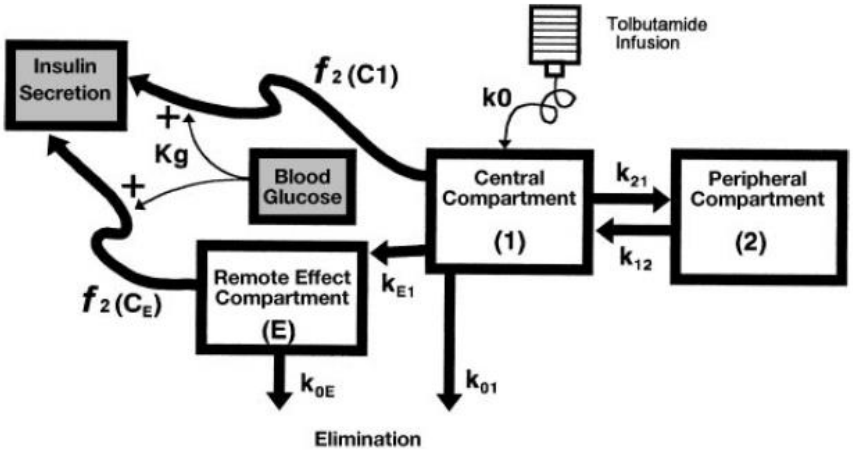

Fig. 15.

PK/PD model used to describe the biphasic insulinergic effect of tolbutamide [12].

Fig. 15 shows the model used to describe the biphasic insulinergic effect of tolbutamide. The drug is released in the central compartment, with the usual peripheral compartment. Less usual is the remote effect compartment, which has a delayed insulinergic effect on insulin secretion, as opposed to the central one, which has an immediate effect on it. It can be observed that blood glucose has a synergic influence on insulin secretion together with the tolbutamide effects. Instead, Fig. 16 shows the model that describes the influence of tolbutamide on the system managing glucose production and consumption according to the blood glucose levels. It starts from the same assumptions of the Minimal Model [13], except the third: 1) plasma glucose concentration influences inhibition and utilization of glucose itself, 2) insulin has a synergistic influence on these effects of glucose, and 3) the decrease of glucose levels within plasma only depends on insulin present in the remote compartment (lymph), or, like in Rostami-Hodjegan study, it depends on both remote (or peripheral) compartment and serum insulin. It can be observed that tolbutamide effect works in synergy with insulin action.

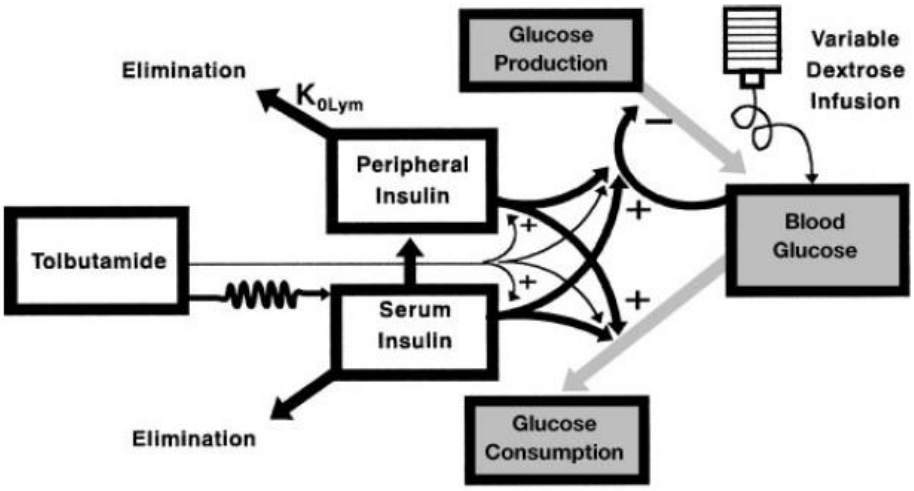

Fig. 16.

Model describing how the combined effect of insulin and tolbutamide on the feedback control of glucose production-consumption by blood glucose concentration [12].

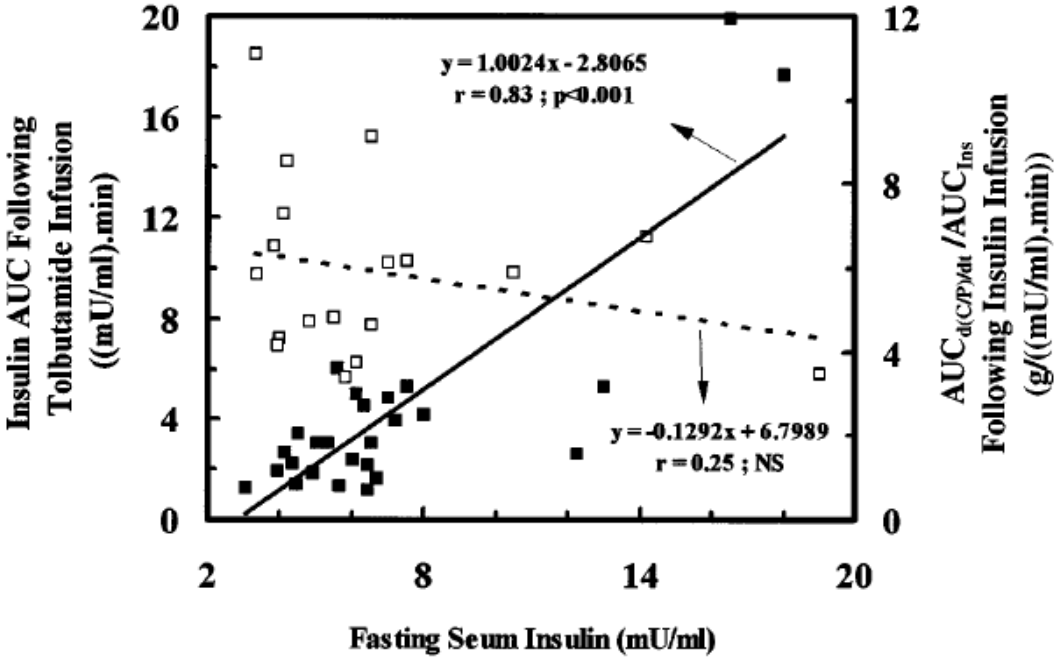

Fig. 17.

The solid line represents the relationship between insulinergic effect of tolbutamide and fasting serum insulin, while the dashed line represents the relationship between the hypoglycemic effect of exogenous insulin and fasting serum insulin [12].

These models successfully characterized PK and PD of tolbutamide, confirming the previous knowledge about the drug. Moreover, it has been highlighted that the covariate insulin sensitivity has a relevant influence on the insulinergic effect of tolbutamide. Indeed, in Fig. 17 it is possible to see those subjects with a higher FSI tend to secrete more insulin, considering the drug concentration as constant. This variability in the tolbutamide effect due to insulin sensitivity is similar to the compensatory insulinergic response to glucose in insulin-resistant subjects. Another point of the study regards the extrapancreatic effects of tolbutamide, consisting in prolongation of insulin effect in the remote compartment (lymph). These effects may be due to tolbutamide itself, or also to the portal-to-peripheral ratio of serum insulin [12].

In conclusion of the bibliographic review, a summary table of the presented studies is shown below (Table 1).

Table 1. Systematic Bibliographic review summary.

Authors, year	Title	Involved subjects	Aim	Model
Hovorka et al., 1998 [7]	Pancreatic β-Cell Responsiveness during Meal Tolerance Test: Model Assessment in Normal Subjects and Subjects with Newly Diagnosed Noninsulin-Dependent Diabetes Mellitus	16 healthy subjects 16 newly diagnosed noninulin-dependent DM	Quantify β-cells responsiveness in response to a meal tolerance test or OGTT.	Two-compartments model of C-peptide secretion and kinetics
Moller et al., 2011 [8]	Mechanism-based population modelling for assessment of L-cell function based on total GLP-1 response following an oral glucose tolerance test	healthy volunteers and T2DM patients	Asses of L-cells function based on GLP-1 response to a standard 75 g OGTT.	Composed by 2 sub-models: a glucose-insulin model and a GLP-1 secretion model. Implemeted with NONMEM VI software.
Fang et al., 2013 [9]	Study reanalysis using a mechanism-based pharmacokinetic/pharmacodynamic model of pramlintide in subjects with type 1 diabetes	25 male T1DM patients and healthy controls	Describe the effects of pramlintide of postprandial glucose regulation in T1DM patients.	Nonlinear mixed-effects two-compartments model of PK/PD of pramlintide. Built on NONMEM VII
Hi et al., 2015 [10]	Target-mediated pharmacokinetic/pharmacodynamic model based meta-analysis and dosing regimen optimization of a long-acting release formulation of exenatide in patients with type 2 diabetes mellitus	T2DM patients and healthy controls. Datasets from 6 papers.	Find the optimal regimen of release of exenatide ER for treatment of T2DM.	Two parts: PK part with FPG and HbA1c compartments; PD part, with 4 transient compartments, an absorption compartment, a central and a peripheral compartment. Nonlinear mixed-effects model built in Monolix.
Bouazza et al., 2016 [11]	Evaluation of the pharmacokinetics of glibenclamide tablet given, off label, orally to children suffering from neonatal syndromic hyperglycemia	18 children with neonatal syndromic hyperglycemia	Asses the capability of glibenclamide, administered orally, to hyperpolarize the β-cells membrane, permitting a normal insulin response.	1-compartment model for the description of the data, a proportional error model for the residual variability and an exponential error model for the inter-subject variability

Authors, year	Title	Involved subjects	Aim	Model
Choy et al., 2016 [5]	Weight-HbA1c-insulin-glucose model for describing disease progression of type 2 diabetes	181 obese T2DM patients treated with diet and exercise for 67 weeks.	Quantify the improvements in insulin sensitivity and overall homeostatic system, employing FPG, FSI and HbA1 as biomarkers of diabetes.	Population model taking into account FPG, FSI, HbA1c (3 transit compartments) and weight.
Rostami-Hodjegan et al., 2020 [12]	Population-based modeling to demonstrate extrapancreatic effects of tolbutamide	2 groups of healthy subjects, differing for insulin sensitivity.	Investigate the PK and PD of tolbutamide (insulinergic) and its extrahepatic effects.	PK/PD model with central, peripheral and remote effect compartments.

3. Population modelling approach for the study of insulin clearance in previous gestational diabetes

3.1 Dataset

Data relative to 141 subjects with an history of gestational diabetes mellitus (pGDM) and 41 healthy subjects (CNT) were provided by the Metabolic Unit of the CNR Institute of Neuroscience which has an agreement with the Department of Information Engineering of Università Politecnica delle Marche. The insulin-modified frequently sampled intravenous glucose tolerance test (IM-IVGTT) procedures were performed after an overnight fast. After baseline blood samples were collected, two intravenous administrations occurred through the antecubital veins of the subjects: dextrose (0,3 g/kg), chemically equal to glucose, at time t=0 for half a minute, and insulin (4 mU/Kg/min), for 5 minutes starting from t=20. Dosages were normalized according to the body weight (BW) of each subject. Blood samples for the measurement of glucose, insulin and C-peptide concentrations were taken at several time instants until 180 min. Insulin concentration was measured at minutes 0, 3, 4, 5, 6, 8, 10, 14, 19, 22, 27, 30, 35, 40, 50, 70, 100, 140, 180 [4].

3.2 Assessment of insulin clearance through a mathematical model

The model presented in this thesis is based on the model of Polidori et. al [4], and it provides a model-based method for the estimation of hepatic and extrahepatic insulin clearance through plasma insulin and C-peptide profiles obtained from the insulin-modified frequently sampled intravenous glucose tolerance test.

The model is shown in Fig. 18. It is a two-compartments model and it is based on the following four assumptions:

1. The endogenous secreted insulin enters the portal vein traveling to the liver before reaching the systemic circulation. The insulin secretion rate (ISR) is obtained by deconvolution starting from C-peptide profiles [14].
2. The rate of delivery of insulin from the systemic circulation to the liver trough the hepatic artery is equal to the product of plasma insulin concentration (P) and the assumed hepatic plasma flow (HPF) rate (whose value was 0.576 L/min/m^2, from the literature [15]).
3. Insulin clearance occurs both in the liver and in extrahepatic tissues, which includes kidney, skeletal muscles and adipose tissue. Extrahepatic clearance is assumed to be proportional to the plasma concentration.

4. Hepatic clearance is modeled using a linear function or a saturable function. Both are tested in each subject, and the one providing the best fit is kept.

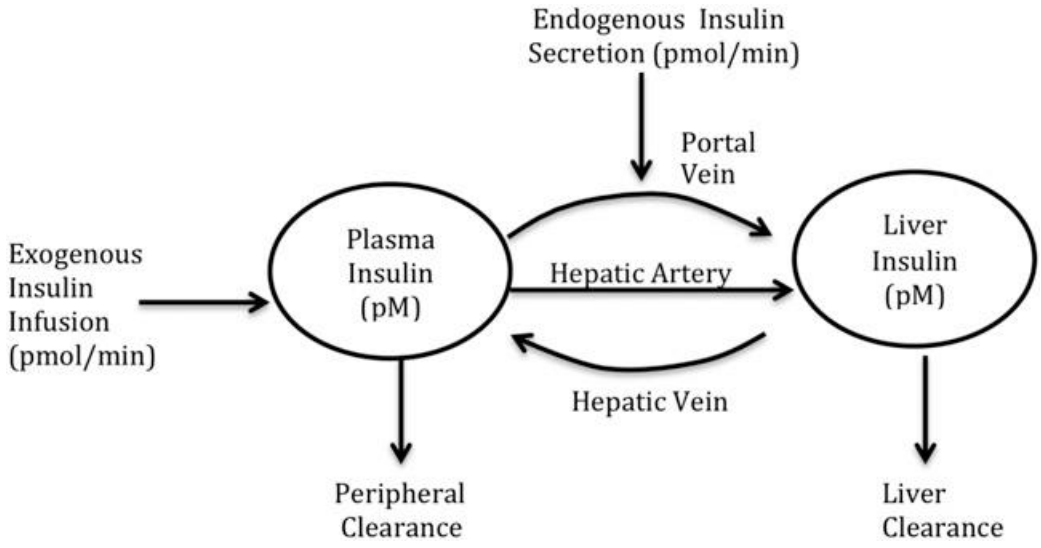

Fig. 18.

Graphical representation of the mathematical model of Polidori et al. The HPF rate used in the equations is the combined plasma flow to the liver from the portal vein and the hepatic artery [4].

The equations and the parameters describing the model are as follows:

$Insulin\ delivery\ to\ liver\ (pmol/min)$:

$$Delivery = ISR + HPF \cdot P$$

$Hepatic\ insulin\ degradation\ (pmol/min)$:

$$Linear\ model = FE_L \cdot Delivery$$

$$Saturabel\ model = \frac{V_{max} \cdot Delivery}{K_m + Delivery}$$

$$Extrahepatic\ insulin\ degradation\ (pmol/min) = CL_P \cdot P$$

where P represents the plasma insulin (pmol/L), ISR is the insulin secretion rate (pmol/min), HPF is the hepatic plasma flow rate (L/min) and CL_P represents the extrahepatic insulin clearance (L/min). FE_L is the hepatic fractional extraction (dimensionless), V_{max} is the maximal hepatic degradation rate

(pmol/min), and K_m is the hepatic insulin delivery rate at which 50% of maximal degradation occurs (pmol/min). Model-identified parameters were normalized by body weight (BW) for comparison across subjects. The differential equations for the linear (first equation) and saturable (second equation) assumptions are as follows:

$$V_P \frac{dP}{dt} = IIR + (1 - FE_L) \cdot ISR - (HPF \cdot FE_L + CL_P) \cdot P$$

$$V_P \frac{dP}{dt} = IIR + ISR - CL_P \cdot P - \frac{V_{max} \cdot Delivery}{K_m + Delivery}$$

V_P is the extrahepatic distribution volume for insulin (L) and IIR is the insulin infusion rate (pmol/min). In order to make possible the comparison with analogous measures of clearance obtained through other experimental methods, these two equations were used to calculate CL_{IV} and CL_{portal}, which respectively are the clearance from a model for an intravenous insulin infusion (as in hyperinsulinemic clamps) and the clearance from a model for a portal infusion of insulin (as in endogenous secretion). For linear model they are:

$$CL_{IV} = CL_P + HPF \cdot FE_L$$

$$CL_{portal} = \frac{CL_{IV}}{1 - FE_L}$$

3.3 Model Implementation

The implementation took place in Monolix. The implemented model is the model of Polidori et al. for the estimation of clearance parameters [4], described in chapter 3.2. Each subject has her own ID, Body weight (BW), height (h), body mass index (BMI), age, basal glucose (G_b) and body surface area (BSA) were used as covariates, while hepatic plasma flow rate (HPF), insulin secretion rate (ISR) and insulin infusion rate (IIR) were employed as regressors, and they represent the input of the model. Plasma insulin (P) is the observed variable. In the model the peripheral insulin clearance (CL_P), the hepatic fractional flow rate (FE_L) and the extrahepatic distribution volume for insulin (V_P) are the variables which are estimated. ISR represents the endogenous insulin secretion from pancreatic β-cells to the liver through the portal vein in response to the glucose infusion. ISR data was obtained by deconvolution of C-peptide profiles, and there is a value for each of the 180 min of measurements. Instead, IIR are the values of the exogenous infusion of insulin occurring from min 20 to min 25. In the rest of the time records the value of IIR is zero. HPF data were computed for each subject as the product of the hepatic plasma flow rate (equal to 0.576 L/min/m^2 [4]) and BSA, which in turn was calculated as:

$$BSA = 0{,}016667 \cdot \sqrt{BW} \cdot \sqrt{H}\,.$$

All the parameters, variables and their measurements units are reported in Table 2.

Table 2 – Model parameters summary.

Abbreviation	Parameter	Measurement Unit	Use in the Model
BW	Body Weight	Kg	continuous covariate
h	Height	cm	continuous covariate
BMI	Body Mass Index	Kg/m^2	continuous covariate
age	Age	years	continuous covariate
Gb	Basal Glucose	pmol/L	continuous covariate
BSA	Body Surface Area	m^2	continuous covariate
HPF	Hepatic Plasma Flow	L/min	regressor
ISR	Insulin Secretion Rate	pmol/L	regressor
IIR	Insulin Infusion Rate	pmol/min	regressor
P	Plasma Insulin	pmol/L	observation
CL_P	Peripheral (extrahepatic) insulin clearance	L/min	estimated parameter
FE_L	hepatic fractional extraction	dimensionless	estimated parameter
V_P	Extrahepatic distribution volume	L	estimated parameter

3.4 Data Analysis

Monolix can rather run single tasks at a time or all at once. They are:

- **POPULATION PARAMETERS**. This first task estimates the population parameters through Stochastic Approximation Expectation-Maximization (SAEM) algorithm. The estimate considers an objective function. This algorithm consists of two phases: an exploratory phase and a smoothing phase, in which convergence is reached.
- **EBs**. The individual parameters are estimated using the conditional mode, representing the most probable values among the individual distributions.
- **CONDITIONAL DISTRIBUTION**. In this task the individual parameters estimation is based on the conditional distribution, representing the uncertainty of the individual parameter values. A Markov chain Monte Carlo (MCMC) algorithm is used for sampling during the computation of the conditional distribution. The individual estimates obtained with the conditional distribution are used in the **Pearson's test** too. This is a statistical test aimed to spot covariates which should be added to the model. It measures the correlation between the random effect of each parameter and each continuous covariate. If some covariate is

categorical, the Pearson's test is substituted with the **ANOVA test**, with the same purpose. If the p-value is particularly low, a correlation is spotted, and the corresponding covariate should be added to the model. Another statistical test which is performed is the **Shapiro Wilk test**, which permits to measure how much a distribution is normal. It may operate on distribution of random effects as well as distribution of individual parameters. If the p-value is particularly low, then the random effects are normally distributed. After the introduction of a covariate for a certain parameter, the of individual distribution normality of such parameter is tested through the **Kolmogorov Smirnov test**. Finally, **Correlation test (t-test)** is a statistical test measuring the correlations between random effects of the parameters.

- **STANDARD ERRORS**. This is the task for the calculation of the Fisher correlation matrix and standard errors. In addition, the eigen values are computed, and the condition number is the ratio between the max eigen values and the minimum one. If the condition number is lower than 100, overparameterization does not occur; if it is comprised between 100 and 1000, there could be overparameterization; if the condition number is greater than 1000, overparameterization is probable. Two methods are proposed for it: linearization method or stochastic approximation method. After running this task, two additional columns appear aside the estimated population parameters: standard error (SE) and relative standard error (RSE%).
- **LIKELYHOOD**. Since the SAEM algorithm does not explicitly compute the objective function, the LIKELYHOOD task is dedicated to this. Indexes as BIC (Bayesian Information Criteria) and AIC (Akaike Information Criteria) give information about the loss of information during the model processing.
- **PLOTs**. Plots are generated.

The employed procedure was:

1. Set 0,5 and 0,5 as initial values of FE_L and CL_P in "Initial Estimates". The initial value of V_P is left equal to 1. These values are close to previous estimate obtained in our laboratory with different tools, and they will be used as starting values for the parameter's estimation. The initial values of the standard deviation of random effects are set to 1, corresponding to the maximum initial dispersion of the parameter distribution, and then it will converge to a smaller value. Starting with this high variability, the algorithm will explore a wider domain of values before converging. For all the simulations, the option MLE (Maximum Likelihood

Estimation) was selected into the "Initial Estimates" options. In this way the estimates will depend on the mean and the variance of the involved distributions.

2. Run all tasks. We chose to consider the individual estimates computed according to the conditional mode, since it refers to the most probable value, differently from the estimates based on the conditional mean. Moreover, stochastic approximation method was selected for all the simulations in this study.
3. Select Covariates and Correlations in the individual model. It is possible to choose which covariates are to be included into the model looking at the Pearson's test, the ANOVA test, the Fisher Correlation Matrix and the corresponding plots. Instead, the correlations can be spotted consulting the results of the Correlation test (t-test). Visualization of which covariates and correlations are suggested for the model is done in the section called "proposed model". In cases of multiple covariates and correlations we performed a selection on the basis of physiological and statistical considerations (i.e., observation of the standard errors, correlation matrices and condition numbers). Run all tasks.

The previously mentioned methodology has been applied by considering: i) the complete dataset (OVP, overall population) and including the group (CNT, pGDM) as a categorical variable; ii) the CNT and pGDM datasets separately.

For each dataset, two estimates have been performed in Monolix. The intermediate estimates provided indications about correlations and covariates to add to the model to improve the quality of final estimates.

Once all the results were obtained, statistical tests were performed on the distributions of individual parameters in matlab, through the two-sample Student's t-test (function "ttest2"). Sets was considered statistically different for p-values $< 0,05$. The sets which were employed for the t-test are the individual estimates in conditional mode. Each distribution was tested with the Shapiro-Wilk test to evaluate if they were normal or not. In case of not normal distributions, the log-transformation of them was given as input for the t-test. Instead, if the individual estimates were normally distributed, no transformation was needed. With the Student's t-test it is possible to assess if two sets of data are statistically different or not.

4. Results

Fig. 19 shows an example of the individual fits for some subjects related to the CNT group.

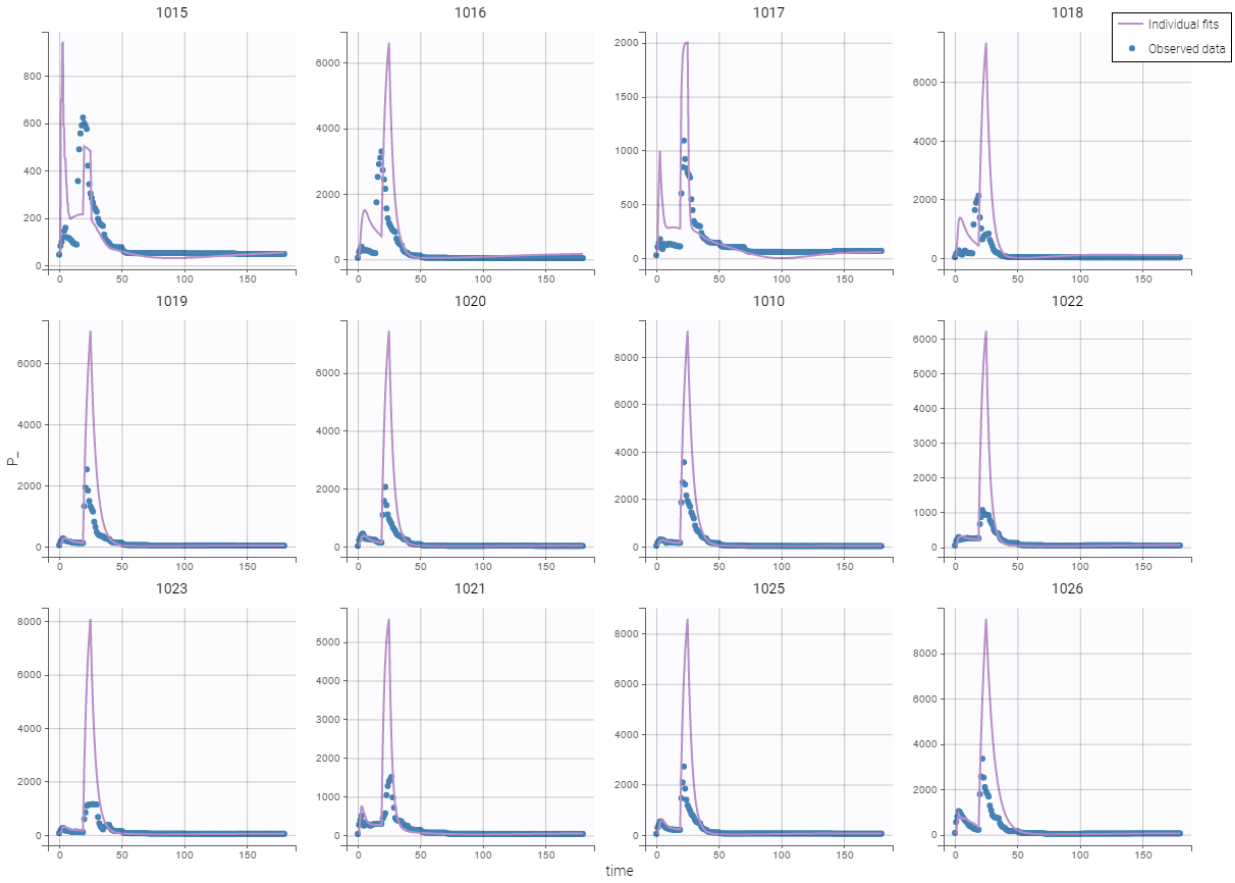

Fig. 19.

Individual fits of plasma insulin concentration (observed variable). Blue dots represent the observed data, while the purple curves represent the fits of the data. It is possible to observe two peaks, one relative to the first glucose infusion (at time zero), and the second due to the second insulin infusion (from min 20 to min 25).

Covariates and correlations suggested in OVP and CNT/pGDM datasets are reported in Table 3. Of note, no covariate, neither correlation, was considered for the estimates using CNT dataset. This means that, for CNT estimates, the final run coincides with the intermediate run. As an example, correlation between the parameter CL_P and the covariate Gb is reported in Figure 20.

Table 3. Results of the correlation and covariate analysis. Correlations and covariates chosen for being considered in the final models are highlighted in bold.

Dataset	Correlation	p-value	Covariates	p-value
OVP	**FE_L, V_P**	0,007	**group (for FE_L)**	0,022
			age (for CL_P)	0,027
CNT	-	-	BSA (for FE_L)	0,007
			BW (for FE_L)	0,007
pGDM	CL_P, V_P	0,005	**Gb (for CL_P)**	0,007

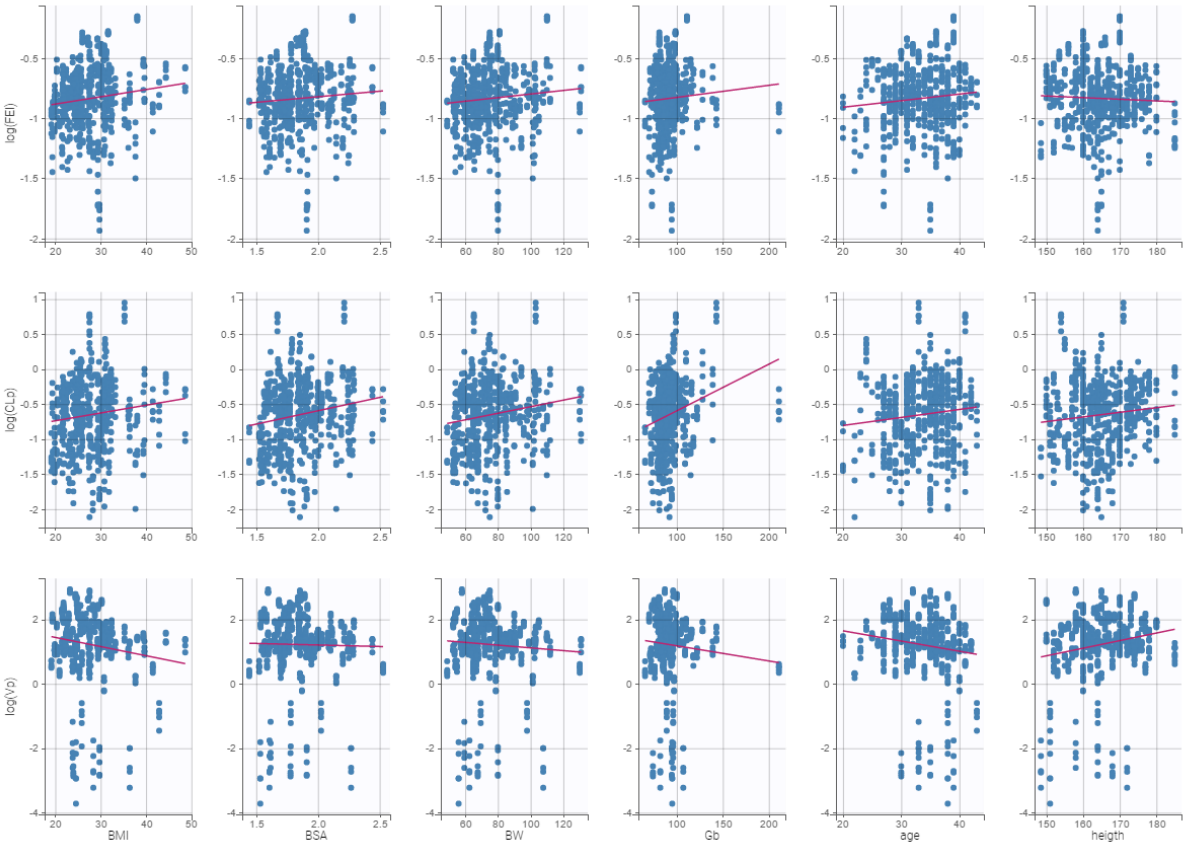

Fig. 20.

Individual parameters vs covariates. It is possible to observe the presence of a correlation between the parameter CL_P and the covariate Gb.

Table 4 reports the values of the estimates of population parameters obtained through SAEM algorithm. Relative standard errors and condition numbers are displayed too.

Table 4. Estimated population parameters in the intermediate phase (before setting correlations and covariates) and in the final phase, with their correspondent relative standard errors.

Dataset	Parameter	Intermediate Population estimate (RSE%)	Condition number	Final population estimate (RSE%)	Condition number
OVP	FE_L	47,7 (2,3)	2,4	39,3 (4,7)	24
	CL_P	0,46 (5,39)		0,59 (5,15)	
	V_P	3,06 (7,91)		3,34 (7,35)	
CNT	FE_L	-	-	29,7 (13,6)	6,9
	CL_P	-		0,91 (16,00)	
	V_P	-		3,76 (17,70)	
pGDM	FE_L	42,8 (2,7)	6,6	44,7 (2,7)	110
	CL_P	0,54 (5,16)		0,32 (26,00)	
	V_P	3,34 (11,4)		3,21 (11,10)	

Legend: "-" is used to express that intermediate and final estimates are equal for CNT dataset, due to the fact that no correlations or parameters were considered for it.

Individual estimates obtained from the analysis of OVP dataset and CNT/pGDM dataset are reported in Table 5.

Table 5. Individual estimates for hepatic and extrahepatic insulin clearance in OVP and CNT/pGDM datasets

Datasets	Parameters	CNT	pGDM	p-value
OVP	FE_L(%)	43,5 [11,6]	47,5 [9,5]*	0,01
	CL_P(L/min)	0,61 [0,28]	0,63 [0,27]	0,11
CNT/pGDM	FE_L(%)	33,0 [10,8]	47,0 [12,8]	<0,001
	CL_P(L/min)	1,04 [0,33]	0,50 [0,15]	<0,001

Data are reported as median [interquartile range]. * statistical significance, p<0,05.

5. Discussion and Conclusions

This study proposed a population modelling approach for the study of insulin clearance in previous gestational diabetes. Estimation of population parameters quantifying hepatic (FE_L) and extrahepatic (CL_P) insulin clearance was provided for healthy women and women with a history of gestational diabetes by exploiting the model proposed by Polidori et al [6] and implementing it in the Monolix software. Monolix is a software providing simple solution for non-linear mixed effects modeling for pharmacometrics. It is based on the SAEM algorithm and provides robust, global convergence even for complex PK/PD models. The implemented Polidori model is a simple model described by a single differential equation with linear dynamics (even though the Polidori model has also a formulation with nonlinear dynamics which was not considered in this study).

As it is possible to see in Table 3, the covariate "group", used for labeling the two sub-populations CNT and pGDM within OVP dataset, was suggested as covariate for FE_L with a p-value of $\simeq$0,02. This result confirmed that the used population modelling approach was able to distinguish the two groups among the overall population OVP thus implying that the history of gestational diabetes may play a role. As shown in Table 3, Monolix suggested additional correlations and covariates for the model, but we decided to only include some of them, according to physiological and statistical reasons, like the observation of the standard errors, correlation matrices and condition numbers given by each simulation.

Looking at the population parameters (Table 4), while the estimates relative to V_P are quite similar in the estimation performed considering different datasets, there is a certain variability for the estimates of the clearance parameters FE_L and CL_P. In fact, with reference to the estimates obtained by the CNT and pGDM datasets, women with an history of gestational diabetes showed a CL_P which is almost three times smaller with respect to control group (0,32 L/min vs 0,91 L/min), indicating a decreased extrahepatic insulin clearance in pGDM population. In the other hand, the population estimate of FE_L was higher for pGDM population with respect to the control group, indicating an increased hepatic insulin clearance (29,7 % vs 44,7 %). Of note, population estimates obtained considering OVP dataset lay in between those obtained with the separate datasets.

In the pGDM dataset the condition number of the final estimation was equal to 110, thus further investigation is required to check the occurrence of overparameterization, which could be present for

values of the condition number comprised between 100 and 1000. However, the RSEs (Table 4) and the correlation matrix (not shown) relative to the last pGDM simulation provided reliable results. Thanks to these factors, together with the fact that this critical condition number is just slightly larger than 100, it is reasonable to conclude that no overparameterization occurred throughout the simulations.

When considering the individual estimates and the difference among groups the FE_L resulted significantly different for the estimations performed on the OVP and on the separate (CNT/pGDM) dataset. Instead, significant differences between the two groups were detected for CL_P only when considering the separate datasets.

In conclusion, the proposed population modelling approach showed its capability to provide population parameter estimates related to hepatic and extrahepatic insulin clearance. Hepatic insulin clearance may be affected by the presence of a history of gestational diabetes, whereas extrahepatic insulin clearance requires further investigation in wider populations.

References

[1] Röder, P. V., Wu, B., Liu, Y., & Han, W. (2016). Pancreatic regulation of glucose homeostasis. *Experimental & Molecular Medicine*, 48(November 2015), e219. https://doi.org/10.1038/emm.2016.6

[2] Chwalba, A., Dudek, A., Otto-Buczkowska, E. (2019). Role of Amylin in Glucose Homeostasis. *Austin Diabetes Research*, 4(1), 1–5.

[3] Morettini, M., Göbl, C., Kautzky-Willer, A., Pacini, G., Tura, A., & Burattini, L. (2020). Former gestational diabetes: Mathematical modeling of intravenous glucose tolerance test for the assessment of insulin clearance and its determinants. *Mathematical Biosciences and Engineering*, *17*(2), 1604–1615. https://doi.org/10.3934/mbe.2020084

[4] Polidori, D. C., Bergman, R. N., Chung, S. T., & Sumner, A. E. (2016). Hepatic and Extrahepatic Insulin Clearance Are Differentially Regulated : Results From a Novel Model-Based Analysis of Intravenous Glucose Tolerance Data. 65(March), 1556–1564. https://doi.org/10.2337/db15-1373

[5] Choy, S., Kjellsson, M. C., Karlsson, M. O., & De Winter, W. (2016). Weight-HbA1c-insulin-glucose model for describing disease progression of type 2 diabetes. *CPT: Pharmacometrics and Systems Pharmacology*, *5*(1), 11–19. https://doi.org/10.1002/psp4.12051

[6] Diabetes, D. O. F. (2006). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, *29*(SUPPL. 1).

[7] Hovorka, R., Chassin, L., Luzio, S. D., Playle, R., & Owens, D. R. (1998). Pancreatic β-cell responsiveness during meal tolerance test: Model assessment in normal subjects and subjects with newly diagnosed noninsulin- dependent diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism*, *83*(3), 744–750. https://doi.org/10.1210/jc.83.3.744

[8] Møller, J. B., Jusko, W. J., Gao, W., Hansen, T., Pedersen, O., Holst, J. J., Overgaard, Rune V., Madsen, Henrik, Ingwersen, Steen H. (2011). Mechanism-based population modelling for assessment of L-cell function based on total GLP-1 response following an oral glucose tolerance test. *Journal of Pharmacokinetics and Pharmacodynamics*, *38*(6), 713–725. https://doi.org/10.1007/s10928-011-9216-2

[9] Fang, J., Landersdorfer, C. B., Cirincione, B., & Jusko, W. J. (2013). Study reanalysis using a mechanism-based pharmacokinetic/pharmacodynamic model of pramlintide in subjects with type 1 diabetes. *AAPS Journal*, *15*(1), 15–29. https://doi.org/10.1208/s12248-012-9409-7

[10] Li, H., Xu, J., & Fan, X. (2015). Target-mediated pharmacokinetic/pharmacodynamic model based meta-analysis and dosing regimen optimization of a long-acting release formulation of exenatide in patients with type 2 diabetes mellitus. *Journal of Pharmacological Sciences*, *127*(2), 170–180. https://doi.org/10.1016/j.jphs.2014.12.004

[11] Bouazza, N., Djerada, Z., Gozalo, C., Busiah, K., Beltrand, J., Berdugo, M., Urien, S., Treluyer, J. M., & Polak, M. (2016). Evaluation of the pharmacokinetics of glibenclamide tablet given, off label, orally to children suffering from neonatal syndromic hyperglycemia. *European Journal of Clinical Pharmacology*, *72*(11), 1373–1379. https://doi.org/10.1007/s00228-016-2119-9

[12] Rostami-Hodjegan, A., Peacey, S. R., George, E., Heller S. R., Tucker G. T. (2020). Population-based modeling to demonstrate extrapancreatic effects of tolbutamide. Journal of Applied Physiology, 82, 2045–2053.

[13] Bergman, R. N., Phillips, L. S., & Cobelli, C. (1981). Physiologic evaluation of factors controlling glucose tolerance in man. Measurement of insulin sensitivity and β-cell glucose sensitivity from the response to intravenous glucose. Journal of Clinical Investigation, 68(6), 1456–1467. https://doi.org/10.1172/JCI110398

[14] Van Cauter, E., Mestrez, F., Sturis, J., & Polonsky, K. S. (1992). Estimation of insulin secretion rates from C-peptide levels: Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes, 41(3), 368–377. https://doi.org/10.2337/diabetes.41.3.368

[15] Caumo, A., Florea, I., & Luzi, L. (2007). Effect of a variable hepatic insulin clearance on the postprandial insulin profile: Insights from a model simulation study. Acta Diabetologica, 44(1), 23–29. https://doi.org/10.1007/s00592-007-0237-7