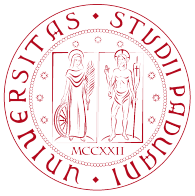

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In Silico Testing of Artificial Pancreas and New Type 1 Diabetes Treatments: Model Development and Assessment

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Contents

Abstract

English

In healthy subjects, glucose regulation relies on a complex hormonal control system that maintains the blood glucose level in a safe range. Impairment of this regulatory system is the cause of several metabolic disorders, such as type 1 diabetes (T1DM), characterized by the absolute deficiency of insulin production, leading to a chronic hyperglycemia that, if not treated, can result in severe microvascular and macrovascular complications. Currently, the best therapy for T1DM management makes use of a continuous subcutaneous insulin pump (CSII) coupled with a subcutaneous continuous glucose monitoring sensor (CGM), the so-called sensor-augmented-pump therapy (SAP). Nevertheless, to ease T1DM subject's life condition, in the last decade, the researchers have been focused on developing an automatic closed-loop control system for insulin infusion, the so-called Artificial Pancreas (AP), which aims to maintain the glucose level within the euglycemic range.
In this regards, simulation models allowed important steps forward in the AP research, enabling the possibility to perform several in silico tests, with relevant time- and cost- savings. In particular, in 2008 the US Food and Drug Administration accepted the T1DM simulator developed by Universities of Virginia (UVA) and Padova as a substitute for preclinical trials for certain insulin treatments, including closed-loop algorithms. This dramatically accelerated the process for the approval of human trials. The UVA/Padova simulator (S2008) is based on a rather complex model of glucose dynamics

that was identified on a data set of 204 healthy subjects for which not only plasma glucose and insulin measurements but also estimates of glucose fluxes were available. The simulator, equipped with 100 in silico adults, 100 adolescents, and 100 children, spanning the variability observed in the real type 1 diabetic population, has been updated in 2013 in order to better describe the distribution of glucose concentration observed in clinical trials (S2013). However, at the beginning of this project, the simulator validity was never been validated against clinical data. In addition, nowadays, the frontier of the AP research is the development of control algorithm effective for weekly or monthly use. However, the T1DM simulator was not fully adequate for the long-term testing, since its domain of validity was limited to a single-meal scenario.

The first aim of this research is thus to assess the simulator validity using data of the available clinical trials. The second objective is to extend the domain of validity of the simulator, making it suitable for simulating long-term clinical trials. Finally, a third scope is to illustrate the possible uses of the simulator, including setting up a paradigm for in silico trials for testing of new insulin treatments.

To achieve the first objective, a database of 24 T1DM subjects was first considered, who received dinner and breakfast in two occasions, for a total of 96 post-prandial glucose traces. Measured plasma glucose profiles were compared with those obtained with both S2008 and S2013, by replicating in 100 in silico adults the same experimental condition of the data (i.e. same meal amount and insulin delivery). The Continuous Glucose-Error Grid Analysis was used to assess the validity of the simulated traces, and the most common clinical outcome metrics, obtained in silico, were compared with the experimental ones. The results were satisfactory, proving that the virtual adults of the S2013 are representative of an age-matched T1DM population observed in a clinical trial.

Then, the T1DM model has been validated on 47 T1DM subjects who received dinner, breakfast and lunch, in three admissions, for a total of 23 hours per session. In particular, given the complexity of the model and the availability of glucose and insulin measurements only, a Bayesian approach has

been adopted for model identification, considering, as a priori information, the parameter distribution included in the simulator for the generation of in silico subjects. Variability of model parameters describing glucose absorption and insulin sensitivity (SI, i.e. the ability of insulin to stimulate glucose disposal and suppress endogenous glucose production) was allowed, assuming that meal composition may be different at breakfast, lunch, and dinner (resulting in different absorption rate), and that SI may vary throughout the day. The model well described glucose traces and the posterior distribution of model parameters was similar to that included in the simulator; absorption parameters at breakfast were significantly different from those at lunch and dinner, reflecting more rapid dynamics of glucose absorption; on the other hand, insulin sensitivity varies in each individual but without a specific pattern. These results suggested the need of a time-varying simulator to better describe the glucose variability in the long-term.

In this regard, a model of intra-day variability of insulin sensitivity has been developed, by using data of a recent multiple tracer experiment performed in 20 T1DM subjects, which revealed the existence of diurnal patterns of SI, with SI lower at breakfast than lunch and dinner, on average. This difference was not statistically significant, both due to the small population size and the high inter-subject variability. In particular, seven SI daily patterns were identified, and their probabilities were estimated from the data. This information has been translated into the simulator by associating each in silico subject to one of the seven variability patterns, and modeling its SI with time-varying parameters. To test the goodness of the model, the same experimental protocol of the 20 T1DM subjects was replicated in silico: the comparison of simulated glucose against the data was satisfactory, showing that the simulated plasma glucose level was higher at breakfast than lunch and dinner, as the clinical data.

Finally, two case studies, illustrating the simulator employment, are presented. An AP adaptive control algorithm was tested first. The performance was evaluated in silico in a 1-month scenario: in particular, being able to provide a realistic time-varying behavior of the system, it was possible to prove evidence of the improved glucose control achievable with the adaptive

control with respect to a non-adaptive AP algorithm. Then, the simulator was employed to evaluate the pharmacological effect of a novel inhaled insulin: in particular, the simulator have served to evaluate the post-prandial glucose in response to different insulin dosing regimens, thus allowing to determine, for each in silico subject, the best insulin pattern to optimally control post-prandial glucose.
In conclusion, in this work, the UVA/Padova T1DM simulator was assessed against clinical T1DM data; then, the T1DM model was identified on a 24-hour scenario, proving that a time-varying model was required to well describe the daily glucose variability; a model of intra-day variability of SI was thus developed and incorporated into the simulator. Finally, the use of the simulator was illustrated in two examples, i.e. the preclinical testing of an adaptive AP algorithm and the design of optimal dosing regimen of a novel inhaled insulin. In both cases, the simulator proved to be a useful tool for the in silico testing of T1DM treatments.

Italiano

Il metabolismo del glucosio nei soggetti sani è regolato da un complesso sistema di controllo ormonale che mantiene la concentrazione plasmatica di glucosio nel range di sicurezza. Alterazioni di tale sistema sono causa di diverse malattie metaboliche, tra cui il diabete mellito di tipo 1 (T1DM), una malattia autoimmune che, a causa della totale incapacità del pancreas di secernere insulina, è caratterizzata da una iperglicemia cronica che, se non trattata, porta a complicanze cardiovascolari. Ad oggi, la miglior terapia per la gestione del T1DM è la cosiddetta Sensor-Augmented-Pump, che impiega un microinfusore di insulina (Continuous Subcutaneous Insulin Infusion, CSII) e un sensore per la misura quasi continua della glicemia (Continuous Glucose Monitoring, CGM), entrambi con accesso sottocutaneo. Tuttavia, allo scopo di migliorare ulteriormente le condizioni di vita dei pazienti diabetici, nell'ultimo decennio la ricerca si è orientata allo sviluppo del cosiddetto pancreas artificiale (AP), ossia un sistema automatico di infusione di insulina, atto a mantenere la glicemia il più possibile nel range euglicemico.
In questo contesto, i modelli di simulazione permettono di testare, velocemente e in tutta sicurezza, le performance degli algoritmi di controllo in diverse condizioni sperimentali. Tra questi, il simulatore di soggetti T1DM sviluppato dalle Università di Padova e Virginia (UVA/Padova) è stato accettato nel 2008 dalla U.S. Food and Drug Administration come sostituto alla sperimentazione preclinica animale per il test di trattamenti insulinici, tra cui gli algoritmi di controllo utilizzati nel AP. Questo ha permesso di velocizzare notevolmente il processo di approvazione alla sperimentazione nell'uomo. Il simulatore UVA/Padova (S2008) si basa su un modello matematico del sistema glucosio-insulina, che è stato identificato su un dataset di 204 soggetti non diabetici, in cui erano a disposizione sia le misure plasmatiche di glucosio e insulina che le stime dei flussi metabolici di glucosio; esso è dotato inoltre di una popolazione di soggetti virtuali, composta da 100 adulti, 100 adolescenti e 100 bambini, che rispecchia la variabilità di una popolazione diabetica reale. Nel 2013, il simulatore è stato aggiornato (S2013) per migliorare la descrizione della glicemia e renderla più simile a quella osservata nei trial

clinici. Tuttavia, all'inizio di questo progetto, il simulatore non era ancora stato validato su dati clinici. Inoltre, il limite di validità del simulatore era vincolato a scenari tempo-invarianti, rendendolo, di fatto, poco adeguato al test degli algoritmi di AP di ultima generazione, che mirano al controllo glicemico nel lungo periodo.
Pertanto, lo scopo di questa tesi è innanzitutto validare il simulatore su dati clinici di soggetti T1DM, e successivamente estendere il suo dominio di validità a scenari tempo-varianti più realistici, permettendo quindi un test più robusto degli algoritmi di AP nel lungo periodo. Infine sono descritti due esempi di applicazione del simulatore, tra cui il test in silico di insuline di nuova generazione.
Per quanto riguarda il primo obbiettivo, è stato utilizzato un dataset di 96 tracce post-prandiali di glucosio, relative a 24 soggetti T1DM studiati in due occasioni, a cena e colazione. Per validare il simulatore dal punto di vista clinico, le popolazioni adulte di entrambe le versioni, S2008 e S2013, sono state confrontate con quella reale, sottoponendo i soggetti in silico alle stesse condizioni sperimentali dei soggetti reali (vale a dire stesse quantità di carboidrati e insulina somministrati). Le glicemie simulate sono state quindi confrontate con i dati utilizzando la Continuous Glucose-Error Grid Analysis e analizzando le più comuni metriche di quantificazione del controllo glicemico. I risultati ottenuti hanno dimostrato che la popolazione adulta della versione S2013 è rappresentativa di una popolazione T1DM adulta studiata in un trial clinico.
Il modello del simulatore è stato successivamente validato su un dataset di 47 soggetti T1DM studiati in tre sessioni da 23 ore ciascuna, in cui venivano somministrati tre pasti (cena, colazione, pranzo). Data la complessità del modello e la disponibilità delle sole misure di glucosio e insulina, l'identificazione del modello è stata effettuata ricorrendo a un approccio di stima Bayesiano, in cui l'informazione a priori utilizzata (prior) è la distribuzione congiunta dei parametri del modello utilizzata nel simulatore per la generazione dei soggetti in silico. Per ottenere un buon fit del modello, è stato necessario introdurre una variabilità inter-individuale nei parametri che descrivono l'assorbimento del glucosio legato al pasto e nella sensibilità insulinica (SI, un indice che quanti-

fica la capacità dell'insulina nell'inibire la produzione endogena di glucosio e nel promuoverne l'utilizzazione da parte dei tessuti), ipotizzando l'esistenza di variazioni relative alla composizione dei pasti tra colazione, pranzo e cena, e variazioni di SI durante la giornata. I risultati hanno mostrato come il modello sia in grado di descrivere le tracce glicemiche, e la distribuzione delle stime dei parametri è risultata simile alla distribuzione inclusa nel simulatore. Come ci si attendeva, i parametri di assorbimento a colazione sono risultati significativamente differenti rispetto a quelli di pranzo e cena, denotando una dinamica di assorbimento più rapida a colazione; al contrario, i risultati mostrano che la SI varia da un pasto all'altro, ma senza evidenziare un pattern significativo. Questi risultati hanno evidenziato che, per riuscire a descrivere adeguatamente la variabilità glicemica durante la giornata, è necessario un simulatore tempo-variante.
È stato quindi sviluppato un modello della variabilità diurna di SI, sfruttando i risultati di un esperimento con traccianti multipli condotto in 20 soggetti T1DM. In questo studio la SI stimata a colazione è risultata, in media, più bassa rispetto alle stime di SI a pranzo e cena. Tale differenza, tuttavia, non è risultata statisticamente significativa a causa del modesto numero di soggetti studiati e dell'alta variabilità inter-individuale. Pertanto, i soggetti sono stati classificati in base ai valori di SI assunti durante la giornata; ciò ha permesso di identificare sette classi di variabilità, ognuna caratterizzata da una certa probabilità. Tale informazione è stata quindi implementata nel simulatore, assegnando ogni soggetto in silico a una delle sette possibili classi, e descrivendo quindi la SI del soggetto come un segnale che varia nel tempo. Il modello di variabilità di SI è stato quindi validato simulando lo stesso protocollo sperimentale dei dati e confrontando la variabilità glicemica ottenuta in simulazione con quella osservata nei dati: in particolare, le simulazioni sono risultate confrontabili con i dati clinici, mostrando escursioni post-prandiali a colazione più ampie rispetto a quelle osservate a pranzo e cena.
Infine, sono stati illustrati due esempi di utilizzo del simulatore. In un caso, il simulatore è stato impiegato per il test in silico di un algoritmo di controllo adattativo di AP. Nello specifico, la performance di controllo è stata

testata simulando uno scenario di un mese in cui, grazie all'integrazione del modello di variabilità di SI, è stato possibile apprezzare come l'approccio adattativo permetta un miglior controllo glicemico rispetto a un controllore non adattativo. Nel secondo esempio, l'uso del simulatore ha permesso di testare gli effetti farmacologici di una nuova tipologia di insulina inalata: in particolare, è stato possibile valutare l'escursione glicemica post-prandiale in risposta a diversi regimi di somministrazione del farmaco, e determinare per ogni soggetto virtuale il regime di somministrazione più adatto a garantire il miglior controllo glicemico post-prandiale.
In conclusione, in questa tesi è stato innanzitutto validato il simulatore del diabete di tipo 1 UVA/Padova utilizzando dati clinici di soggetti T1DM; successivamente, il modello del simulatore è stato identificato su dati di soggetti T1DM studiati in uno scenario di 24 ore, evidenziando la necessità di un modello tempo-variante per descrivere correttamente l'andamento della glicemia durante la giornata; il simulatore è stato quindi aggiornato, integrando un modello di variabilità diurna di SI. Infine, sono stati illustrati due casi di studio, in cui il simulatore è stato impiegato per il test di un algoritmo di controllo adattativo e per l'ottimizzazione del regime di somministrazione di una nuova tipologia di insulina inalata. I risultati hanno dimostrato l'utilità del simulatore UVA/Padova per il test di trattamenti terapeutici per la gestione del diabete.

Glossary

AP	Artificial Pancreas
BG	Blood Glucose
BW	Body weight
CF	Correction factor
CHO	Carbohydrates
CR	Carbohydrate-to-insulin ratio
CSII	Continuous Subcutaneous Insulin Infusion
CGM	Continuous Glucose Monitoring
CG-EGA	Continuous Glucose-Error Grid Analysis
CV	Coefficient of Variation
CVGA	Control Variability Grid Analysis
EGP	Endogenous glucose production
FDA	Food and Drug Administration
MAP	Maximum a Posteriori
MMPC	Modular Model Predictive Control
PK	Pharmacokinetic
R2R	Run-to-Run
$\mathbf{Ra_{meal}}$	Rate of meal glucose appearance
S2008	First version of UVA/Padova T1DM simulator
S2013	Updated version of UVA/Padova T1DM simulator
SAP	Sensor-Augmented-Pump
SD	Standard deviation
SI	Insulin Sensitivity
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TDI	Total daily insulin
U	Glucose utilization
UVA	University of Virginia

1 Introduction

1.1 Background

1.1.1 Glucose metabolism and diabetes

It is crucial that blood glucose level does not decrease under 70 mg/dL since glucose is the predominant metabolic fuel for the brain which cannot store more than a few minutes supply as glycogen, or quickly increase its extraction of glucose from the circulation. The glucose concentration in blood is thus regulated by a complex internal feedback systems that keep blood glucose level within a narrow range around its basal value (target blood glucose range: $70 - 180$ mg/dL). Hypoglycemia is identified when plasma glucose concentration goes below 70 mg/dL, while hyperglycemia occurs when glucose concentration raises over 180 mg/dL [99]. Prevention of hypoglycemia is critical to survival. On the other hand, the chronic hyperglycemia leads to micro- and macro-vascular complications which include limb loss, blindness, ischemic heart disease, and end-stage renal disease [99–101].
Comprehension of the mechanisms that regulate plasma glucose have significantly evolved since the discovery in the 1920s of the peptide hormone insulin, which was considered the main responsible of glucose homeostasis. Insulin is secreted by β-cells in the islets of Langerhans in response to high levels of plasma glucose, promotes the glucose utilization by tissues and inhibits the

endogenous glucose production by liver and kidney. In the 1950s pancreatic α-cells hormone glucagon was discovered. It is secreted in response to a fall in plasma glucose concentration below the hypoglycemic threshold, and acts by stimulating hepatic glycogenolysis and gluconeogenesis, thus raising endogenous glucose production and resulting in an increase of plasma glucose concentration. Then, other hormones have been discovered to contribute to glucose regulation, such as amylin, incretin hormones (e.g. GLP1), growth hormone, epinephrine, and cortisol, although their effects is modest if compared with insulin and glucagon (Figure 1.1). In these regards, the glucose regulation can be meant as a bi-hormonal system, with insulin representing the key regulatory hormone of glucose disappearance, and glucagon the major regulator of endogenous glucose release.

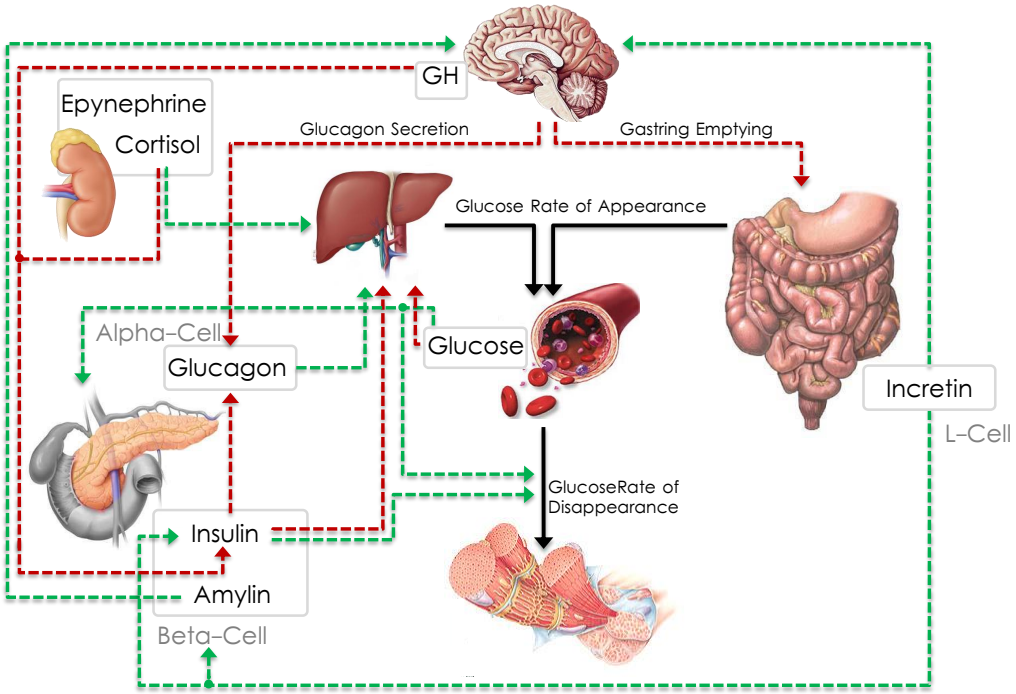


Figure 1.1: Glucose homeostasis in healthy individuals: roles of the major hormones. Black lines represent fluxes, red dotted lines represent inhibition control signals and green dotted lines represent promotion control signals.

Impairment of the glucose regulatory system is the cause of several metabolic disorders, such as diabetes. Diabetes is a chronic disease, characterized by the inability of the body to well control the blood glucose concentration,

mainly due to a total deficiency in insulin secretion (type 1 diabetes, T1DM) or to both an impairment in insulin action and the inability of β-cells to compensate for that (type 2 diabetes, T2DM), which lead to a chronic hyperglycemia. In particular, type 1 diabetes is the result of immune-mediated destruction of the pancreatic β-cells, i.e. the site of insulin production and secretion. Despite T1DM represents about only the 5%-10% percent of the world diabetic population (while the remaining 90% is T2DM), it has a strong impact on patient's life: it usually occurs in childhood and adolescence (although it can occur at all ages), and, due to the absolute insulin deficiency, the insulin therapy to control hyperglycemia is required for the entire life.

1.1.2 Diabetes treatments

Since its discovery in the 1920s, insulin has been the only pharmacological treatment usable for the T1DM management. Advances in insulin therapy have included not only improving the source and purity of the hormone, but also developing more physiological means of delivery, from the first auto syringe to the more sophisticated insulin pump, i.e. the continuous subcutaneous insulin infusion (CSII) systems [38, 61, 72, 103]. Paralleling, technological advances in glucose measurement devices allowed to pass from the first portable glucometers in the 1970s, rather bulky and cumbersome, to the recent subcutaneous continuous glucose monitoring (CGM) systems, i.e. minimally invasive glucose sensors which provide the glucose levels almost in real-time [29, 41, 88].
Nowadays, the best approach to the T1DM management is the so-called sensor-augmented-pump therapy (SAP), which makes use of a subcutaneous insulin pump coupled with a CGM sensor. Several clinical studies have demonstrated the impact of SAP therapy in glucose control, with benefits for patients' life, e.g. [5, 81, 83]. Nevertheless, a lot of actions are still left to subject's self management, which definitely affect his life condition. For example, a single meal involves the estimation of carbohydrate content, the measurement of pre-prandial glycemia, the calculation and injection of the insulin meal bolus, and, if needed, the calculation and administration of a

post-prandial correction bolus.

Thus, in order to improve patients' life condition, recently the research addressed its efforts on the development of the so-called Artificial Pancreas (AP), i.e. an automated system combining a glucose sensor, a closed-loop control algorithm, and an insulin infusion device. AP research has been active for the last 50 years: after the pioneering work by Kadish [39] in 1964, there were some works reporting closed-loop control results between 1974 and 1978, [1, 52, 62, 71, 82], with the commercialization, in the 1977, of the first AP device, i.e. the Biostator [11]. However, the hardware setup consisted of glucose sensors and insulin infusion systems with intravenous access, thus being not suitable for the use in daily life. It is right thanks to the recent introduction of CGM and CSII systems and the incorporation of AP control algorithm into portable platforms that it was possible to perform an increasing number of clinical trials, moving from an inpatient to an more real-life-resambling outpatient setup, with experiments lasting some days [8, 17, 25, 27, 28, 49, 54, 77]. Examples of AP systems different hardware technology are shown in Figure 1.2.

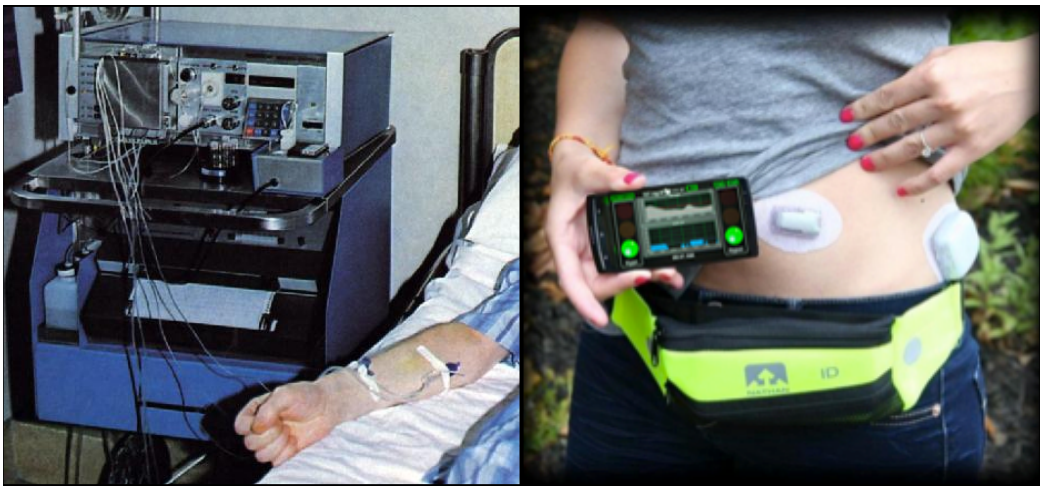


Figure 1.2: Technology evolution in AP research: the Biostator [11] (*Left panel*); a portable AP in 2010s, i.e. Diabetes Assistant (DiAs) [40] (*Right panel*).

However, despite the technological improvements of the hardware setup, there are several drawbacks related to the closed-loop controllers that need to be enhanced, such as the therapy optimization during particular conditions (like physical exercise) or the meal bolus management. Moreover, it is worth noting that the current employed rapid acting insulin analogs are suitable for the insulin basal daily coverage, but represent the bottleneck for the optimal meal control: in fact, the rapid rise of postmeal glucose is difficult to avert because of the inherent delays in subcutaneous insulin absorption and action [87]. Hence, alterative routes of insulin administration, such has intradermal, intraperitoneal and pulmonary, are currently under study.

1.1.3 Simulation models

Simulation models allowed important steps forward in testing the performance of AP algorithms or different insulin infusion routes, enabling the possibility to perform several in silico tests, with relevant time- and cost-saving. Simulation models are widely employed in physics and engineering, where system structure and behavior are generally known, and thus the dynamics can be mathematically represented by using first principles. These powerful tools are used whenever a certain system results too difficult, expensive, dangerous, unethical or impossible to reproduce in a laboratory.
In the last 40 years, several simulation models of the glucose system have been developed, e.g. [2, 14, 15, 18, 31, 56, 78, 84, 86]. However, the impact of these models in the AP research was modest: these "old generation" simulators, despite providing a detailed description of the system, are based on "average models", i.e. they are able to describe only the average dynamics of the system in a populations, but not the inter-individual variability, which actually is crucial to perform realistic in silico trials and appropriately test the robustness of control algorithms. In addition, the "old generation" simulators were all based on plasma glucose and insulin concentrations only, and given model complexity, such information is not sufficient to properly describe the system.

In the last decade, "new generation" T1DM simulators have been developed, aiming to reproduce inter-subject variability of glucose dynamics in a type 1 diabetic population. Among them, a simulator environment has been proposed by the Cambridge group, which is based on a high order model of glucose dynamic that was identified on glucose and insulin data of T1DM subjects monitored in a clinical trial [35]. Given the large number of model parameters, some of them have been fixed to population values available in the literature [36, 37, 57], in order to make the model identifiable. However, being the model identified on plasma glucose and insulin concentrations, the compensation among the parameters was very likely.

The UVA/Padova T1DM simulator

A different approach was employed in developing the T1DM simulator proposed by the University of Padova in collaboration with the University of Virginia (UVA) [43],[20]. In particular, the UVA/Padova T1DM simulator is based on a rather complex model of glucose dynamics that was identified using not only plasma glucose and insulin measurements but also estimates of the glucose fluxes, i.e. plasma glucose rate of appearance after a meal (Ra_{meal}), endogenous glucose production (EGP) by liver and kidney, glucose utilization (U) by tissues, available in a large population of healthy subjects studied with multiple tracer [3]. This represents the peculiarity of the UVA/Padova T1DM simulator, that allowed to generate 100 in silico adults, 100 adolescents, and 100 children, which span the variability observed in the real type 1 diabetic populations. More details on model developments are provided in *Chapter* 2.

A first version of the simulator was proposed in 2008 (S2008, [43]), and was accepted by the U.S. Food and Drug Administration (FDA) as a substitute for preclinical trials for certain insulin treatments, including closed-loop control algorithms. In these years, the simulator has been extensively used by several institutions, academies and pharmaceutical companies. In 2013, the simulator was updated (S2013), in order to account for counteregulation system and include a better description of glucose dynamics in hypoglycemia

[20].

At the beginning of this project, despite the improvements introduced in the last version, the UVA/Padova T1DM simulator still presented some limitations. First of all, it was never been validated against a T1DM population, despite this step was required to evaluate whether the simulator was representative of real T1DM subjects, since, as it will be explained in *Chapter* 2.2.2, its model was based on a dataset of healthy subjects [3].
Another important point to take into account is that the simulator was time-invariant: in fact, being it developed on single meal data [3], the simulator was unable to describe glucose variations that can occur in a day. In this regard, with the increasing outpatient long-term clinical trials [53, 55], the need to extend the domain of validity of the simulator has become crucial, in order to improve the ability of the simulator to predict glucose variability in the long period, thus providing more realistic scenarios for long-term in silico trials. Once these limitations are overcome, the simulator can be an useful tool not only in AP context but also for testing new insulin molecules and different insulin delivery routes.

1.2 Aim

The aim of this thesis is to overcome the limitations of the UVA/Padova T1DM simulator described above, thus making it a suitable framework for the in silico testing of AP control algorithms and new T1DM treatments. To achieve this goal one has:

- To assess the simulator validity against T1DM data of the now available clinical trials.
- To develop an intra-subject variability model to extend the domain of validity of the simulator, thus making the simulator suitable for long-term AP clinical trials.
- To show examples of simulation use, for testing both AP controllers and novel insulin treatments.

1.3 Outline of the thesis

The thesis is articulated as follows:
Chapter 2 presents an overview on the UVA/Padova T1DM simulator, illustrating its main characteristics, the improvements introduced with the 2013 update, and the main drawbacks that motivated the present research.
Chapter 3 describes the experimental data used to develop and validate the model.
Chapter 4 describes the methodology carried out to clinically assess the simulator against a T1DM population observed during a clinical trial.
Chapter 5 describes the approach adopted to validate the model of the T1DM simulator on a large T1DM population studied on a 24-hour scenario. This will highlight the need of a time-varying simulator in order to well describe the glucose variability in the long-term.
Chapter 6 presents a model of intra-day variability of insulin sensitivity to be incorporated into the simulator, in order to improve the description of diurnal glucose variability. This will provide a new time-varying simulator.
Chapter 7 presents two applications of the simulator: i) for testing an adaptive control algorithm; ii) for assessing the best dosing of a novel insulin treatment.
Specific comments are reported at the end of *Chapters* 4–6. The results obtained in this work as well as emerged open questions and future research directions are discussed in *Chapter* 8.

2 The UVA/Padova T1DM simulator

2.1 Introduction

In this chapter, a detailed description of the UVA/Padova T1DM Simulator is provided. The first version of the simulator (S2008) [43], accepted in 2008 by FDA as a substitute to animal trials for the preclinical testing of control strategies in artificial pancreas studies [42], is presented first. Then, the modifications introduced in 2013 (S2013) to fix some of the main drowbacks of S2008, are described.

2.2 T1DM simulator 2008

The original version of the UVA/Padova T1DM Simulator (S2008) consists of a model of glucose-insulin dynamics during a meal, a population of 300 virtual patients and an user interface.

2.2.1 The model

The matematical model describing glucose dynamics in T1DM subjects derives from a model built on data of healthy subjects (as explained below). In fact, model structure is the same in healty and T1DM except for some equations. Thus, the model of healthy subject is presented first, then the modification needed to simulate T1DM subjects are introduced.

Healthy model

The model of healthy subject, schematically shown in Figure 2.1 (*upper panel*), was proposed by Dalla Man and colleagues in 2007 [22]. Briefly, the model assumes that the glucose and insulin subsystems are linked one to each other by the action of insulin on glucose utilization and endogenous production, and the control of glucose on insulin secretion by the β-cells.

The glucose subsystem consists of a two-compartment model describing glucose kinetics [93]:

$$\begin{cases} \dot{G}_p(t) = EGP(t) + Ra_{meal}(t) - U_{ii}(t) - E(t) - k_1 \cdot G_p(t) + k_2 \cdot G_p t(t) \\ G_p(0) = G_{pb} \\ \dot{G}_t(t) = -U_{id}(t) + k_1 \cdot G_p(t) - k_2 \cdot G_p t(t) \\ G_t(0) = G_{tb} \\ G(t) = G_p(t)/V_G \\ G(0) = G_b \end{cases} \tag{2.1}$$

where G_p and G_p (mg/kg) are glucose masses in plasma and rapidly equilibrating tissues, and in slowly equilibrating tissues, respectively; G (mg/dL) is the plasma glucose concentration; suffix b denotes basal state; EGP is the endogenous glucose production (mg/kg/min); Ra_{meal} is the glucose rate of appearance in plasma (mg/kg/min); E is renal excretion (mg/kg/min); U_{ii} and U_{id} (mg/kg/min) are the insulin-independent and -dependent glucose utilizations, respectively; V_G is the distribution volume of glucose (dL/kg); and k_1 and k_2 are the rate parameters.

The insulin subsystem also consists of two compartments, describing the insulin in the liver and the plasma [30]:

$$\begin{cases} \dot{I}_p(t) = -(m_2 + m_4) \cdot I_p(t) + m_1 \cdot I_l(t) + S(t) \\ I_p(0) = I_{pb} \\ \dot{I}_l(t) = -(m_1 + m_3) \cdot I_l(t) + m_2 \cdot I_p(t) \\ I_l(0) = I_{lb} \\ I(t) = I_p(t)/V_I \\ I(0) = I_b \end{cases} \tag{2.2}$$

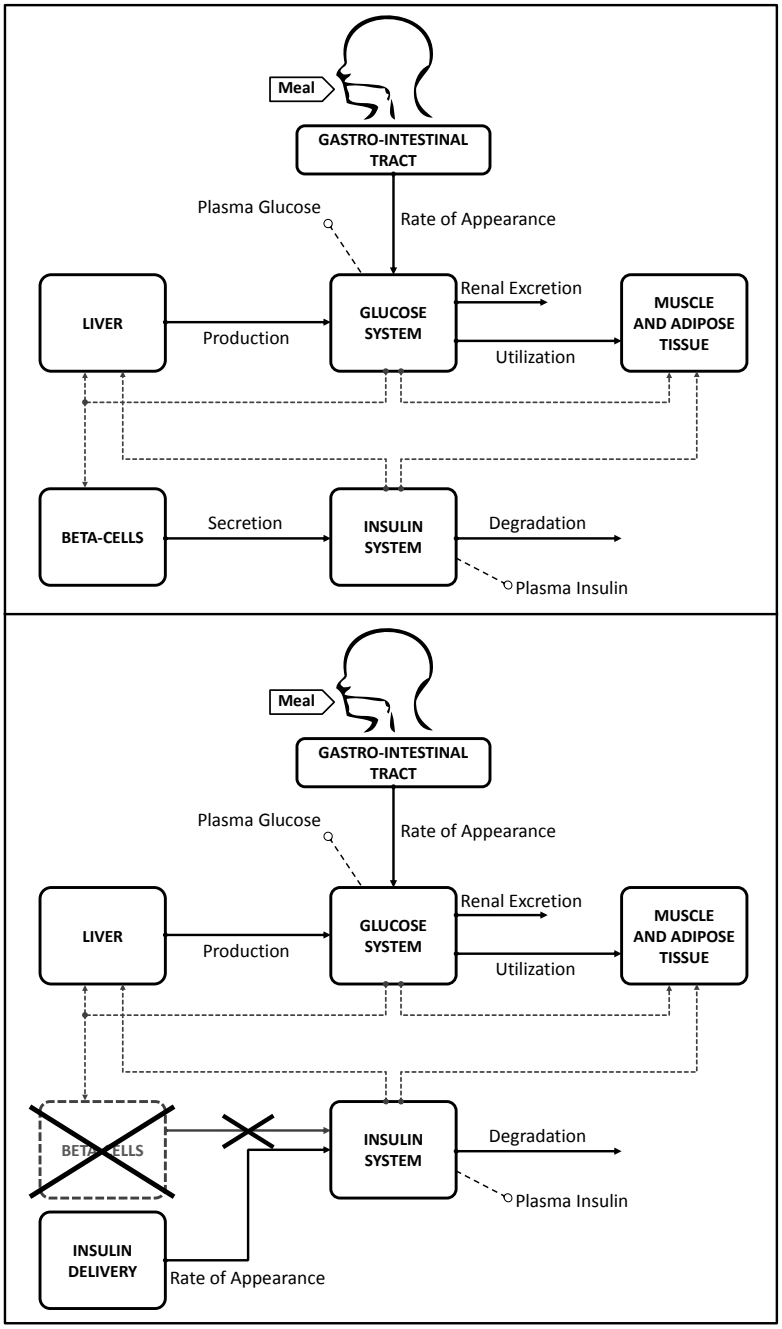


Figure 2.1: Scheme of the glucose-insulin model in healthy (*upper panel*), and model of S2008 T1DM simulator (*lower panel*). In the latter, endogenous insulin secretion is replaced by an exogenous insulin administration.

where I_l and I_p (pmol/kg) are insulin masses in plasma and in liver, respectively; I (pmol/L) is the plasma insulin concentration; suffix b denotes basal

state; S is the insulin secretion (pmol/kg/min); V_I is the distribution volume of insulin (L/kg); m_1, m_2 (min^{-1}) are rate parameters; finally, degradation (D) occurs both in the liver (through m_3) and in the periphery (linearly through m_4). Hepatic insulin extraction (HE), i.e. the insulin flux leaving the liver irreversibly divided by the total insulin flux leaving the liver, is time varying, depending on S:

$$HE(t) = -m_5 \cdot S(t) + m_6 \quad HE(0) = HE_b \tag{2.3}$$

so that

$$m_3(t) = \frac{HE(t)}{1 - HE(t)} \cdot m_1 \tag{2.4}$$

Parameters m_2 and m_4 are derived from equation (2.2) at basal state and considering that the liver is responsible for 60% of insulin clearance in the steady state:

$$m_2 = \left(\frac{S_b}{I_{pb}} - \frac{m_4}{1 - HE_b} \right) \cdot \frac{1 - HE_b}{HE_b} \tag{2.5}$$

$$m4 = \frac{2}{5} \cdot \frac{S_b}{I_{pb}} \tag{2.6}$$

where S_b is the basal insulin secretion, and HE_b is assumed fixed to 0.6.

Endogenous glucose production, glucose rate of appearance, and glucose utilization are the most important model unit processes. Suppression of EGP (mg/kg/min) is assumed to be linearly dependent on plasma glucose mass and a delayed insulin action on the liver (X^L) [23]:

$$EGP(t) = k_{p1} - k_{p2} \cdot G_p(t) - k_{p3} \cdot X^L(t) \qquad EGP = EGP_b \tag{2.7}$$

$$\dot{X}^L(t) = -k_i \cdot [X^L(t) - I'(t)] \qquad X^L(0) = I_b \tag{2.8}$$

$$\dot{I}'(t) = -k_i \cdot [I'(t) - I(t)] \qquad I'(0) = I_b \tag{2.9}$$

where X^L is obtained with a chain of two compartments; k_{p1} (mg/kg/min) is the extrapolated EGP at zero glucose and insulin, k_{p2} (min^{-1}) is the liver

glucose effectiveness, k_{p3} (mg/kg/min per pmol/L) is a parameter governing amplitude of insulin action on the liver (i.e. the insulin sensitivity on glucose prouction), and k_i is the rate parameter accounting for delay between insulin signal and insulin action.

Glucose intestinal absorption describes the glucose transit through the stomach and intestine by assuming the stomach to be represented by two compartments (one for solid and one for liquid phase), while a single compartment is used to describe the gut [19]:

$$\begin{cases} Q_{sto}(t) = Q_{sto1}(t) + Q_{sto2}(t) \\ Q_{sto}(0) = 0 \\ \dot{Q}_{sto1}(t) = -k_{gri} \cdot Q_{sto1}(t) + D \cdot \delta(t) \\ Q_{sto1}(0) = 0 \\ \dot{Q}_{sto2}(t) = -k_{empt}(Q_{sto}) \cdot Q_{sto2}(t) + k_{gri} \cdot Q_{sto1}(t) \\ Q_{sto2}(0) = 0 \\ \dot{Q}_{gut}(t) = k_{abs} \cdot Q_{gut}(t) + k_{empt}(Q_{sto}) \cdot Q_{sto2}(t) \\ Q_{gut}(0) = 0 \\ Ra_{meal}(t) = \dfrac{f \cdot k_{abs} \cdot Q_{gut}(t)}{BW} \\ Ra_{meal}(0) = 0 \end{cases} \tag{2.10}$$

with Q_{sto} (mg) the amount of glucose in the stomach (solid, Q_{sto1} and liquid phase Q_{sto2}), and Q_{gut} (mg) the glucose mass in the intestine; k_{gri} is the rate of grinding; k_{empt} is the rate constant of gastric emptying, described as a nonlinear function of Q_{sto}:

$$\begin{aligned} k_{empt}(Q_{sto}) = k_{min} + \frac{k_{max} - k_{min}}{2} \cdot \\ \cdot \left\{ \tanh[\alpha(Q_{sto} - \beta \cdot D)] - \tanh[\beta(Q_{sto} - c \cdot D)] + 2 \right\} \end{aligned} \tag{2.11}$$

k_{abs} is the rate constant of intestinal absorption; f is the fraction of intestinal absorption which actually appears in plasma; D (mg) is the amount of ingested glucose; BW (kg) is the body weight.

Glucose utilization U is based on the literature [63, 75, 76, 102], and is

the sum of two terms: a constant insulin-independent utilization (U_{ii}), which takes place in the first compartment, representing glucose uptake by the brain and erythrocytes (F_{cns}), and an insulin-dependent utilization (U_{id}), which occurs in a remote compartment, representing peripheral tissues and depending nonlinearly (as Michaelis-Menten) from glucose in the tissues [102]:

$$U_{ii}(t) = F_{cns} \tag{2.12}$$

$$U_{id}(t) = \frac{[V_{m0} + V_{mx} \cdot X(t)] \cdot G(t)}{K_{m0} + G_t(t)} \tag{2.13}$$

$$\dot{X}(t) = -p_{2U} \cdot X(t) + p_{2U} \cdot [I(t) - I_b] \qquad X(0) = 0 \tag{2.14}$$

where V_{mx} (mg/kg/min per pmol/L) and X (pmol/L) are, respectively, the insulin sensitivity and the insulin action on glucose utilization; p_{2U} is the rate constant of insulin action on the peripheral glucose utilization.

Glucose renal excretion (E) by the kidney occurs if plasma glucose exceeds a certain threshold and can be modeled by a linear relationship with plasma glucose:

$$E(t) = \begin{cases} k_{e1} \cdot [G_p(t) - k_{e2}] & \text{if } G_p(t) > k_{e2} \\ 0 & \text{if } G_p(t) \leq k_{e2} \end{cases} \tag{2.15}$$

where k_{e1} (min^{-1}) is the glomerular filtration rate and k_{e2} (mg/dL) is the renal threshold of glucose.

Insulin secretion (S) by β-cells is described by a model proposed by Toffolo and colleagues [91]:

$$S(t) = \gamma \cdot I_{po}(t) \tag{2.16}$$

$$\dot{I}_{po}(t) = -\gamma \cdot I_{po}(t) + S_{po}(t) \quad I_{po}(t) = I_{pob} \tag{2.17}$$

$$\dot{S}_{po}(t) = \begin{cases} Y(t) + K \cdot \dot{G}(t) + S_b & \text{for } \dot{G}(t) > 0 \\ Y(t) + S_b & \text{for } \dot{G}(t) \leq 0 \end{cases} \tag{2.18}$$

$$\dot{Y}_{po}(t) = \begin{cases} -\alpha \cdot \big[Y(t) - \beta \cdot \big(G(t) - h\big)\big] & \text{if } \beta \cdot \big(G(t) - h\big) \geq -S_b \\ -\alpha \cdot \big[Y(t) + S_b\big] & \text{if } \beta \cdot \big(G(t) - h\big) < -S_b \end{cases} \qquad Y(0) = 0 \tag{2.19}$$

where γ (min^{-1}) is the transfer rate constant between portal vein and liver, K (pmol/kg per mg/dL) is the pancreatic responsivity to the glucose rate of change, α (min^{-1}) is the delay between glucose signal and insulin secretion, β (pmol/kg/min per mg/dL) is the pancreatic responsivity to glucose, and h (mg/dL), set to G_b to guarantee system steady state in basal condition, is the threshold level of glucose above which the β-cells secrete new insulin.

T1DM model

As already discussed in *Chapter* 1.1.1, in T1DM subjects an external insulin ifusion is required to supply the deficiency in β-cell insulin secretion. Thus, in the model of the T1DM subject, insulin secretion is replaced by a model of external insulin delivery (Figure 2.1, *lower panel*). In particular, subcutaneous insulin kinetics is described as a variation of the model proposed in [64]

$$\begin{cases} \dot{I}_{sc1}(t) = -(k_d + k_{a1}) \cdot I_{sc1}(t) + IIR(t) \\ I_{sc1}(0) = I_{sc1ss} \\ \dot{I}_{sc2}(t) = k_d \cdot I_{sc1}(t) - k_{a2} \cdot I_{sc2}(t) \\ I_{sc2}(0) = I_{sc2ss} \end{cases} \tag{2.20}$$

where I_{sc1} is the amount of nonmonomeric insulin in the subcutaneous space, I_{sc2} is the amount of monomeric insulin in the subcutaneous space, IIR (pmol/kg/min) is the exogenous insulin infusion rate, k_d (min^{-1}) is the rate constant of insulin dissociation, and k_{a1} and k_{a2} are the rate constants of nonmonomeric and monomeric insulin absorption, respectively. Thus, equation (2.2) becomes

$$\begin{cases} \dot{I}_p(t) = -(m_2 + m_4) \cdot I_p(t) + m_1 \cdot I_l(t) + Ra_I(t) \\ I_p(0) = I_{pb} \\ \dot{I}_l(t) = -(m_1 + m_3) \cdot I_l(t) + m_2 \cdot I_p(t) \\ I_l(0) = I_{lb} \\ I(t) = I_p(t)/V_I \\ I(0) = I_b \end{cases} \tag{2.21}$$

where Ra_I is the rate of appearance of external insulin in plasma

$$Ra_I(t) = k_{a1} \cdot I_{sc1}(t) + k_{a2} \cdot I_{sc2}(t) \tag{2.22}$$

The T1DM model of equations (2.1), (2.3)-(2.15), (2.21), (2.22), has 26 free parameters, among which the most important are hepatic and peripheral insulin sensitivity (k_{p3}, V_{mx}), representing the ability of plasma insulin to inhibit endogenous glucose production and enhance glucose disposal, respectively.

2.2.2 Identification strategy and joint parameters distribution

As already stated in *Chapter* 1.1.3, the key strength of the UVA/Padova simulator is the availability of a robust joint distribution of model parameters, i.e. the covariance matrix used for the generation of the in silico subjects. In other words, a reliable correlation among the model parameters allows to well describe the inter-subject variability without the risk of compensation among the model parameters. This was achievable by identify the simulation model on both plasma glucose and insulin concentrations and estimates of postprandial fluxes, i.e. Ra_{meal}, EGP, U, and, in healthy subjects, S. However, at the time of simulator conception, such information was available uniquely in a large non-diabetic population [3], in which 204 subjects underwent a triple-tracer mixed meal study. Briefly, this technique [4] is able to minimize fluctuations in tracer-to-tracee ratio, allowing an accurate measurement of glucose turnover. In particular, in [3], one oral tracer was added to the meal, while two other tracers were intravenously infused to mimic, respectively, the expected Ra_{meal} and EGP time courses; the estimation of U was then obtained from glucose rate of change through principles of mass balance; finally, S was reconstructed by deconvolution [73].

The model of equations (2.1)-(2.19) [22] was thus identified on this available data by decomposing the model into its single processes and using a forcing functions strategy, as illustrated in Figure 2.2. Unit process models were identified for each subject, thus providing the distribution of model

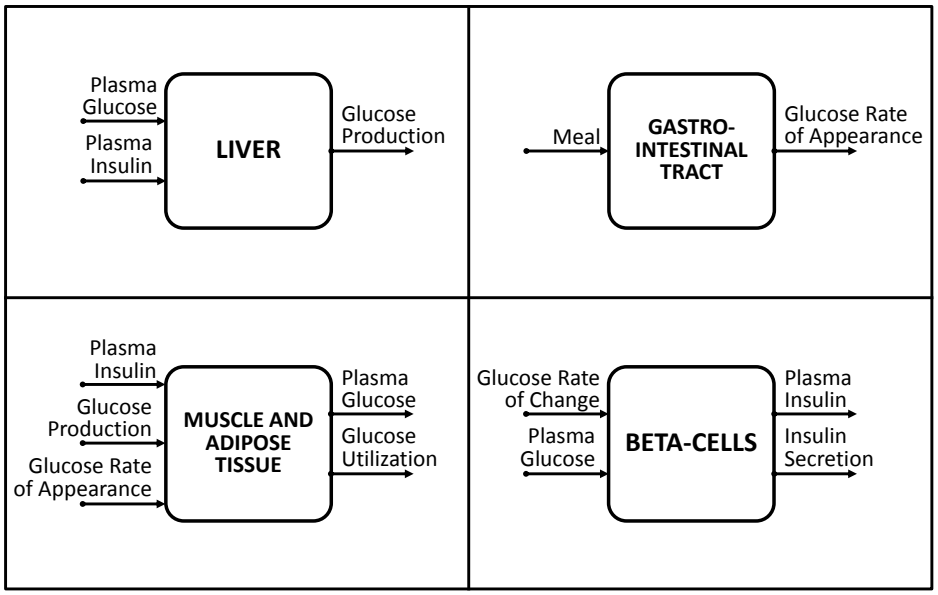


Figure 2.2: Unit process models and forcing function strategy: EGP (*top left panel*); Ra_{meal} (*top right panel*); U (*bottom left panel*); S (*bottom right panel*). Entering arrows represent forcing function variables, outgoing arrows are model output.

parameter estimates in the population. Both the average vector and the covariance matrix were calculated from the parameter estimates, thus defining the joint distribution of model parameters. The rationale is illustrated in Figure 2.3. It is worth noting that model parameters describing insulin secretion are substituted by k_d, k_{a1} and k_{a2} (appearing in equations (2.20) and (2.22)), which are assumed uncorrelated from the other model parameters, since they are not estimated but fixed to population values taken from the literature [21]. This is not in contrast with the physiology of the system (subcutaneous insulin absorption in T1DM, at variance with insulin secretion in healthy, is independent on subject's insulin sensitivity or other parameters determining glucose control).

2.2.3 In silico populations

The in silico T1DM population is made up of 100 adults, 100 adolescents, and 100 children. Each in silico subject is represented by a vector containing subject-specific model parameters, which has been generated by randomly

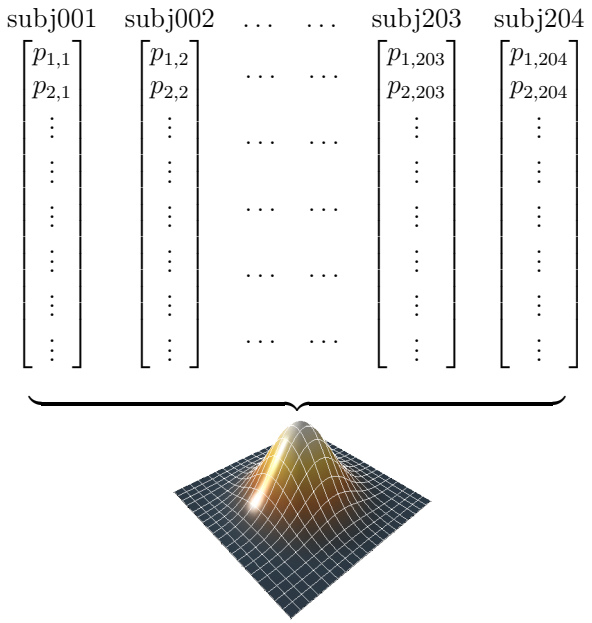


Figure 2.3: Scheme of the procedure used to generate the joint parameter distribution: the identification of all the unit processes provide a vector of model parameters for each subject; the set of all parameter vectors constitutes the parameter distribution, from which both the average vector and the covariance matrix are extracted.

extracting one realization of the model parameter vector from the parameter joint distribution described above.

In addition to the model parameters, to each virtual subject is associated an optimal insulin therapy, described by three key parameters: the carbohydrate-to-insulin ratio (CR), the insulin basal rate, the total daily insulin (TDI), and the insulin correction factor (CF). CR (g/U) determines the amount of insulin required to cover the charbohydrates (CHO) contained into a certain meal. It is calculated as the largest bolus in insulin units per grams of CHO that does not create a drop in plasma glucose lower than 95% of fasting plasma glucose after a meal containing 50 g of CHO. Insulin basal rate (U/hr) is the rate of insulin infusion required to maintain the subject at basal state during fasting conditions. TDI is computed based on a 200 g CHO daily diet, using a basal insulin infusion rate maintaining fasting glucose and the CHO ratio.

2.3 T1DM simulator 2013

The updated version of the UVA/Padova T1DM simulator (S2013) presents some important improvements with respect to S2008, both on the model on which the simulator is based, but also on the joint parameter distribution, the definition of clinically relevant parameters, and the strategy for in silico subjects generation.

2.3.1 Model improvements

With respect to the previous version, S013 model, showed in Figure 2.4, includes some modifications. A model of glucagon kinetics, secretion, and action, is added in order to account for counterregulation. In particular,

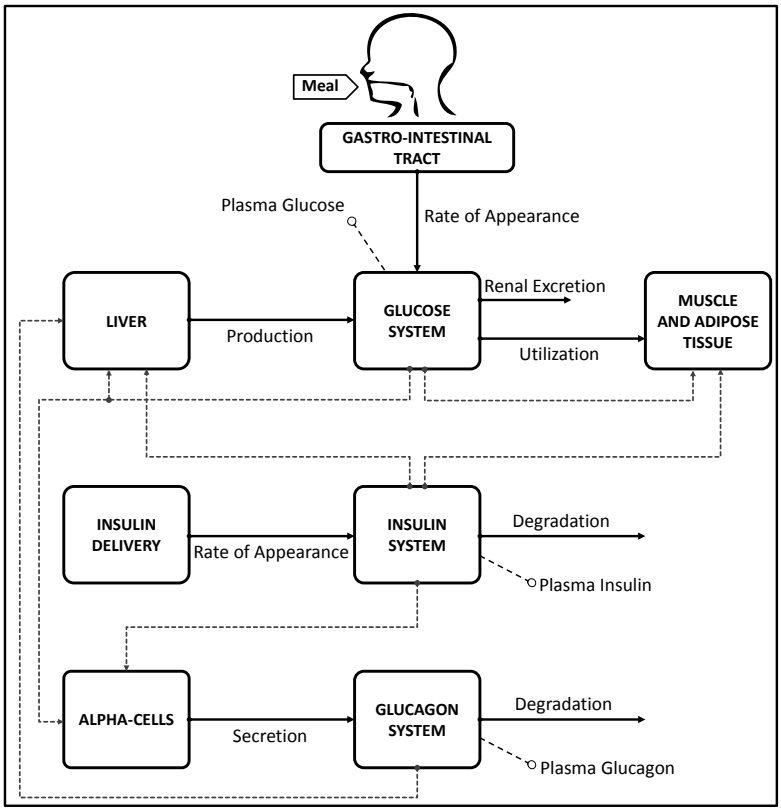


Figure 2.4: Scheme of the S2013 T1DM simulator.

glucagon kinetic consists of a single-compartment model:

$$\dot{H}(t) = -n \cdot H(t) + SR_H(t) \qquad H(0) = H_b \tag{2.23}$$

where H (ng/L) is the plasma glucagon concentration; suffix b denotes basal state; SR^H (ng/L/min) is the glucagon secretion; and n (min^{-1}) is the clearance rate. Glucagon secretion is described as the sum of two components, i.e. one static and one dynamic:

$$SR_H(t) = SR^s_H(t) + SR^d_H(t) \tag{2.24}$$

Static secrection (SR^s_H, ng/L/min) is described as follows:

$$\dot{SR}^s_H(t) = \begin{cases} -\rho \cdot \left[SR^s_H(t) - \max(\sigma_2[G_{th} - G(t)] + SR^b_H, 0) \right] & \text{if } G(t) \geq G_b \\ -\rho \cdot \left[SR^s_H(t) - \max\left(\frac{\sigma[G_{th} - G(t)]}{I(t)+1} + SR^b_H, 0 \right) \right] & \text{if } G(t) < G_b \end{cases} \tag{2.25}$$

where G and I are the plasma glucose and insulin concentrations; suffix b denotes basal state; σ and σ_2 (ng/L/min per mg/dL·L/pmol) are α-cell responsivities to glucose level, ρ (min^{-1}) is the rate parameter accounting for the delay between static glucagon secretion and plasma glucose. In this way, static secretion is stimulated when $G < G_b$ (but modulated by insulin) and inhibited when $G \geq G_b$. On the other hand, dynamic secretion (SR^d_H, ng/L/min) is related to the glucose rate of change:

$$SR^d_H(t) = \delta \cdot \max\left(-\frac{dG(t)}{dt}, 0 \right) \tag{2.26}$$

where δ (ng/L·mg/dL) is the α-cell responsivity to glucose rate of change. It is worth noting that, in real life, glucagon secretion is almost certainly dependent on insulin level in the α-cells (paracrine effect), not in the circulation. However, due to the difficulty to model the intrapancreatic levels, the use of plasma insulin, even if not perfectly physiologic, appears the the best surrogate. The model of EGP has been updated, so that equation (2.7) becomes:

$$EGP(t) = k_{p1} - k_{p2} \cdot G_p(t) - k_{p3} \cdot X^L(t) + \xi \cdot X^H(t) \qquad EGP = EGP_b \tag{2.27}$$

$$\dot{X}^H(t) = -k_H \cdot X^H(t) + k_H \cdot \max[(H(t) - H_b), 0] \qquad X^H(0) = 0 \tag{2.28}$$

where X^H (ng/L) is the delayed glucagon action on EGP, ξ (mg/kg/min per ng/L) is the liver responsivity to glucagon, and k_H (min^{-1}) is the rate parameter accounting for the delay between glucagon concentration and action.

Another important change in the simulator model concerns the description of glucose dynamics in hypoglycemia. In fact, the model of glucose utilization of equations 2.13 and 2.14 is unable to well describe the hypoglycemic range likely due to an inadequate description of insulin action, which paradoxically increases when glucose decreases under a certain threshold. This phenomenon has been described during hyperinsulemic clamps in T1DM [47, 51]. Based on this observation, a new model has been developed, which assumes that insulin-dependent utilization U_{id} increases when glucose decreases below a certain threshold, following the blood glucose risk function [50]:

$$U_{id}(t) = \frac{[V_{m0} + V_{mx} \cdot X(t) \cdot (1 + r_1 \cdot risk)] \cdot G(t)}{K_{m0} + G_t(t)} \tag{2.29}$$

$$risk = \begin{cases} 0 & \text{if } G \geq G_b \\ 10 \cdot [f(G)]^2 & \text{if } G_{th} \leq G < G_b \\ 10 \cdot [f(G_{th})]^2 & \text{if } G < G_{th} \end{cases} \tag{2.30}$$

$$f(G) = \log\left(\frac{G}{G_b}\right)^{r_2} \tag{2.31}$$

where G_b is the basal glucose, G_{th} is the hypoglycemic threshold (set at 60 mg/dL), and r_1 and r_2 are risk model parameters.

2.3.2 New joint distribution of model parameters

The incorporation of glucagon model into the simulator required also to update the parameter joint distribution. In particular, a different database

was considered [76], in which non-diabetic subjects received an intravenous insulin bolus with the aim to bring them in hypoglycemia (usually not achievable in healthy under physiological conditions), and EGP was estimated using a procedure similar to that described in *Chapter* 2.2.2. It is to note that covariance matrix related to counterregulation was added to the original one of S2008, maintaining them uncorrelated, since they were generated from different datasets.

2.3.3 New in silico population

The generation of model parameters was performed paralleling what described in *Chapter* 2.2.3. Some refinements have been introduced in the calculation of relevant clinical parameters. In particular, at variance with S2008, CR was determined with the following simulation, which mimics, as much as possible, the criterion used to empirically determine it in real patients: each subject receives 50 g of CHO, starting from his basal level. The optimal insulin bolus is determined so that: (1) glucose concentration, measured 3 hours after the meal, is between 85% and 110% of its basal level; (2) the minimum glucose concentration is above 90 mg/dL; and (3) the maximum glucose concentration is between 40 and 80 mg/dL above the basal level. CR is then calculated as the ratio between the amount of ingested CHO and the optimal insulin bolus:

$$\mathrm{CR} = \frac{\text{ingested CHO}}{\text{optimal bolus}} \tag{2.32}$$

CF was determined with the so-called 1700 rule [24],

$$\mathrm{CF} = \frac{1700}{\mathrm{TDI}} \tag{2.33}$$

where TDI is the total daily insulin, determined for each virtual patient, using optimal CR and basal infusion rate, and assuming an average diet of 180 g of CHO for adolescents and adults and 135 g of CHO for children.

Finally, also new criteria for the generation of the in silico subjects have been introduced. In particular: (1) CR $\leq$ 30 g/U for adult and adolescents

and CR $\leq$ 40 g/U for children; (2) steady state glucose in absence of insulin infusion $>$ 300 mg/dL; and (3) Mahalanobis distance [65] lower than that corresponding to the 95% percentile.

2.4 Limitations of S2013

As already stated at the end of *Chapter* 1.1.3, even though the UVA/Padova T1DM simulator was largely used for the in silico testing of AP algoritms, when the present research started, there were some criticisms that had to be addressed.

First of all, as described in *Chapter* 2.2.2, the simulator core was not directly derived from T1DM data, i.e. the parameter joint distribution was extracted from non-diabetic individuals [3]; in this regard, *Chapters* 4 and 5 describe the validation of the simulator against T1DM populations observed in clinical trials.

Secondly, the simulator was developed on single meal data. So far, AP controllers have been successfully tested in the short period, so that this did not represent a big issue. Nevertheless, at the beginning of this thesis, the AP research envisioned the possibility to extend AP testing in long-term trials – and, indeed, it was recently achieved, as described in [53, 55]. Hence, the simulator has to be able to well describe the possible variations that can occur during the day, in order to provide an appropriate test bed for the preclinical phase. In other words, to better describe the physiology, the simulator must be time-varying (Figure 2.5).

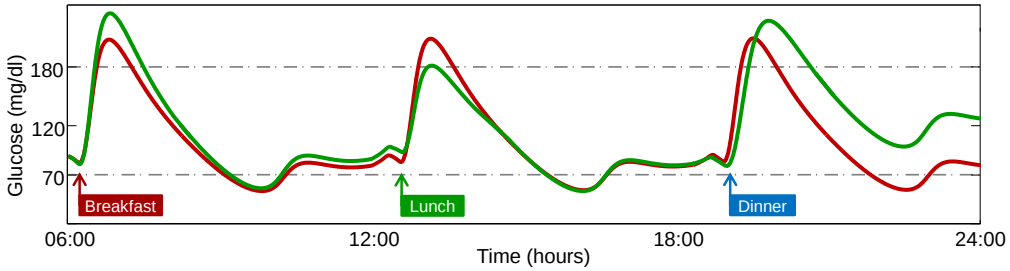


Figure 2.5: Comparison of a time-invariant (*red line*) vs. a time-varying (*green line*) simulation scenario, obtained in an illustrative subject undergoing three identical meals. As it can be observed, with the current time-invariant simulator the glucose dynamic exhibits the same pattern during the day. On the contrary, a time-varying simulator would allow the introduction of a certain glycemic variability, similarly to what occurs in real life.

3 Databases and protocols

3.1 Introduction

In this chapter the data bases and protocols used in this work are described.
In particular, three different datasets have been used, each one having the characteristics needed for a particular purpose: *Database1* is employed for the clinical assessment of the state-of-art simulator (*Chapter* 4); *Database2* is employed for model validation (*Chapter* 5); *Database3* is used to develop the intra-subject variability model (*Chapter* 6).

3.2 Database 1

The database used for model assessment consists of 24 T1DM adult subjects (14 males, age = 42.8±11.9 years, BW = 74.8±13.6 kg) [44], recruited at the Universities of Virginia, Charlottesville ($N = 11$), Padova, Italy ($N = 7$), and Montpellier, France ($N = 6$).
Each patient had two 22-hour hospital admissions (from 3:00 p.m. to 1:00 p.m. on the following day), one in open- and one in closed-loop, respectively. During the open-loop session, the subject-specific basal-bolus therapy was used with the personal insulin pump. During the closed-loop session, an

OmniPod® Insulin Management System (Insulet Corp.) was used, and the insulin infusion and the pre-meal bolus were managed by a control algorithm that was initiated at 5:00 p.m. in a data-collection mode and used from 9:30 p.m. to 12:00 a.m..

In both admissions subjects received dinner (70.7 ± 3.3 g of CHO) between 6:00 p.m. and 7:00 p.m. and breakfast (52.9 ± 0.1 g of CHO) between 7:00 a.m. and 8:00 a.m.. For each subject, meals were the same in the two admissions. A scheme of the protocol design is shown in Figure 3.1. Throughout the admissions, venous blood samples were collected every 30 min right after each meal ingestion for measurements of plasma glucose and insulin concentrations, or every 15 min if hypoglycemia was occurring or imminent.

Plasma glucose was measured using YSI^{TM} 2300 STAT Plus Analyzer (YSI Inc., Yellow Springs, OH, USA). As it will be discussed in *Chapter* 4.1, for the specific purpose of the thesis to be achieved using this dataset (i.e. the clinical assessment of the simulator, see *Chapter* 4), it is not needed to keep the data of each admission separately, since the results will be not dependent from the algorithm used in the trial. Thus, average time courses of plasma glucose and insulin are shown aggregated in Figure 3.2. A detailed description of the clinical protocol is reported in [44].

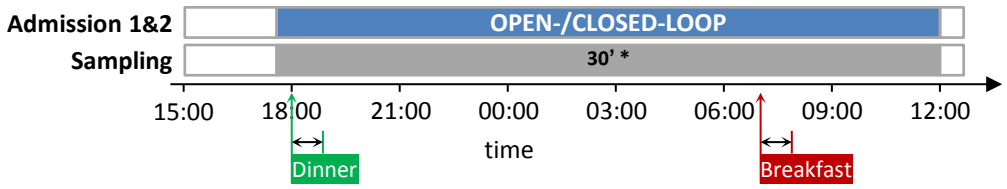


Figure 3.1: Overview of the admission day. Patients underwent open- or closed-loop and were served dinner and breakfast.
*: sampling time reduced to 15 min if hypoglycemia was occurring or imminent.

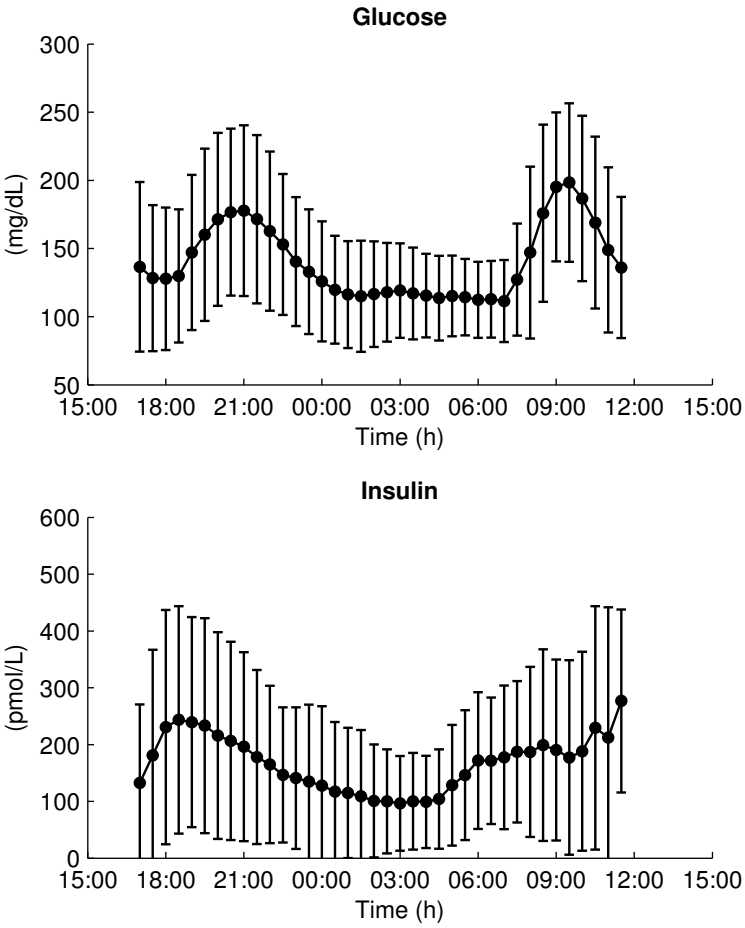


Figure 3.2: Average time courses of plasma glucose (*upper panel*) and insulin (*lower panel*) concentrations measured in T1DM subjects (N = 48). Vertical bars represent the standard deviation SD.

3.3 Database 2

Database used to validate the T1DM model consists of 47 T1DM subjects (33 males; age = 42.0 ± 10.1 years, BW = 77.5 ± 13.4 kg) [58], recruited in six clinical centers (Academic Medical Center Amsterdam, NL (N=7); CHRU Montpellier, FR (N=8); Medical University of Graz, AT (N=8); Profil Institute for Metabolic Research GmbH, GER (N=8); University of Cambridge, UK (N=8); University of Padova, IT (N=8)), within the AP@home FP7-EU project. Subjects underwent three randomized 23-hour admissions, i.e. one open- and two closed-loop sessions. During the open-loop admission, subjects had their usual insulin therapy through an insulin pump, while the insulin infusions were managed by a control algorithm during the closed-loop admissions. For each admission, subjects received standard solid meals at dinner D (7:00 p.m., Day 1), breakfast B (8:00 a.m., Day 2) and lunch L (12:00 a.m., Day 2), respectively containing 80g, 50g and 60g of CHO, and did a moderate physical activity session (3:00 p.m., Day 2). A scheme of the protocol design is shown in Figure 3.3. Throughout the admissions, venous blood samples were collected for measurements of plasma glucose and insulin concentrations every 15 min in the first 2 hours after each meal, every 1 hour at night and every 30 min elsewhere.
Plasma glucose was measured using YSI^{TM} 2300 STAT Plus Analyzer (YSI Inc., Yellow Springs, OH, USA) and plasma insulin was measured using an insulin chemiluminescence assay (Invitron Ltd, Monmouth, UK) (The Institute of Life Sciences, Swansea University, S. Luzio). A detailed description of the clinical protocol is reported in [58]. It is worth noting that the analysis conducted with this dataset to validate the T1DM model (see *Chapter* 5) is not dependent from the admissions. Thus, also in this case, average measures of plasma glucose and insulin are plotted together in Figure 3.4.

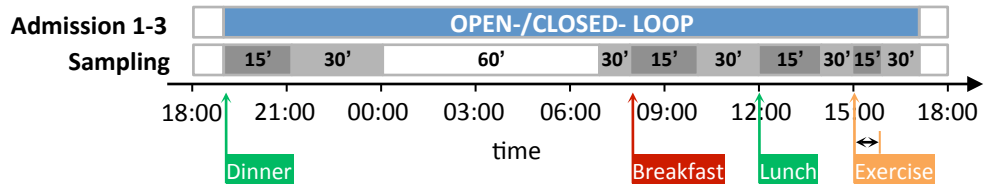


Figure 3.3: Overview of the admission day. Patients underwent open- or closed-loop and received dinner, breakfast and lunch. A 30-min session of physical exercise started at 3:00 p.m..

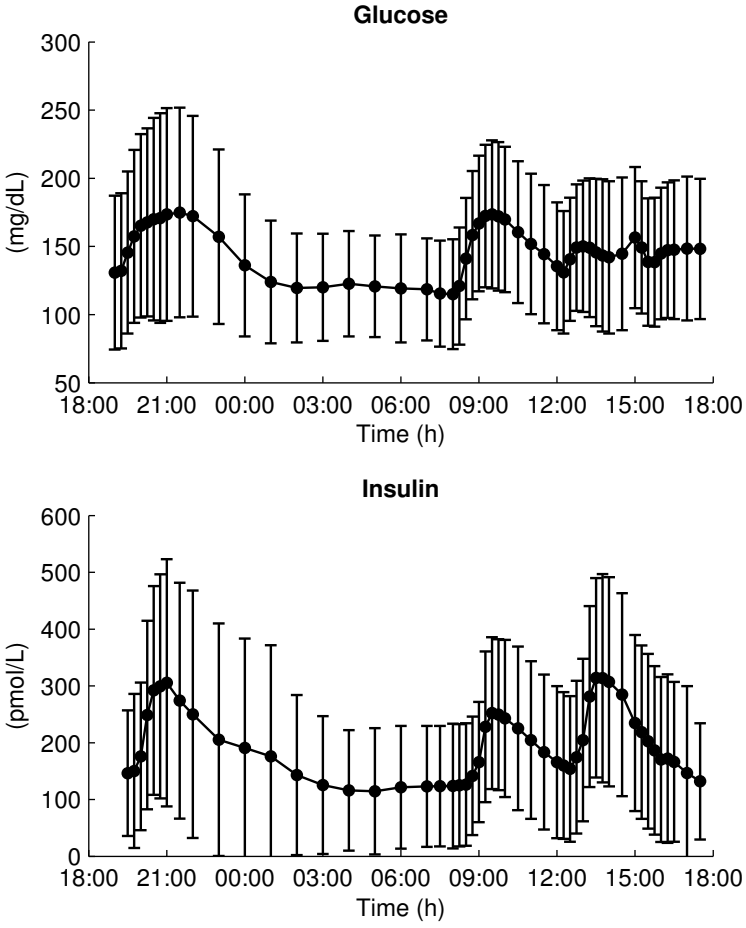


Figure 3.4: Average time courses of plasma glucose (*upper panel*) and insulin (*lower panel*) concentrations measured in T1DM subjects (N = 141). Vertical bars represent the standard deviation SD.

3.4 Database 3

The database employed to develop the model of intra-subject diurnal variability of insulin sensitivity consists of 20 T1DM subjects (11 males; age = 42.9 ± 14.4 years; BW = 74.7 ± 15.4 kg; CR = 8.6 ± 2.1 g/U) [34], who were admitted for a 3-day study in the Clinical Research Unit of the Mayo Center for Clinical and Translational Science (Rochester, MN).
In brief, once a day, a triple-tracer mixed-meal study protocol [4] was performed during breakfast (B), lunch (L), or dinner (D) in a randomized Latin square design (see Table 3.1 for an example), with identical meal composition (50 g of CHO). Blood samples were collected at t = -180, -30, 0, 5, 10, 20, 30, 60, 90, 120, 150, 180, 240, 300, and 360 min, with $t = 0$ corresponding to the timing of the meal, for measurement of plasma glucose and insulin concentrations.
Plasma glucose was measured using YSI^{TM} 2300 STAT Plus Analyzer (YSI Inc., Yellow Springs, OH, USA) and plasma insulin was measured by a two-site immunoenzymatic assay performed on the DxI automated immunoassay system (Beckman Coulter Inc., Chaska, MN). A detailed description of the clinical protocol is reported in [34]. Since this dataset will be used to assess the intra-subject diurnnal variability of insulin sensitivity (see *Chapter* 6), it is important to highlight the possible differences existing in plasma concentrations measured at B, L and D. Thus, in this case, average time courses of plasma glucose and insulin at B, L and D are shown separately in Figure 3.5.

Table 3.1: Latin Square Randomization

	Day 1	Day 2	Day 3
Breakfast		§	
Lunch			§
Dinner	§		

Randomization order in an illustrative subject: "§" means that the triple-tracer approach is used. In this example, the triple-tracer is used at dinner of Day 1, breakfast of Day 2, and lunch of Day 3.

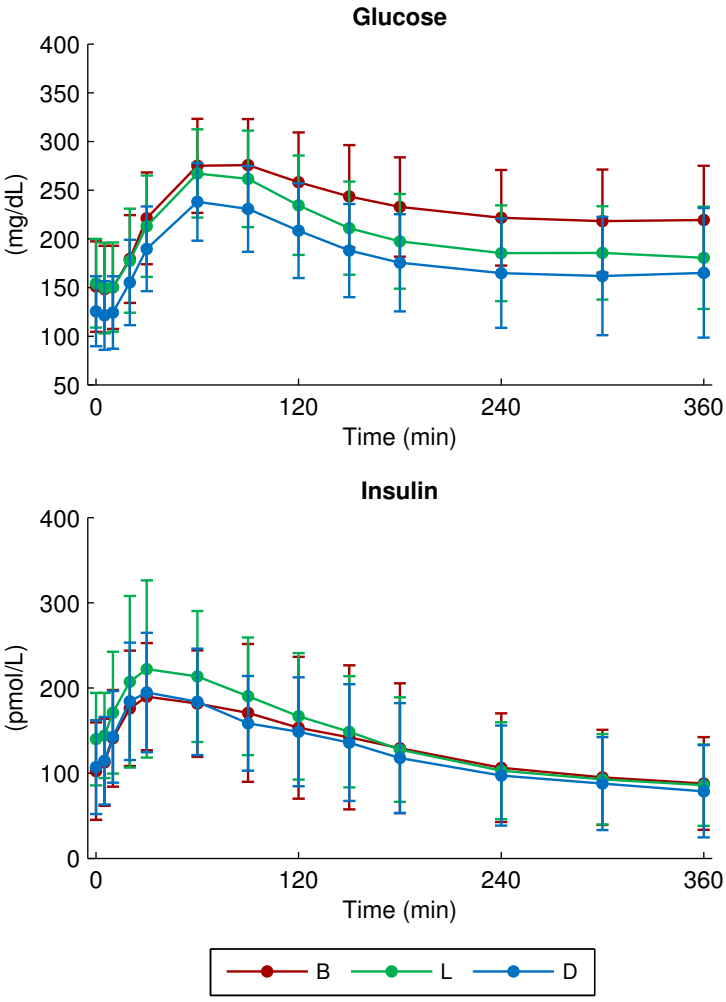


Figure 3.5: Average time courses of plasma glucose (*upper panel*) and insulin (*lower panel*) measured at breakfast (B), lunch (L), and dinner (D) in T1DM subjects (N = 20). Vertical bars represent the standard deviation SD.

4 Clinical Assessment of the T1DM Simulator

4.1 Introduction

As already stated in *Chapter* 1.1.3, the UVA/Padova T1DM Simulator has been successfully used by several research groups in academia, as well as by companies active in the field of T1DM. However, its validity was never been validated against T1DM data obtained in clinical trials. In this chapter, the clinical assessment of the UVA/Padova T1DM Simulator is presented. Briefly, both the first (S2008) and the last (S2013) versions of the simulator were assessed against a real T1DM population by undergoing the in silico adults to the same protocol of the real subjects, i.e. the virtual subjects received the same meal and insulin delivery of the real ones. The procedures are described in details in the following sections.

The experimental data used for the clinical assessment of the simulator are those of *Database1* described in *Chapter* 3.2. In particular, because the virtual subject receives the same insulin amount used by the real subject, the results are in principle independent from the algorithm used in the trial, so that no difference between study admissions are considered. Moreover, each glucose trace is subdivided into post-dinner (from dinner ingestion to 7 h later), overnight (from 5 h after dinner to the beginning of breakfast), and post-breakfast (from breakfast ingestion to 5 h later) portions, so that a total of 96 post-meal traces are considered.

4.2 Model assessment

The simulator assessment aims to prove the following statements:

1. For each real T1DM subject, a virtual subject exists who, if undergoing the same experimental scenario (i.e., same meal CHO amount, insulin boluses, and basal pattern, given at the same time), behaves similarly to the real one from a clinical point of view, i.e. it shows a similar pattern and lies in the same clinically relevant zones, as illustrated in Figure 4.1. In particular, the clinical relevant zones are the hypo-, eu-, and hyper-glycemc zones, for which blood glucose levels are < 70 mg/dL, between $[70 - 180]$ md/dL, and > 180 mg/dL, respectively.
2. The distribution of the most important clinical outcome metrics in the simulated traces reproduces those observed experimentally.

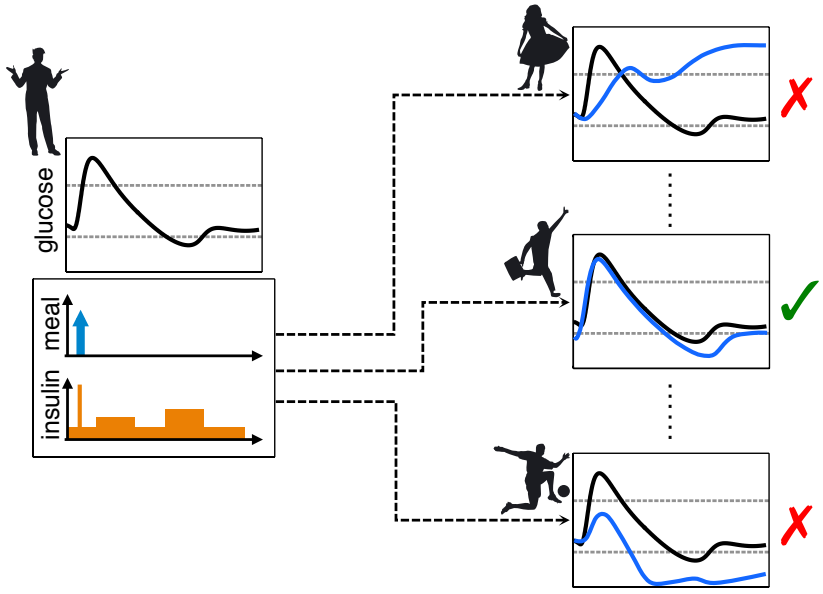


Figure 4.1: Matching criterion: a real T1DM subject, receiving a certain amount of CHO with a meal and insulin basal infusion and bolus, exhibits a hyperglycemic glucose peak after meal and a hypoglycemic event during the second half of the experiment (*left side*). Among the 100 in silico subjects receiving the same meal and insulin amounts (*right side*), the one showing the best agreement in exploring the clinical zones is selected as best match.

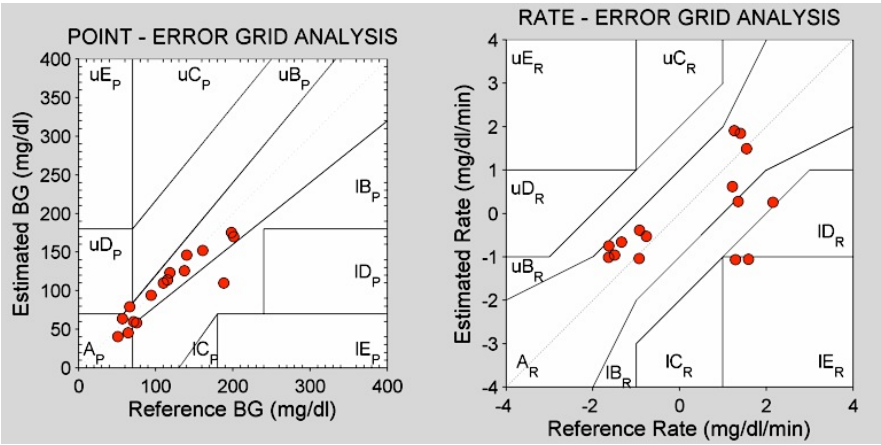
To test the first requirement, each measured plasma glucose profile is compared with those simulated in the 100 in silico adults, who undergo the same experimental scenario (meals, basal insulin, and boluses). In particular, for each pair real-virtual subject an index (FIT), quantifying the agreement between real and virtual glucose trace, is calculated as:

$$\text{FIT} = 1 - \sqrt{\frac{\sum_{k=1}^{N}(G^{meas}(t_k) - G^{sim}(t_k))^2}{\sum_{k=1}^{N}(G^{meas}(t_k) - G^{mean}(t_k))^2}} \tag{4.1}$$

where G^{meas} is the measured and G^{sim} is the simulated blood glucose concentration, G^{mean} is the average measured glucose, and N is the number of samples. Among the 100 simulated profiles, the one providing the best FIT is selected and compared with the real glucose profile by using the Continuous Glucose Error Grid Analysis (CG-EGA) [48]. The CG-EGA method was originally developed for the clinical evaluation of CGM sensors in terms of both accurate glucose readings and accurate direction and rate of glucose fluctuations. In brief, CG-EGA compares the CGM profile with the reference blood glucose (BG) and provides a point-error grid analysis (P-EGA) [10], combined with a rate-error grid analysis (R-EGA), and an error matrix (EM). The P-EGA and the R-EGA plot CGM vs. BG and CGM rate of change vs. BG rate of change, respectively, on a plane divided into specific zones, which accounts for the dangerousness of erroneous readings in relation to the actual glucose level [48][46]. The EM summarizes the results of the analysis, reporting the percentage of accurate readings, benign errors, and erroneous readings of the P-EGA and R-EGA. Here this tool is used to compare glucose measurements (G^{meas}) against the simulations (G^{sim}). An example is reported in Figure 4.2.

To test the second requirement, the distributions of the most popular clinical outcome metrics obtained in real and simulated experiments, are compared. In particular, glucose means and intrasubject interquartile range (IQR), Low and High Blood Glucose Indices (LBGI and HBGI, representing two risk statistics related to the individual glycemic control in the hypo- and hyper- glycemic range [46],[45]), the percentage of BG < 70 mg/dL (hy-

poglycemia) and BG > 180 mg/dL (hyperglycemia), the percentage of time in which BG < 70 mg/dL (hypoglycemia) and BG > 180 mg/dL (hyperglycemia), the area between 70 mg/dL and the glucose curve when BG < 70 mg/dL (hypoglycemia), the area between 180 mg/dL and the glucose curve when BG > 180 mg/dL (hyperglycemia), and the number of hypoglycemic and hyperglycemic events are evaluated. Two sample comparisons are performed using the paired t test, for normally distributed variables, and the Wilcoxon signed rank test, for variables not-normally distributed, both with the significance level set at $P = 0.05$.



Rate Error - Grid Zones	Point Error - Grid Zones										
	Hypoglycemia			Euglycemia			Hyperglycemia				
	A	D	E	A	B	C	A	B	C	D	E
A	75.0%	0.0%	0.0%	87.5%	0.0%	0.0%	33.4%	0.0%	0.0%	0.0%	0.0%
B	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	33.3%	33.3%	0.0%	0.0%	0.0%
uC	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
lC	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
uD	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
lD	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
uE	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
lE	25.0%	0.0%	0.0%	12.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Figure 4.2: PP-EGA and R-EGA in an illustrative case (*upper panel*): as it can be observed, P-EGA provides all points residing in A zone except for one, which is in the low-B zone; R-EGA provides two points in low-E zone, two in low-B, and the remaining in A zone; the resulting EM is provided in *lower panel*, where accurate (in white), benign (light blue), and erroneous points (dark blue) are shown.

4.3 Results

Figure 4.3 shows the comparison between plasma glucose data measured in one T1DM subject and simulated profiles obtained with the S2013 (*left panel*) and S2008 (*right panel*) in one illustrative case. The S2013 well reproduces the glucose patterns observed in clinical trials in all clinical zones, in particular the rapid falls in glucose levels and the hypoglycemic episodes. The simulated profiles obtained with the S2008 reproduce quite well the data in euglycemia and hyperglycemia but not the hypoglycemic episodes.

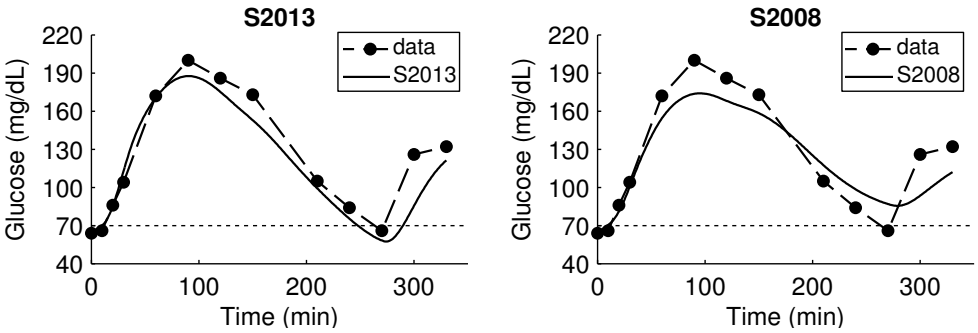


Figure 4.3: Data Vs. simulations in one representative subject: S2013 (*left panel*) vs. S2008 (*right panel*). Hypoglycemic threshold (70 mg/dL) is depicted as dotted line.

The average results of CG-EGA of the S2013 (Table 4.1, *first row*) are very good in hypo-, eu-, and hyper-glycemia (percentage in accurate + benign zones: 86.4% in hypoglycemia, 99.9% in euglycemia and 99.2% in hyperglycemia). The results of the S2008 (Table 4.1, *second row*) show that the performance is similar to that of S2013 in eu- and hyper-glycemia (percentage in accurate + benign zones: 99.8% in euglycemia and 99.5% in hyperglycemia) but worse in hypoglycemia (percentage in accurate + benign zones: 40.7%). The improvement presented above is confirmed by the analysis of the distribution of outcome metrics, reported in Table 4.2. The outcome metrics of S2013 simulations (Table 4.2, *second column*) are not statistically different from those obtained from the data, except for the percentage of time in hypoglycemia and the area under the BG curve for hypoglycemia. For the S2008 (Table 4.2, *third column*), most of the outcome metrics are significantly lower in simulation ($P < 0.05$).

Table 4.1: Continuous Glucose Error Grid Analysis: S2013 vs. S2008

	Hypoglycemia			Euglycemia			Hyperglycemia		
	Accurate	Benign	Bad	Accurate	Benign	Bad	Accurate	Benign	Bad
S2013	85.9%	0.5%	13.5%	98.8%	1.1%	0.1%	99.2%	0.0%	0.8%
S2008	40.7%	0.0%	59.3%	98.3%	1.5%	0.2%	99.5%	0.0%	0.5%

Average results of continuous glucose error grid analysis on measurements and simulations obtained with the two simulators.

Table 4.2: Outcome Metrics

		Simulator	
	Data	S2013	S2008
Blood glucose (mg/dL)			
Mean	156.9 ± 41.3	157.3 ± 43.3 (NS)	155.9 ± 42.4 (NS)
IQR	71.2 [50.9-96.1]	66.0 [47.8-89.2] (NS)	57.3 [33.1-82.5] (<0.001)
LBGI	0.59 [0.02-2.22]	0.36 [0.00-3.00] (NS)	0.23 [0.00-1.20] (<0.001)
HBGI	4.85 [1.79-8.34]	4.63 [1.60-8.24] (NS)	4.23 [1.19-7.86] (<0.001)
Percentage of values in			
Hypoglycemia	6.47 ± 10.19	7.98 ± 13.21 (NS)	3.59 ± 9.04 (0.001)
Hyperglycemia	28.78 ± 24.75	27.64 ± 24.93 (NS)	27.49 ± 26.98 (NS)
Percentage of time in			
Hypoglycemia	4.04 ± 7.93	6.22 ± 11.81 (0.006)	2.52 ± 7.99 (0.020)
Hyperglycemia	33.90 ± 29.02	33.44 ± 30.99 (NS)	32.71 ± 32.28 (NS)
Area under the curve (mg·h/dL)			
Hypoglycemia	2.65 ± 8.99	5.00 ± 14.50 (0.019)	1.05 ± 3.97 (0.023)
Hyperglycemia	83.23 ± 111.15	80.82 ± 112.44 (NS)	70.96 ± 110.43 (<0.001)
Number of events			
Hypoglycemia	37	32	19
Hyperglycemia	72	67	61

For the comparison between data and simulations, values are expressed as mean ± SD for normally distributed variables (P value from the paired t-test) or median [interquartile range (IQR)] for non-normally distributed variables (P value from the Wilcoxon signed rank test). Low Blood Glucose Index (LBGI) and High Blood Glucose Index (HBGI) are dimensionless. NS, not significant ($P > 0.05$).

4.3.1 Comments to results

The results presented above proves the validity of the S2013 model in terms of ability to reproduce the glucose variability observed during a clinical trial. However, there are some aspects that have to be taken into account. First of all, the proposed method finds the in silico subject that best matches (from a clinical point of view) the observed glucose trace, without any concern about the similarity of physiological parameters, such as the body weight or the CR. In these regards, the percent difference between subject and virtual parameters was 18% on average for body weight and 67% for CR. The higher difference found for CR is due to the fact that the virtual CR (CR^{sim}) is the "optimal" one for a given subject. Thus, when doing the comparison, one should take into account the postprandial glycemic control obtained in the experiment: for instance, if CR^{sim} is higher than the real one (CR^{real}), but (CR^{real}) makes the subject experience hypoglycemia, one should have administered less insulin (i.e., applying a higher CR for that meal); conversely, if the CR^{sim} is lower than the real one, but the (CR^{real}) makes the subject experience hyperglycemia, one should have administered more insulin (i.e., applying a lower CR for that meal). This analysis revealed that a difference between real and virtual CR grater than 50% was found in 23 subjects. However, 14 of them would have benefitted by using a CR closer to the virtual one, potentially avoiding hypo- or hyper-glycemia (an illustrative case is shown in Figure 4.4).

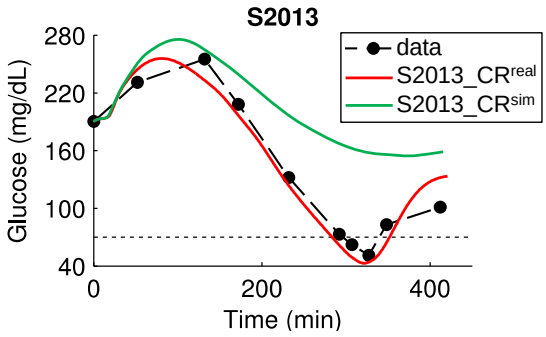


Figure 4.4: Glucose response using CR^{real} (red) vs. CR^{sim} (green) in one illustrative case. By applying the real insulin amount, i.e. using CR^{real} (= 7 g/U), the subject experiences hypoglycemia. Conversely, the subject would have benefitted by using CR^{sim} (= 15 g/U), avoiding hypoglycemia.

The matching criterion was also applied limiting the search to the in silico subjects with CR similar to the real one. In this case a match was always found except for eight traces, which, however, presented hypoglycemia, and thus the corresponding CR were too agressive, as explained above. This procedure also provided similar results in terms of CE-EGA.
Moreover, the assessment was done by assuming no relation between the post-prandial portion of the same subject (i.e. post-dinner and post-breakfast, in both study admissions). Indeed, a single in silico subject was unable to simultaneously match post-prandial glucose profile at dinner and breakfast. This was in some sense expected, because the model of the simulator was, at the time of the development of this method, not able to describe glucose variability in a multiple-meal scenario. This outcome suggests that different meal composition results in different glucose traces and/or time varying parameters (e.g. insulin sensitivity) should be included in the simulator to better fit the physiology. These are specifically the aims of the following two chapters.

The method to assess the simulator against T1DM data, as well as the obtained results, have been published in [95].

5 Validation of the T1DM model

5.1 Introduction

The clinical assessment of the simulator, presented in *Chapter* 4, showed that, for each T1DM patient of *Database1*, an in silico subject exists who clinically matches the real data, thus proving that the virtual subjects are representative of a T1DM population observed in a clinical trial. This was an important feature of the simulator to be used in preclinical trials. However, this does not demonstrate the goodness of the model nor the adequacy of the joint parameter distribution included into the simulator. In addition, the fact that two different in silico subjects were required to well match the post-dinner and post-breakfast glucose traces of the same real subject means that a good simulation model should at least take into account that different meal compositions may product different glucose profiles. Moreover, accounting for circadian variability of important parameters, like insulin sensitivity (SI), i.e. a parameter that quantifies the effect of insulin to stimulate the whole-body (liver and periphery) glucose disposal and inhibit the glucose production, may be needed.

In this chapter, the simulator model is validated by fitting it, for the first time, against data of a large number of T1DM subjects, who underwent three 23-hour admissions and received breakfast, lunch and dinner containing different amounts of CHO (*Database2*, *Chapter* 3.3). However, the available

data consists of only plasma glucose and insulin measurements and meal CHO contents. This precludes the possibility to identify the model with the approach described in *Chapter* 2.2.2: in fact, given the lack of glucose fluxes, i.e. meal glucose rate of appearance (Ra_{meal}), endogenous glucose production (EGP), glucose utilization (U), renal excretion (E), it is not possible to univocally identify all the single processes of the model with weighted least squares or maximum likelihood identification techniques. On the other hand, Bayesian parameter estimation techniques can be used in such conditions, since they exploit a certain a priori statistical knowledge to supply the lack of data. As a matter of fact, in the following a Bayesian method to identify the S2013 model is presented. The natural choice was to exploit the parameter joint distribution employed for the generation of the in silico subjects of the simulator and use it as a priori knowledge.

5.2 The Bayesian estimator Maximum a Posteriori

Given the complexity of the model, the sole availability of plasma glucose and insulin data makes it impossible to reliably identify the model by using a weighted least squares or maximum likelihood estimator. In fact, one can obtain a good description of plasma glucose and insulin data with many different descriptions of the metabolic fluxes (i.e. Ra_{meal}, EGP, U and E); in other words, a good model fit can be achieved with several combinations of model parameters. To overcome this problem, a Bayesian approach is adopted, i.e. the estimation of the model parameter vector p takes account of both the information provided by the data vector z, i.e. the a posteriori information, and the knowledge on the a priori joint distribution of p, assumed independent form z. In particular, the Maximum a Posteriori (MAP) Bayesian estimator provides a point estimate p so that, once fixed z, the a posteriori probability density of p is maximum:

$$\hat{p}_{MAP} = \underset{p}{\operatorname{argmax}}\, f_{(p|z)}(p|z) \tag{5.1}$$

where

$$f_{(p|z)}(p|z) = \frac{f_{(z|p)}(z|p) f_p(p)}{f_z(z)} \tag{5.2}$$

with $f_p(p)$ denoting the a priori probability density of p, considered random, $f_z(z)$ the a priori probability density of z, and $f_{(z|p)}(z|p)$ the a posteriori probability density of z. Assuming that z is affected by measurement error v, Gaussian, with zero mean and covariance Σ_v, and p is extracted from a Gaussian distribution with mean μ_p and covariance Σ_p, equation (5.1) can be rewritten as:

$$\hat{p}_{MAP} = \underset{p}{\operatorname{argmin}} \left\{ [z - G(p)]^T \Sigma_v^{-1} [z - G(p)] + [p - \mu_p]^T \Sigma_p^{-1} [p - \mu_p] \right\} \tag{5.3}$$

with $G(p)$ denoting the model prediction. In other words, the first term in equation (5.3) represents the model fit, while the second term is the distance of the estimated parameters from their joint distribution. To guarantee the non-negativity of model parameter estimates, here the parameter distribution is assumed to be lognormal:

$$s = \log(p) \tag{5.4}$$

Therefore, the estimated parameter vector can be expressed as

$$\hat{p} = \exp(\hat{s}) \tag{5.5}$$

where

$$\hat{s} = \underset{s}{\operatorname{argmin}} \left\{ [z - G(\exp(s))]^T \Sigma_v^{-1} [z - G(\exp(s))] + [s - \mu_s]^T \Sigma_s^{-1} [s - \mu_s] \right\} \tag{5.6}$$

with μ_s and Σ_s denoting the mean and the covariance matrix in logaritmic form. More details on MAP estimation can be found in [12].

5.3 Model identification

The T1DM simulator does not yet account for the effect of physical activity on glucose dynamics. Thus, the model identification is performed using data of *Database2* [58] excluding physical exercise portions, which however occurred at the end of the experiment. Hence, the model is identified on 20-hour plasma glucose data using the MAP estimator. In order to avoid local minima, the minimization of the objective function is performed using a cascade of a direct search method followed by a gradient-based algorithm. Measurement error is assumed to be additive, Gaussian, with zero mean and constant coefficient of variation (CV) of 2%. Plasma insulin concentration is the model forcing function and is assumed to be known without error. Moreover, being glucagon measurements not available, the average glucagon model parameters are used. Interestingly, model identification provides, in addition to model parameters, an estimation of the main glucose fluxes, i.e. Ra_{meal} (equation (2.10)), EGP (equation (2.27)), U (equations (2.12),(2.29)) and E (equation (2.15)). However, when presenting the results, the net rate of disappearance Rd_{net} (defined as $Rd_{net} = U + E - EGP$) is shown since without glucose tracers, even with a strong prior, it could be difficult to separately estimate U, E and EGP contributes.

The a priori information in equation (5.6) is the joint parameter distribution used to generate the in silico adult population included into the T1DM simulator. To describe post-prandial glucose pattern at different times of the day, variability of parameters describing glucose absorption (i.e. k_{abs}, k_{max}, k_{min} in equations (2.10),(2.11)) is permitted, under the assumption that different meal composition at breakfast B, lunch L, and dinner D results in different absorption parameters. The other parameters are maintained constant throughout the day. However, with this setting, model fit to the data was not satisfactory (not shown). Therefore, variation in parameters describing SI (i.e. k_{p3} and V_{mx} in equations (2.27),(2.29)) is permitted. In particular, k_{p3} and V_{mx} are allowed to be different at B with respect to L and D, while L and D are set to be equal. These assumptions are based on the findings of a recent study [34], in which T1DM subjects exhibited a SI trend lower at

B compared to L and D. It is worth noting that, at variance with [34], here SI parameters at L and D are assumed to be the same in each subject, due to the small amount of samples collected between lunch and physical exercise. This was necessary to guarantee that k_{p3} and V_{mx} at L are estimated with good precision. As a matter of fact, this dataset was not adequate to test intra-day variability of SI parameters, due to both the short duration of the post-lunch data portion and the possible confounding effect introduced by different compositions and CHO contents of the meal (the assessment of daily variation of insulin sensitivity is tackeled in *Chapter* 6). The intra-day variability is implemented for each time-varying parameter as an almost step-wise-line signal that varies three times a day.

5.4 Results

5.4.1 Fit, Parameters and Fluxes

The model is identified in all the available 141 glucose traces, and well fits the glucose data, as proved by the average weighted residuals time course, shown in Figure 5.1. An example of the glucose model fit in a representative subject is shown in Figure 5.2, *upper panel*. In addition, the model-derived time courses of Ra_{meal} and net rate of disappearance Rd_{net} are provided (Figure 5.2 *middle* and *bottom panels*).

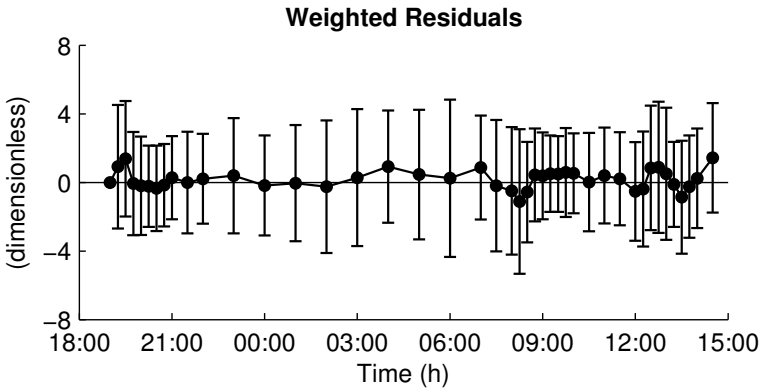


Figure 5.1: Average weighted residuals (vertical bars represent standard deviation (SD)) of model fit on plasma glucose data (N = 141).

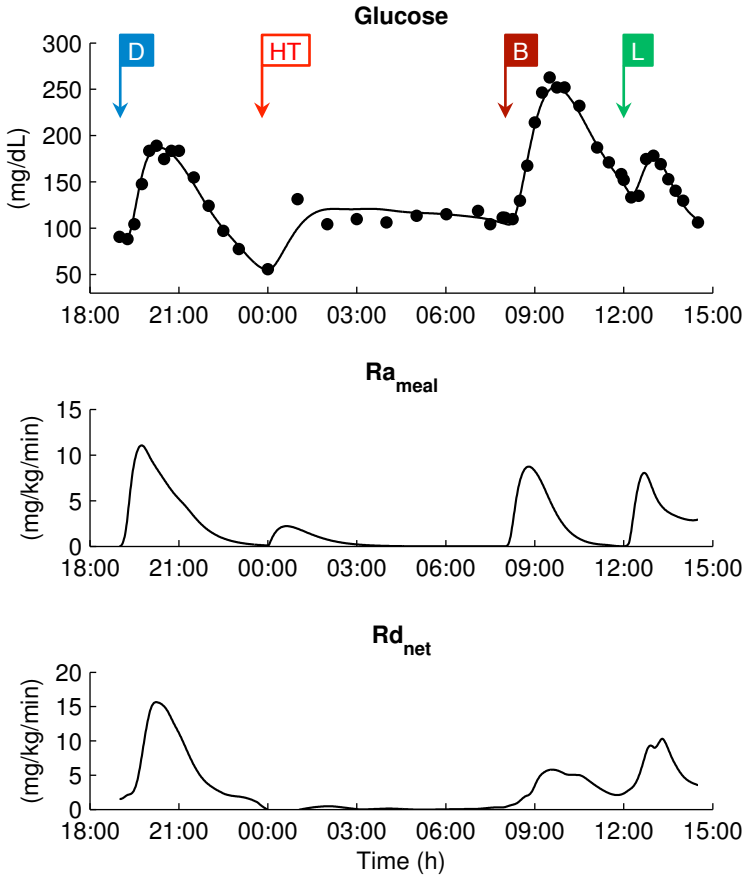


Figure 5.2: Model prediction vs. plasma glucose concentration (*upper panel*) and the corresponding glucose fluxes, i.e. Ra_{meal} (*middle panel*) and Rd_{net} (*lower panel*), provided by the model in an illustrative subject. Data are indicated with dots, while model predicted plasma glucose and fluxes are shown with continuous lines. B, L, D, HT indicate breakfast (50 CHO grams), lunch (60 CHO grams), dinner (80 CHO grams) and hypoglycemia treatment (10 CHO grams), respectively.

In order to assess the goodness of the model fit, the coefficient of determination (R^2) and the index FIT (already defined in *Chapter* 4.2) have been calculated. In this regard, mean $\pm$ SD (min $-$ max) of R^2 and FIT are $0.962 \pm 0.027(0.854 - 0.996)$ and $0.812 \pm 0.066(0.615 - 0.934)$, respectively. The precision of parameter is evaluated based on the coefficient of variation (CV, defined as the ratio between the standard deviation of the estimated parameter and the parameter value), which is related to how much a variation of a specific parameter influences the model prediction (the lower the CV, the higher the sensitivity of model prediction to the parameter). As

expected, having resorted to a Bayesian approach, model parameters are estimated with good precision (CV = $1.3\% \pm 0.2\%$), despite the complexity of the model with respect to the available data.

5.4.2 Intra- and Inter-Subject Variability

The a posteriori distribution of model parameters is generally in agreement with that included in the UVA/Padova simulator. However, some differences occur. In particular, glucose gastric emptying parameters at B are significantly different from those at L and D (Figure 5.3), reflecting a more rapid glucose dynamics at B, likely due to the different meal compositions of the B vs. L and D. On the other hand, insulin sensitivity varies during the day in a subject-specific fashion, without showing a consistent pattern in the population (Figure 5.4), in agreement with what already reported in [34]. The complete lists of glucose absorption and insulin sensitivity parameter estimates are reported in Table 5.1 and Table 5.2 (page 52). The comparison among parameter distributions included in the simulator and those obtained at B, L, and D in this study are shown in Figure 5.5 and 5.6, for the glucose absorption (i.e. k_{abs}, k_{max}, k_{min}) and the insulin sensitivity parameters (V_{mx}, k_{p3}), respectively. In particular, concerning the absorption parameters (Figure 5.5), the mean for L and D are significantly lower than those of B and the simulator ($P < 0.0001$ when comparing L and D against both B and the simulator prior), except for k_{abs}, which does not exhibit significant differences among B, L and D. On the other hand, distributions of insulin sensitivity parameters at B, L, D, and the simulator (Figure 5.6) are not statistically different ($P > 0.05$ from Wilkoxon Signed Rank Test when comparing L&D against both B and the simulator prior).

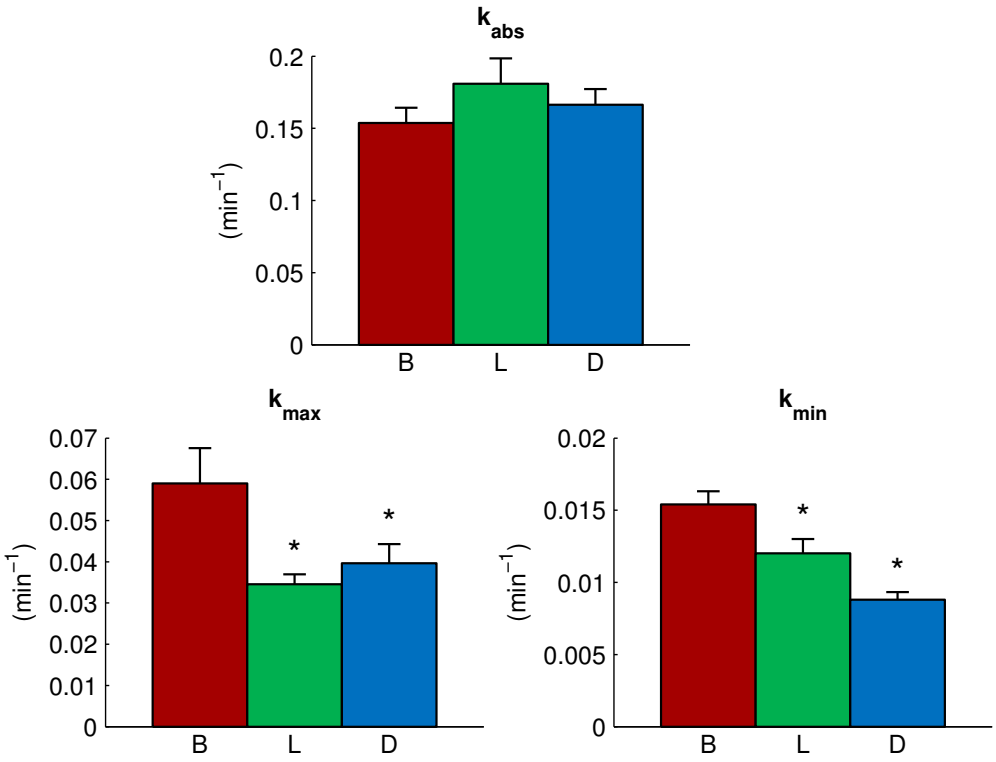


Figure 5.3: Glucose absorption parameters k_{abs}, k_{max} and k_{min} estimated at breakfast B (red), lunch L (green), and dinner D (blue). Vertical bars represent standard error (SE). $^*P < 0.05$ with respect to B, from Wilcoxon Signed Rank Test.

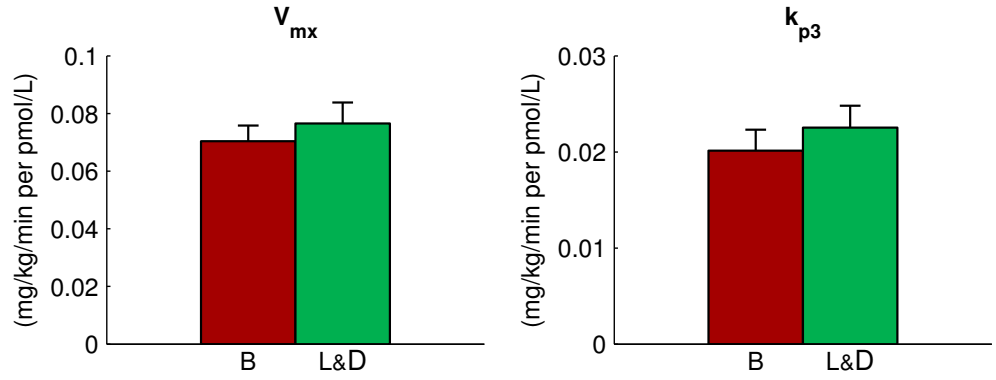


Figure 5.4: Insulin sensitivity parameters V_{mx} and k_{p3} estimated at breakfast B (red) and lunch&dinner L&D (green). Vertical bars represent standard error (SE). $^*P < 0.05$ with respect to B, from Wilcoxon Signed Rank Test.

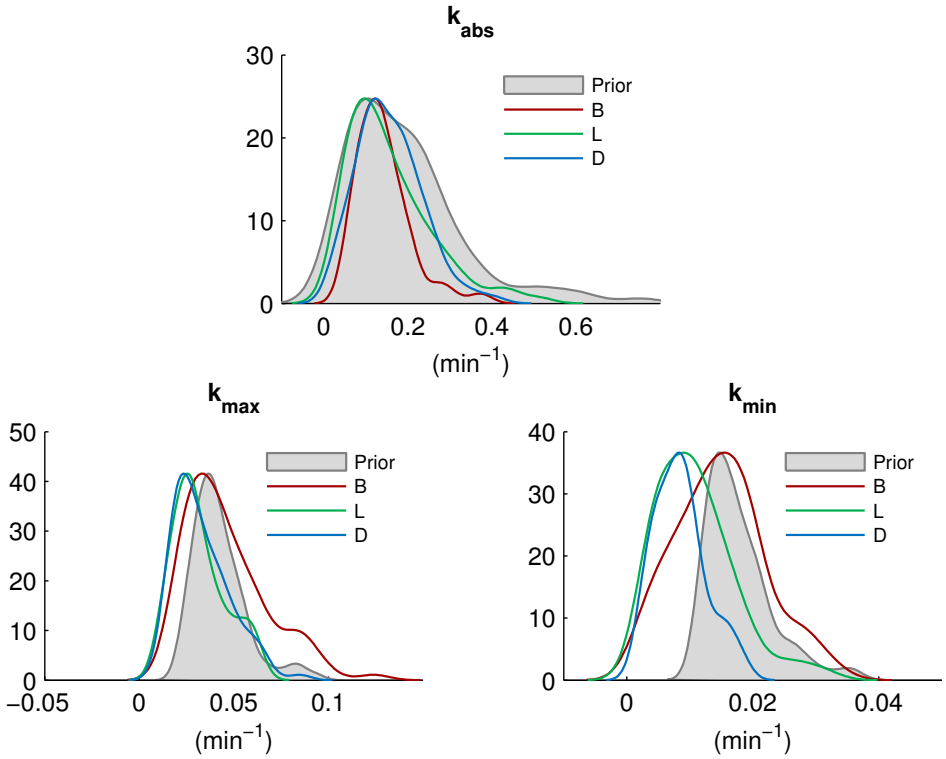


Figure 5.5: Distribution of glucose absorption parameters k_{abs}, k_{max} and k_{min} at breakfast B (red), lunch L (green), and dinner D (blue) compared to the prior (grey area).

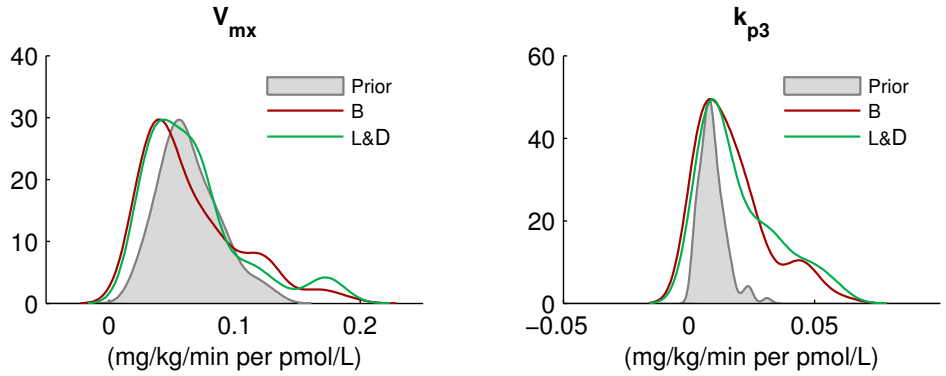


Figure 5.6: Distribution of insulin sensitivity parameters V_{mx} and k_{p3} at breakfast B (red) and lunch&dinner L&D (green) compared to the prior (grey area).

Table 5.1: Glucose Absorption Parameters

Parameter	B	L	D
k_{abs} (min^{-1})	0.130 [0.092-0.174]	0.130 [0.076-0.216] (NS)	0.147 [0.098-0.209] (NS)
k_{max} (min^{-1})	0.040 [0.027-0.059]	0.028 [0.021-0.041] (<0.0001)	0.030 [0.021-0.043] (<0.0001)
k_{min} (min^{-1})	0.015 [0.009-0.019]	0.010 [0.005-0.015] (<0.0001)	0.008 [0.005-0.011] (<0.0001)

Values are reported as median [IQR] (P value with respect to B, from Wilcoxon Signed Rank Test). NS, not significant ($P > 0.05$).

Table 5.2: Insulin Sensitivity Parameters

Parameter	B	L&D
V_{mx} (mg/kg/min per pmol/l)	0.051 [0.034-0.090]	0.058 [0.037-0.080] (NS)
k_{p3} (mg/kg/min per pmol/l)	0.015 [0.006-0.025]	0.014 [0.008-0.033] (NS)

Values are reported as median [IQR] (P value with respect to B, from Wilcoxon Signed Rank Test). NS, not significant ($P > 0.05$).

5.4.3 Assessment of a Priori Parameter Distribution

The Bayesian MAP estimation technique is a very powerful tool to identify models whenever the available data are scarce in comparison to model complexity. However, the choice of the prior distribution is critical and influences the final parameter estimates. If the prior is too informative (small variance), the risk is that parameter estimates collapse into the prior and the model does not fit the data well. On the other hand, if the prior is not informative enough (large variance), the parameter estimates may be imprecise. Ideally, the a priori information and the a posteriori information should be well balanced. This is reasonably achieved if the fit of the data is good and the parameter estimates are precise. However, this performance does not guarantee that the a priori parameter distribution is representative of our type 1 diabetic population. To investigate the agreement between prior and posterior and the stability of the solution, the parameter distributions are compared using the Wilkoxon Signed Rank test and the iterative two-stage method. For sake

of convenience, let's call *Stage1* the model identification performed using the prior included into the simulator, *Stage2* the model identification performed using the posterior obtained from *Stage1* as new prior, and so on. The posteriors are thus compared with the priors after each iteration, i.e. *Stage2* vs. *Stage1*, *Stage3* vs. *Stage2*, etc. In particular, for each subject, the absolute relative difference $\delta\mathbf{p}_j$ is calculated as:

$$\delta\mathbf{p}_j = [\delta p_{1,j}, \ldots, \delta p_{n,j}], \quad j \geq 2 \tag{5.7}$$

with

$$\delta p_{i,j} = \left| \frac{p_{i,j} - p_{i,j-1}}{p_{i,j-1}} \right|, \quad i \in [1, \ldots, n] \tag{5.8}$$

where $p_{i,j}$ is the value of i-th parameter estimated at the j-th stage, and n is the number of model parameters. The lower the $\delta p_{i,j}$ the better the agreement between prior and posterior.

After the first iteration, the posteriors obtained at *Stage1* and *Stage2* are not statistically different except for k_{abs} at breakfast ($P = 0.03$). As concerns the absolute relative difference, mean $\pm$ SD of $\delta\mathbf{p}_2$ is 0.059 ± 0.044, mean $\pm$ SD of $\delta\mathbf{p}_3$ is 0.045 ± 0.035 and mean $\pm$ SD of $\delta\mathbf{p}_4$ is 0.034 ± 0.028.

5.4.4 Comments to results

The obtained results prove that the Bayesian approach is able to cope with the identification of the T1DM model: in fact, despite the large number of parameters, by using the a priori information included into the UVA/Padova simulator, it is possible to well predict the glucose time courses of 141 experiments in T1DM subjects. Model parameters are estimated with good precision and their posterior distribution is in agreement with that included in the simulator. By using an iterative two-stage approach it has been demonstrated that the relative differences in model parameters are modest, getting smaller and smaller at each iteration, lower than 5% already after the second iteration. Similarly, the posterior distributions are statistically identical ex-

cept for some of the absorption parameters. This result was in some sense expected, since these parameters have to vary more than the others, since the meal composition in the *Database2* (solid meal) is different from that used for generating the prior of the simulator (Jell-O meal, see *Chapter* 2.2.2, [3]). Taken together, these results confirm that the model included into the simulator is adequate to describe T1DM glucose dynamics, and that the choice of the prior is key. Here the choice has been to exploit the parameter distribution included into the simulator, which was derived from multiple tracer data [3, 22]. In principle, other distributions can be used, and it is likely that different a priori parameter distributions would lead to different parameter estimates, and thus to different a posteriori distributions. In this regard, the prior included into the simulator is meant adequate, since it provides repeatable parameter solutions: in fact, the distribution of constant parameters remains the same among the subsequent iterations, and, at the same time, the fitting procedure is sensitive enough to capture the variation of the important parameters, e.g. those related to the meal composition and insulin sensitivity.

Concerning the intra- and inter-subject variability, it is important to point out that the significant differences observed in distributions of glucose absorption parameter (Figure 5.5) were expected: in fact, the parameter distributions of the simulator were obtained from subjects who received a mixed meal containing rapidly absorbed carbohydrates. Hence, it is reasonable that the posterior distribution estimated at B is virtually superimposable to the prior, since, usually breakfast contains fast absorbing CHO, while the posterior distributions estimated at L and D are statistically different from the original ones, since usually those meals are characterized by a slower absorption. This, again, supports the notion that the information content in the data is rich enough to observe this important variation in model parameters and that the prior is not too constraining, so that model parameters are allowed to move away from it, if data say so.

On the other hand, results found in insulin sensitivity (Figure 5.6) confirm what already reported in Hinshaw et al. [34], even if, as already anticipated in paraghraph 5.3, this dataset is not fully adequate to draw strong conclu-

sions in this regard.
These results prove the validity of the model and the prior included into the T1DM simulator, provided that variations of some key parameters are allowed during the day, as already dicussed in other similar works, e.g. [32]. Further analysis will have to be done in order to include in the simulator a robust model of glucose absorption depending on meal composition, e.g exploiting data of a recent experiment on complex carbohydrate metabolism [79]. Conversely, a model of daily variation of insulin sensitivity is proposed in the following *Chapter* 6.
The proposed Bayesian method has proved to be a valid approach to identify the T1DM simulation model, so that it could be applied to exploit the huge amount of data available from the clinical trials, and thus it could allow understanding how the model parameters change in response to specific experimental conditions. In this regard, further developments are required in order to make the method usable with CGM sensor and insulin pump data, such as including the insulin kinetic subsystem and using some appropriate signal preprocessing, e.g. retrospective fitting [26], able to reliably estimate the original blood glucose from CGM sensor measurement.

The Bayesian approach and the results presented above have been included in a manuscript, currently under revision [94].

6 Model of the intra-day variability of insulin sensitivity

6.1 Introduction

As anticipated in *Chapter* 4, the time-invariant characteristic of the UVA/Padova T1DM simulator limits its validity to a single meal scenario. In *Chapter* 5, it was found that the variability of meal CHO content, and thus the variability of the meal rate of appearance, is not sufficient to completely explain different glucose pattern at breakfast, lunch and dinner, and variations in insulin sensitivity (SI) parameters are also needed.

In this chapter, a model of intra-day SI variability is developed and incorporated in the simulator. To do that, data of [34] are employed, i.e. *Database3* described in *Chapter* 3.4, consisting of 20 T1DM subjects studied in three separated occasions, respectively at breakfast (B), lunch (L) and dinner (D). In particular, in [34] SI was estimated from plasma glucose and insulin concentrations at B, L and D with the oral minimal model [13]. The protocol was suitable for that purpose, since identical meals were administered at B, L and D, thus avoiding confounding effects of CHO content and meal composition on SI estimation. As reported in that work, the results proved the existence of diurnal patterns of SI, with SI lower at B than L and D, on average (Figure 6.1a). However, this difference was not statistically

significant, due to both the small population size and the high inter-subject variability (Figure 6.1b).
The idea is thus to use these data to analyze the possible pattern of SI intra-day variability for each subject, to assess the occurrence of each pattern in the population, and to incorporate this information into the simulator. This procedure allows extending the domain of validity of the simulator, making it suitable to perform longer realistic in silico trials.

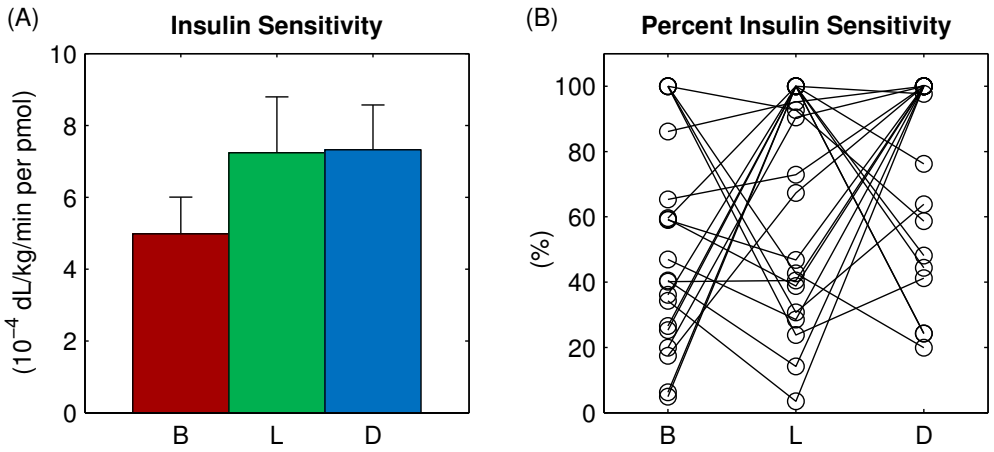


Figure 6.1: (a) Average insulin sensitivity at different time of the day (breakfast [B], lunch [L], and dinner [D]). Data are mean ± SE values. (b) Percentage intra-day insulin sensitivity variation at B, L, and D.

6.2 Classification of insulin sensitivity pattern

To highlight the characteristic SI pattern in each subject, each SI value is first normalized to the maximum observed in the same subject:

$$\mathrm{SI}_j = \frac{\mathrm{SI}_j}{\max([\mathrm{SI_B}, \mathrm{SI_L}, \mathrm{SI_D}])} \qquad j = [B, L, D] \tag{6.1}$$

Then, a threshold is chosen, for which values above threshold are labeled as high (h), whereas values below threshold are labeled as low (l). The threshold value is determined on the basis of the precision of the SI estimates: more

precisely, in [34] SI was estimated with a precision of 20%, on average; assuming SI maximum at B (i.e. 100%), the SI values at L and D are considered to be different from that at B if they are outside the "two-sigma" (i.e. 95%) confidence interval (i.e. below $100\% - 2 \times 20\% = 60\%$). By this consideration, the threshold is fixed to 60%, therefore a 40% difference is assumed to be imputable to a fortuitous variation and is subsequently modeled as a random effect.
With this definition, there are seven possible empirical classes, each one associated with a particular diurnal pattern of SI at B, L, and D (Figure 6.2a):

- Class 1: *h*-*h*-*h* (equivalent to *l*-*l*-*l*)
- Class 2: *h*-*h*-*l*
- Class 3: *h*-*l*-*h*
- Class 4: *h*-*l*-*l*
- Class 5: *l*-*h*-*h*
- Class 6: *l*-*h*-*l*
- Class 7: *l*-*l*-*h*

Each subject is univocally associated with one of the above classes. The probability of each class is then calculated as:

$$P(\text{Class } i) = N_i/N_{tot} \tag{6.2}$$

where N_i is the number of subjects belonging to the i-th Class and N_{tot} is the total number of subjects.

The classification results are shown in Figure 6.2b, in particular:

- Two subjects into Class 1 $\rightarrow P(\text{Class } 1) = 0.1$
- One subject into Class 2 $\rightarrow P(\text{Class } 2) = 0.05$
- One subject into Class 3 $\rightarrow P(\text{Class } 3) = 0.05$
- Two subjects into Class 4 $\rightarrow P(\text{Class } 4) = 0.1$
- Four subjects into Class 5 $\rightarrow P(\text{Class } 5) = 0.2$
- Four subjects into Class 6 $\rightarrow P(\text{Class } 6) = 0.2$
- Six subjects into Class 7 $\rightarrow P(\text{Class } 7) = 0.3$

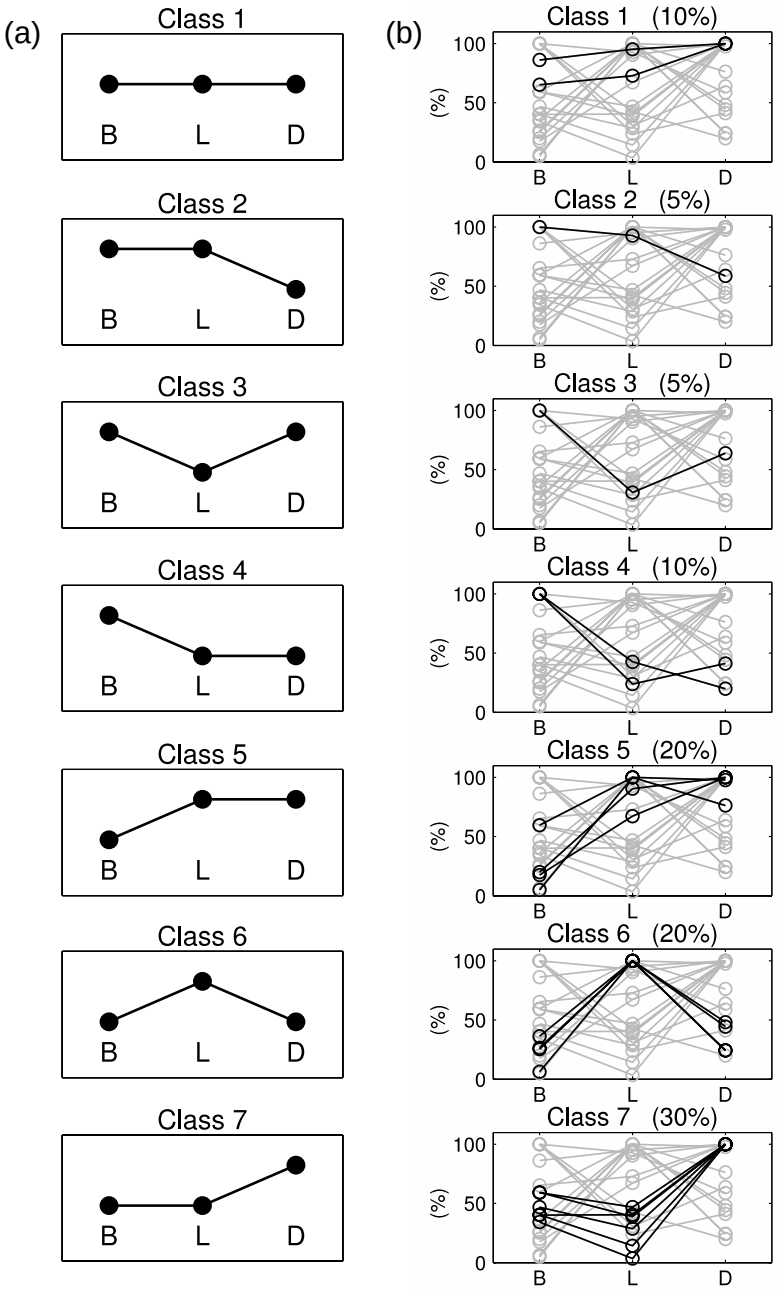


Figure 6.2: (a) The seven possible classes of insulin sensitivity diurnal pattern. B, breakfast; L, lunch; D, dinner. (b) Percentage intra-day insulin sensitivity variation at breakfast (B), lunch (L), and dinner (D), clustered among the seven variability classes. Percent values reported on the top of each panel represent the percentage of the population belonging to the respective variability class.

6.3 Incorporation of the intra-day variability of insulin sensitivity into the T1DM simulator

In order to implement the intra-day variability of SI into the T1DM simulator, each in silico subject is randomly assigned to one of the previously defined seven classes, according to the estimated probability (Figure 6.2b). The fact that a subject belongs to the i-th Class means that the SI daily pattern of that subject, described by parameters k_{p3} and V_{mx} (model equations 2.27, 2.29), is on average the one associated to the i-th Class. For instance, if the j-th subject, characterized by insulin sensitivity (k_{p3}^j, V_{mx}^j), belongs to Class 5 (*l-h-h*), its parameters will be, on average, $(\alpha k_{p3}^j, \alpha V_{mx}^j)$, (k_{p3}^j, V_{mx}^j), and (k_{p3}^j, V_{mx}^j), respectively, at B, L, and D, with $\alpha < 1$.
Parameter α is set to 0.4, based on the fact that, in each class, "low" SI values are (mean $\pm$ SD) $40\% \pm 23\%$ of the "high". However, deviations from the nominal profile are allowed, by modulating the nominal pattern with a multiplicative random noise v, described by a normal distribution $\mathcal{N}(\mu, \sigma^2)$, with $\mu = 1$ and $\sigma = 0.2$. Parameter σ is chosen in order to explain a random effect deviation up to 40% of the maximum. The actual V_{mx} and k_{p3} patterns are then transformed in the corresponding time-varying parameters $V_{mx}(t)$ and $k_{p3}(t)$ (i.e., an almost stepwise-line signal that varies three times a day). For example, for the j-th subject belonging to Class 5 the step-wise $\hat{V}_{mx}(t)$ signal at the i-th day is defined as:

$$\hat{V}_{mx}(t) = V_{mx}^j \cdot \begin{cases} v_{D,i-1} & \text{if } t < t_{B,i} \\ \alpha \cdot v_{B,i} & \text{if } t_{B,i} \leq t < t_{L,i} \\ v_{L,i} & \text{if } t_{L,i} \leq t < t_{D,i} \\ v_{D,i} & \text{if } t \geq t_{D,i} \end{cases} \tag{6.3}$$

where $t_{B,i}$, $t_{L,i}$, $t_{D,i}$ deonte the transition times at which $\hat{V}_{mxD,i-1} \rightarrow \hat{V}_{mxB,i}$, $\hat{V}_{mxB,i} \rightarrow \hat{V}_{mxL,i}$, $\hat{V}_{mxL,i} \rightarrow \hat{V}_{mxD,i}$, while $v_{D,i-1}$, $v_{B,i}$, $v_{L,i}$, $v_{D,i}$ are the random noise values related to $\hat{V}_{mxD,i-1}$, $\hat{V}_{mxB,i}$, $\hat{V}_{mxL,i}$, $\hat{V}_{mxD,i}$, respectively (Figure 6.3, *dashed orange line*). Then, $V_{mx}(t)$ is obtained from $\hat{V}_{mx}(t)$, by smoothing

the step variation. For example, the transition $\hat{V}_{mxB,i} \rightarrow \hat{V}_{mxL,i}$ at the i-th day is defined as:

$$V_{mx}(t) = \hat{V}_{L,i} + \frac{\hat{V}_{B,i} - \hat{V}_{L,i}}{2} \cdot \frac{-\tanh[\beta \cdot (t - c \cdot \Delta t] + 1}{0.5 \cdot \{-\tanh[\beta \cdot (-c \cdot \Delta t)] + 1\}} \quad t_{B,i} \leq t < t_{L,i} \tag{6.4}$$

where $\Delta t = t_{L,i} - t_{B,i}$, c is fixed to 0.2, and $\beta = 5/(2 \cdot c \cdot \Delta t)$ (Figure 6.3, *continuous red line*). The same procedure is followed to obtain $k_{p3}(t)$. Figure 6.3 shows the procedure described above for parameter V_{mx} in an illustrative subject of Class 5.

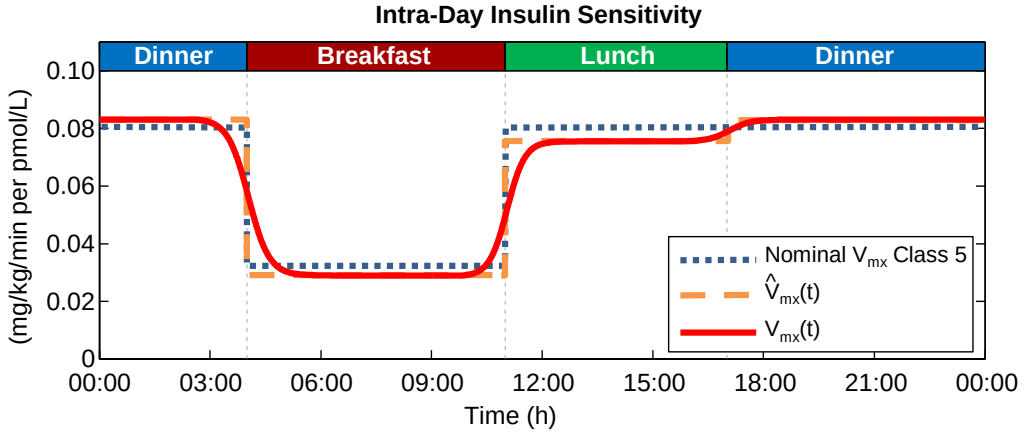


Figure 6.3: Intra-day insulin sensitivity parameter (V_{mx}) profile for a virtual subject belonging to Class 5: V_{mx} nominal pattern is depicted as pointed blue line, of which the 100% is the original time-invariant value (here set at 0.08 mg/kg/min per pmol/L); step-wise $\hat{V}_{mx}(t)$ is reported as continuous red line; $V_{mx}(t)$ is depicted as continuous red line.

6.3.1 In silico subjects update: time-varying carbohydrate-to-insulin ratio

After the incorporation of the time-varying insulin sensitivity into the T1DM simulator, three CR values (for B, L and D, respectively) have been calculated for each in silico subject, using the procedure explained in *Chapter* 2.3.3. In particular, each subject received 50 g of CHO in two occasions, i.e. with V_{mx} and k_{p3} parameters set to, respectively, 100% or 40% of their originally constant values; then, CR has been determined for both the occasions (respectively CR_{100} and CR_{40}), through the same procedure explained in

Chapter 2.3.3; finally, the sequence of three CR values has been properly obtained by combining CR_{100} and CR_{40} according to the average nominal SI daily pattern (e.g. for subjects belonging to Class 5 the CR sequence is CR_{40}, CR_{100}, and CR_{100}, at B, L, and D, respectively). Consequently, also the total daily insulin (TDI) and the correction factor (CF) have been re-calculated (similarly to what explained in *Chapter* 2.3.3). Distributions of V_{mx}, k_{p3} and CR at B, L, and D are shown in Figure 6.4: as it can be noted, V_{mxB} is, on average, lower than V_{mxL} and V_{mxD} (similarly, k_{p3B} is, on average, lower than V_{p3L} and V_{p3D}); consisently, CR values are lower, i.e. more aggressive, at B compared to L and D.

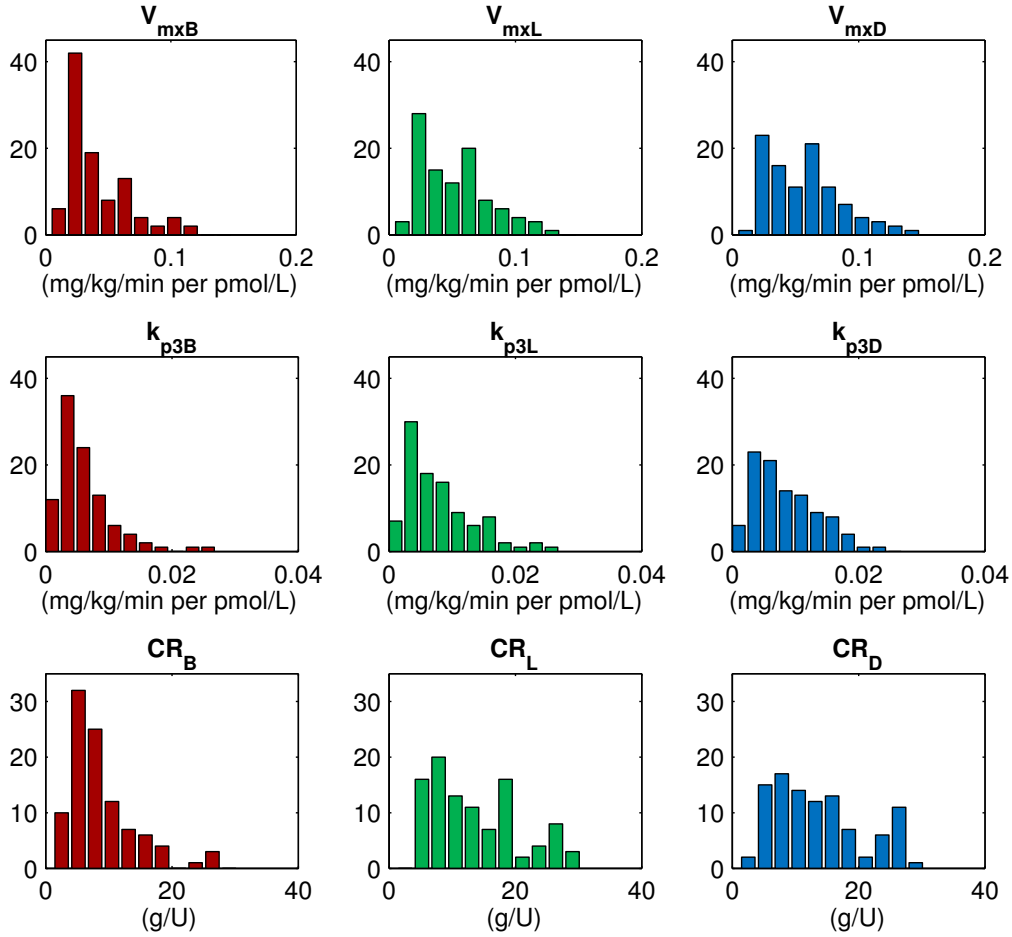


Figure 6.4: Distributions of V_{mx} (*upper*), k_{p3} (*middle*) and CR (*lower panels*) at breakfast (B), lunch (L), and dinner (D).

6.4 In silico experiments

In order to assess the intra-day variability model included into the simulator, the ability to reproduce in silico the glucose variability observed in the data is tested. Therefore the experimental scenario of *Database3* (described in *Chapter* 3.4) is reproduced in silico. More specifically, the 100 in silico subjects received B at 7:00 a.m., L at 1:00 p.m., and D at 7:00 p.m., with the appropriate amount of ingested carbohydrates (50 g of CHO). The optimal basal insulin is infused in each virtual subject. In contrast, the pre-meal insulin bolus is reduced, on average, by 2U with respect to the one calculated with the patient optimal CR. In fact, by definition, with the optimal CR, plasma glucose would have returned to its target level within the 3 hours, whereas the data show that the plasma glucose level was still considerably above target at $t = 360$ min (see Figure 3.5). It is worth noting that, with this reduction, the average amount of insulin administered with the bolus is similar in the real and virtual populations (3.93 ± 0.22 U vs. 3.54 ± 0.16 U, respectively). Transition times related to time-varying $V_{mx}(t)$ and $k_{p3}(t)$ are set at 4:00 a.m., 11:00 a.m., and 5:00 p.m. The simulations are thus compared against clinical data. Two-way analysis of variance, including both the main effects and a term for interaction, is used to assess difference between type of data (real vs. simulated) at B, L, and D. In particular, we looked at the significance of the interaction term, which indicates whether or not the differences among B, L, and D are affected by the factor "type of data". A P value of < 0.05 is considered significant.

6.5 Results

Inter-subject glucose variability is provided in Figure 6.5 against the glucose time courses observed in the clinical experiment, proving that the simulator well captures the variability of the data measured after each meal. Figure 6.6 shows the comparison of simulated (*left panel*) vs. above-basal plasma glucose (*right panel*) curves at B, L, and D. Simulated plasma glucose values are, on average, slightly lower and with the peaks occurring somewhat later than the data.

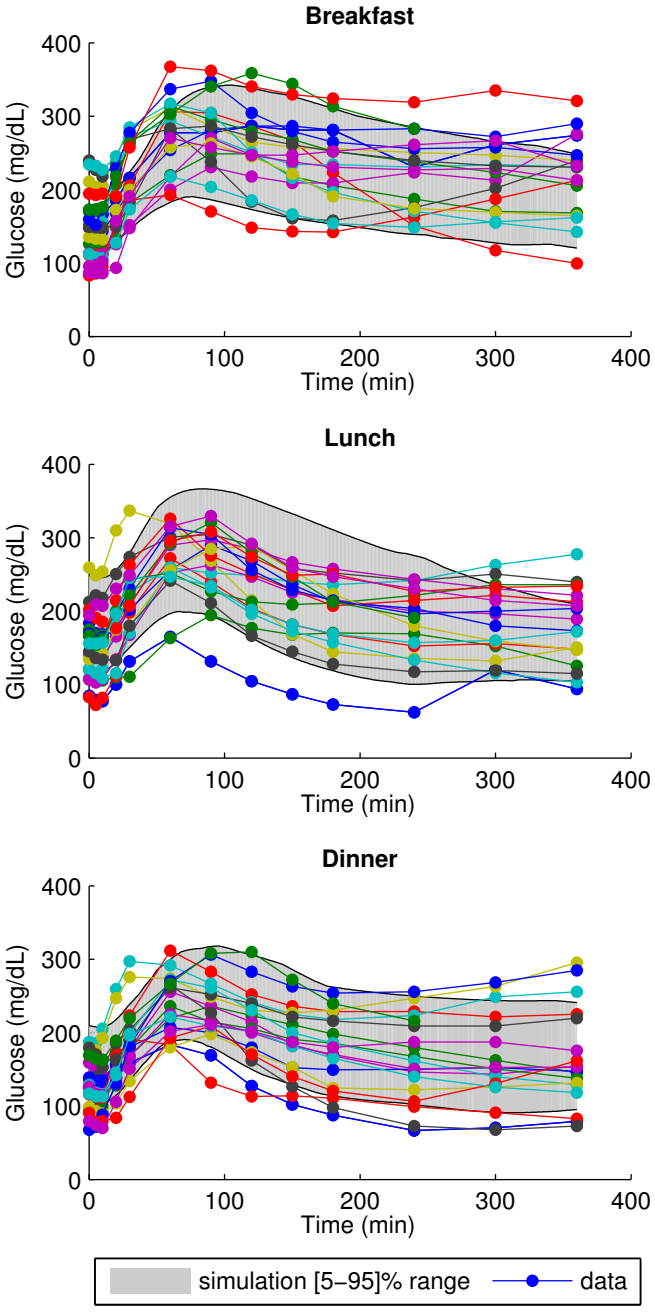


Figure 6.5: Simulator inter-subject variability (*gray area*) is compared with clinical observations (*colored lines with dots*) at breakfast (*upper panel*), lunch (*middle panel*), and dinner (*lower panel*).

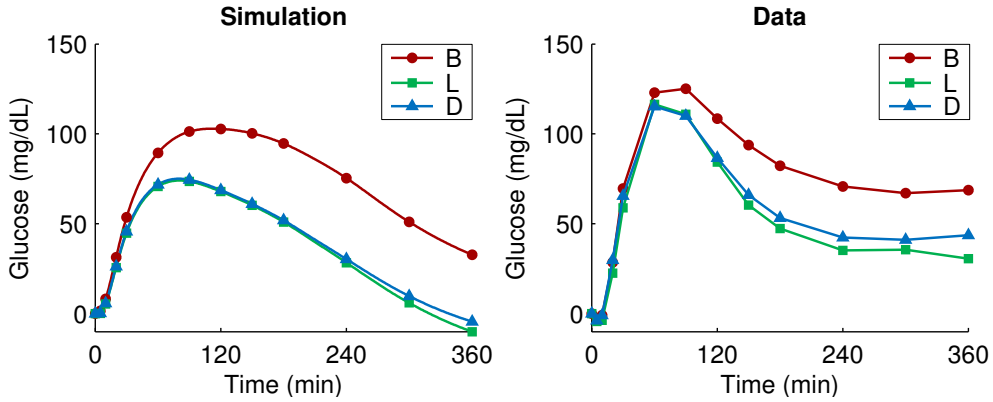


Figure 6.6: Over-basal plasma glucose profiles obtained in simulation (*left panel*) and observed experimentally (*right panel*). B, breakfast; L, lunch; D, dinner.

Values of area under the over-basal glucose curves are 2.60 ± 0.91 (B), 1.38 ± 0.91 (L), and 1.44 ± 1.07 (D) 10^4 mg/dL·min in silico vs. 2.87 ± 1.65 (B), 1.98 ± 1.56 (L), and 2.16 ± 2.00 (D) 10^4 mg/dL·min in vivo. Over-basal peak values are 109 ± 33 (B), 800 ± 29 (L), and 81 ± 30 (D) mg/dL in silico vs. 136 ± 39 (B), 126 ± 37 (L), and 125 ± 48 (D) mg/dL in vivo. The peak times are 125 ± 51 (B), 96 ± 46 (L), and 94 ± 44 (D) min in silico vs. 81 ± 24 (B), 74 ± 21 (L), and 68 ± 24 (D) min in vivo. The interaction term from the two-way analysis of variance is not statistically significant in all the comparisons. Values of area under the over-basal insulin curves are 1.33 ± 0.47 (B), 1.26 ± 0.46 (L), and 1.25 ± 0.46 (D) 10^4 pmol/L·min in silico vs. 1.42 ± 0.98 (B), 0.86 ± 0.67 (L), and 1.10 ± 1.00 (D) 10^4 pmol/L·min in vivo. Over-basal peak values are 94 ± 28 (B), 93 ± 38 (L), and 93 ± 39 (D) pmol/L in silico vs. 116 ± 59 (B), 102 ± 86 (L), and 98 ± 57 (D) pmol/L in vivo. The peak times are 46 ± 9 (B), 45 ± 7 (L), and 45 ± 7 (D) min in silico vs. 60 ± 44 (B), 61 ± 36 (L), and 49 ± 37 (D) min in vivo. The interaction term from the two-way analysis of variance is not statistically significant in all the comparisons except for the peak times ($P = 0.036$).

6.5.1 Comments to results

By replicating in silico the same experimental protocol of *Database3* [34], it has been demonstrated that the simulated above-basal plasma glucose

levels are higher at B with respect to L and D, similarly to what observed in the data. This proves the capability of the simulator, enriched with the time-varying SI parameters (k_{p3}, V_{mx}), to better cope with the diurnal glucose variability.

However, it is worth noting that, on average, the simulated post-prandial excursions are slightly lower, with glucose peaks occurring later than those observed in the study (Figure 6.6). This can be justified by the fact that the T1DM subjects sof *Database3* present, on average, a lower CR with respect to the in silico population ($CR^{real} = 8.6 \pm 2.1$ [range, $6 - 13$] g/U vs. $CR^{sim} = 15.9 \pm 5.3$ [range, $7 - 30$] g/U), hence being less sensitive to insulin. This may explain the existing differences in glucose dynamics.

Nevertheless, it is also important to note that the inter-subject glucose variability observed in clinical data is well captured in simulation at B, L and D (Figure 6.5).

Some considerations have to be taken regarding the transition times and the modulation of the nominal pattern into the variability model. Clearly, the choice of the transition times at which nominal k_{p3} and V_{mx} vary is arbitrary; however, the idea is to set them in order to have a stable values of both $k_{p3}(t)$ and $V_{mx}(t)$ around each meal. Moreover, modulation with the multiplicative random noise would allow a subject to temporarily migrate to another variability class. However, the random noise amplitude of choice permits only migrations to contiguous classes: for example, a subject belonging to Class 3 can temporarily migrate to Class 1, 4, or 5 but not to Class 2.

As reported before, the cutoff value of 60% derives from the precision of SI estimated in [34]. However, different cutoff values have been tested: 50% and 70%. With a 50% cutoff, $P(\text{Class } 2) = 0$, whereas using a 70% cutoff, $P(\text{Class } 4) = 0$. On the other hand, all the classes had nonzero probabilities using the 60% cutoff. In some sense, the small sample size has conditioned the choice of the cutoff level.

In conclusion, the incorporation of an intra-day variability model of insulin sensitivity parameters into the UVA/Padova T1DM simulator has allowed extending its domain of validity from a single-meal time-invariant to a multiple-meal time-varying scenario, making the simulator suitable to perform longer

realistic in silico trials. However, further improvements in this direction will be required to completely describe the glucose variability in the long-term. It would be certainly of interest to investigate the SI variations in a longer scenario (e.g. exploiting the data provided by the recent monthly study in T1DM [53]). In this regard, the method to estimate SI with the oral minimal model is unsuitable because of the availability of only CGM and insulin pump data. However, a recently proposed method for the estimation of SI in T1DM subjects from CGM and insulin pump data [80] could be used, thus allowing a model refinement in the near future.

The proposed model of intra-day variability of insulin sensitivity with the results provided above, have been published in [96].

7 Employment of the T1DM simulator

7.1 Introduction

In this chapter, the application of the UVA/Padova T1DM simulator in two case studies is presented. In particular, a new adaptive artificial pancreas controller is described, in which the simulator served to the in silico testing of the control performance in one month. Then, a second example is shown, in which a pharmacokinetic model of an inhaled insulin is developed and incorporated into the simulator, in order to evaluate in silico the effects of several dosing regimens.

7.2 In silico testing of adaptive artificial pancreas control algorithms

As already discussed in *Chapter* 1.1.2, in the past decade the research has seen unprecedented advances in AP technology, which moved from short-term inpatient studies to short trials at home employing wireless, portable, wearable AP systems. Several studies were conducted in adults, e.g. those using an AP system based on a Modular Model Predictive Control algorithm (MMPC) [70, 85, 90], in gradually less structured and less monitored settings: inpatient first [7], 2-day in hotel settings [26, 27], and, recently, 2-month

evening & night at home [53]. The formerly conducted studies had a limited duration and were restricted to evening and night, thus allowing to neglect the impact of intra- and inter-day glucose response variability of each subject, e.g. to insulin and meals. The latter is a well-known phenomenon and became a major issue with the introduction of longer (week/month) home trials. This large subject-specific variability calls for an adaptive controller. To reliably test such controller in silico, a time-varying simulator is needed, and thus motivated the developement presented in *Chapter* 6.
In the following section, an adaptive AP MMPC algorithm based on the Run-to-Run (R2R) approach is described in summary. The R2R is a well-known learning-type control algorithm [98] that learns information about the control quality from the current run and changes the control variable to apply in the next run. The R2R strategy has already been used for glucose control in T1DM subjects on the basis of a few daily self-monitoring blood glucose (SMBG) measurements [66–69, 104] or using CGM data [33, 89, 92] to adapt day-by-day basal insulin delivery or the insulin meal bolus. R2R in the AP context was introduced in [59], where the aggressiveness of the controller was adapted by using the maximum and minimum glucose values provided by CGM.

In this example, it is described a much more realistic R2R approach for tuning the MMPC algorithm which adapts the basal insulin delivery during the night and the CR during the day, and its in silico test using the new time-varying UVA/Padova T1DM simulator.

7.2.1 Run-to-Run strategy for adaptive MPC tuning

The MMPC algorithm considered here is the linear model predictive control described in [90]. The principal parameters used for control tuning and individualization are the basal insulin delivery, the CR, the CF and the body weight (BW). In particular, the MMPC computes an insulin variation with respect to the basal profile, uses CR and CF (taking into account also the insulin on board, i.e. the amount of insulin, coming from previous

bolus/infusions, that is still active in the body) to compute the insulin reference in the cost function, and BW and CR to tune the control aggressiveness. Thus, the adaptive MMPC aims to: (i) optimize the tuning of the controller parameters; (ii) adapt them to the inter-day variability. Specifically, the R2R strategy is applied to update both the basal insulin delivery and CR parameter (and thus the meal insulin bolus); the update is applied in the next day (run) on the basis of the performance measured during the previous day (run). The choice of the performance indices is a critical point for the success of the R2R. CGM sensors (usually employed in an AP context) allow including clinically relevant indices into the problem, e.g. the percentage of time spent in hypo-, eu-, and hyper-glycemic range, and the average blood glucose (BG). In particular, since a major concern in T1DM therapy is to avoid hypoglycemia, the updating law is primarily designed to lead to 0 the percentage of time spent in hypoglycemia (i.e. BG $<$ 70 mg/dL). Once this primary goal is achieved, a secondary updating law is designed to reduce the percentage of time spent above 180 mg/dL and to lead the average BG to the desired target.

For each run, the variation of the basal insulin rate is proportional to the applied basal delivery and to the performance indices computed during the previous run. In order to give priority in avoiding hypoglycemia, a switching condition depending on the percentage of time spent below 70 mg/dL is introduced. In particular, at run k, the updating law is defined as follows:

$$b(k+1) = \begin{cases} b(k) - \bar{b}k_1^b T_b^b(k) & \text{if } T_b^b(k) > 0 \\ b(k) + \bar{b}\left(k_2^b T_a^b(k) + k_3^b \cdot \dfrac{G_m^b(k) - G_T^b}{G_T^b}\right) & \text{if } T_b^b(k) = 0 \end{cases} \tag{7.1}$$

where b is the basal insulin delivery, the constants k_1^b, k_2^b, k_3^b are the R2R gains, G_T^b is the glycemic target, $\bar{b}$ is the initial basal therapy, and T_b^b, T_a^b, G_m^b are the R2R performance indices associated with the night interval. In particular, T_b^b is the percentage of time spent below 70 mg/dL, T_a^b is the percentage of time spent above 180 mg/dL, and G_m^b is the average glucose concentration in the evaluation interval, which is equal to the night interval

delayed by 3 hours.
A similar updating law is used to optimize the CR values, which are assumed to be constant along n daily intervals $[t_j^B; t_{j+1}^B]$, $j = 1, ..., n$, with $t_{n+1}^B = t_1^B$. In particular, at run k, the updating law for the j^{th} interval is defined as follows:

$$B_j(k+1) = \begin{cases} B_j(k) - \bar{B}_j k_1^B T_b^{B_j}(k) & \text{if } T_b^{B_j}(k) > 0 \\ B_j(k) + \bar{B}_j \left(k_2^B T_a^{B_j}(k) + k_3^B \cdot \dfrac{G_m^{B_j}(k) - G_T^B}{G_T^B} \right) & \text{if } T_b^{B_j}(k) = 0 \end{cases} \tag{7.2}$$

where $B_j(k) = 1/CR_j(k)$ is the inverse of the CR at run k during the interval j, the constants k_1^B, k_2^B, k_3^B are the R2R gains, G_T^B is the glycemic target, constant for all the intervals, $\bar{B}_j = B_j(0)$ is the initial value in the interval j, and $T_b^{B_j}$, $T_a^{B_j}$, $G_m^{B_j}$ are the R2R performance indices associated with the j interval. In particular, $T_b^{B_j}$ is the percentage of time spent below 70 mg/dL, $T_a^{B_j}$ is the percentage of time spent above 180 mg/dL, and $G_m^{B_j}$ is the average glucose concentration collected in the j^{th} evaluation interval. The maximum length of each j^{th} evaluation interval is 7h; it starts from the meal time in the j^{th} interval and is truncated if another meal occurs.
The stability of the proposed strategy can be demonstrated by applying the method described in [89], where a R2R approach for adapting a piecewise basal therapy in an open-loop context is proposed. A key assumption is that disjoint intervals are used to update basal insulin or CR: this is an important requirement, otherwise the problem would move from several scalar to a multivariable framework, with a significant increase of complexity both in terms of algorithm tuning and stability analysis.

7.2.2 In silico testing

The R2R algorithm described above has been tested on the 100 in silico adults of the simulator incorporating the SI intra-day variability model described in *Chapter* 6. A model of CGM sensor error noise, described in [90], is included.

The night interval considered for basal insulin update is [0:00 am; 8:00 am] for all the patients. The n ($n = 3$) time intervals defining the piecewise constant CR are bounded by $t_1^B = 8{:}00$ am, $t_2^B = 1{:}00$ pm, $t_3^B = 8{:}00$ pm for all the patients. R2R gains are fixed to $k_1^b = 0.15$, $k_2^b = 0.175$, $k_3^b = 0.005$, and $G_T^b = 115$ mg/dL, for the basal and to $k_1^B = 0.3$, $k_2^B = 0.05$, $k_3^B = 0.01$, and $G_T^B = 115$ mg/dL, for CR update.
One-month scenario is simulated (more precisely 29 days), in which three meals per day are administered at 8:00 am, 1:00 pm, and 8:00 pm having 40 g, 80 g, and 60 g of CHO, respectively. Moreover, if the BG falls below 65 mg/dL, the protocol prescribes a rescue CHO dose of 16 g (hypotreatment). Two hypotreatments are separated by at least 30 minutes. The simulations are performed twice, either by using the MMPC strategy described in [90] (CL) or by employing the adaptive MMPC enhanced by the R2R strategy (CL_{R2R}).
Performance metrics include average (A) BG and standard deviation (SD), percentage of time spent in euglycemic target range [70-180] mg/dL (T_r), percentage of time spent above 180 mg/dL (T_a), and percentage of time spent below 70 mg/dL (T_b).
The performance of CL and CL_{R2R} is also evaluated by using the Control Variability Grid Analysis (CVGA) [60, 85], which associates the control performance of each in silico subject to a point into a plane. In particular, the plane is divided in four regions, reflecting the control quality and graded from A (good control) to D (bad control), and the point coordinates (x, y) represent the minimum and maximum BG values reached by the in silico subject during the day under analysis.

7.2.3 Results

The average ± SD of simulated BG after one day (Day 2), two weeks (Day 15) and four weeks (Day 29) are shown in Figure 7.1, *left panels*: the postprandial overshoots detected after lunch and dinner (Figure 7.1a) are considerably reduced after two weeks of R2R (Day 15, Figure 7.1b). A further reduction is achieved after four weeks (Day 29, Figure 7.1c), also with a

reduced BG variability. The CVGA plots (Figure 7.1, *right panels*) also confirm the CL_{R2R} improvement, by better populating the center of A and B zones.

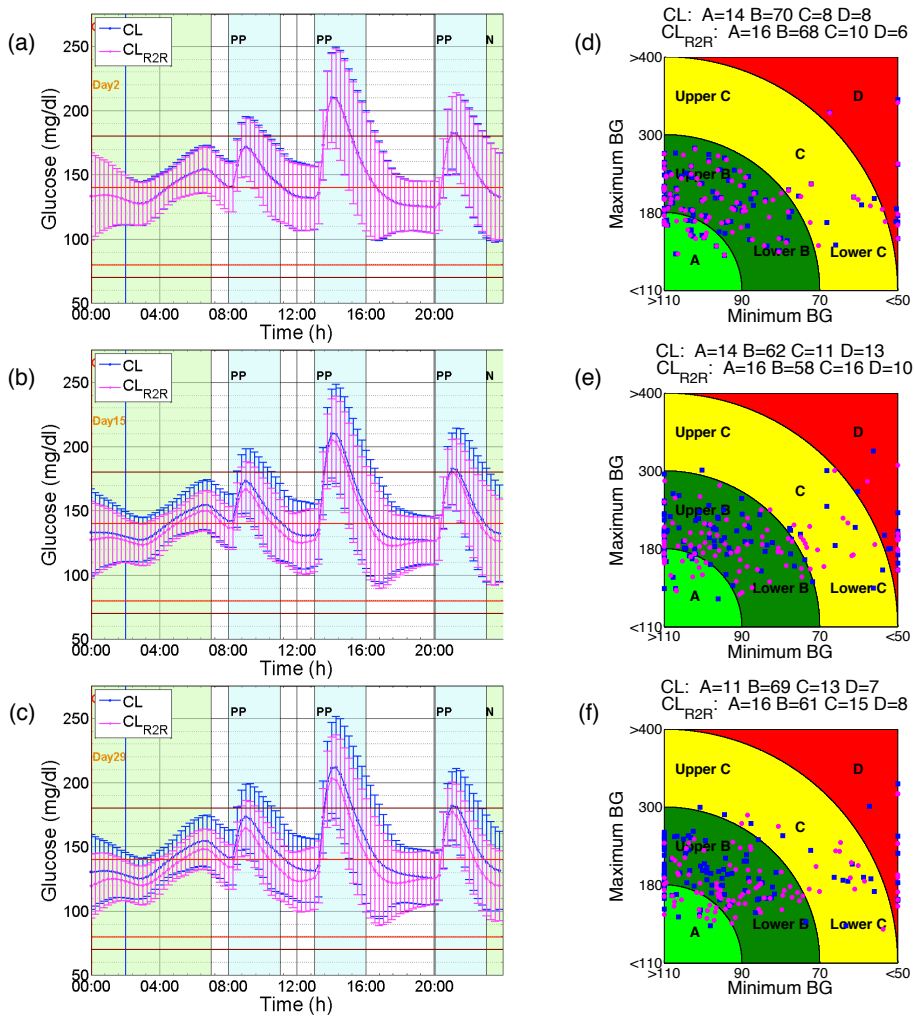


Figure 7.1: *Left panels*: Comparison of average ± SD glucose time courses in CL (blue) vs. CL_{R2R} (magenta) on Day 2 (a), Day 15 (b) and Day 29 (c), respectively. *Right panels*: CVGA of CL (blue square) vs. CL_{R2R} (magenta circle) on Day 2 (d), Day 15 (e) and Day 29 (f). Each point represents the coordinates (x is the minimum and y is the maximum glucose values) associated to a single subject.

Numerical comparison of CL vs. CL_{R2R} on the whole experiment duration is reported in Table 7.1, where the improvement shown by CL_{R2R} is evident. Performance indices show that the improvement of CL_{R2R} vs. CL is modest

after one day; after two weeks (Day 15), the improvements in time in range, time in tight range and time above 180 mg/dL are very much improved with respect to Day 2. This performance is maintained until the end of the experiment (Day 29).

Table 7.1: Performance Metrics Improvements

	Day 2	Day 15	Day 29
M (mg/dL)	0.26%	3.54%	5.87%
Tt (%)	-0.03%	5.82%	5.96%
Ttt (%)	0.45%	11.74%	18.62%
Ta (%)	-0.87%	-32.62%	-39.22%

Percent improvements obtained using the CL_{R2R} algorithm with respect to the sole CL.

7.2.4 Conclusions

In this section, it has been illustrated the use of the time-varying simulator in order to provide a suitable framework to in silico test the performance of an adaptive AP algorithm, i.e. the MMPC based on R2R approach, which uses the percentage of time spent below 70 mg/dL, the percentage of time spent above 180 mg/dL, and the distance of average glucose from a target to adapt the basal insulin delivery during the night and the CR during the day. The encouraging results achieved in silico in a realistic one-month scenario open to an in vivo testing phase, with potential benefits for T1DM subjects.

7.3 Evaluating the pharmacological effects of new insulin molecules

As stated in *Chapter* 1.1.2, the current T1DM therapy (including AP) uses the subcutaneous route for insulin delivery [16]. Indeed, insulin delivered subcutaneously is well-accepted as state of the art, but important limitations, such as delay and variability in insulin absorption represent the major challenges, especially in handling post-prandial glucose excursions. The research on more rapidly absorbed subcutaneous insulin analogs and

other routes of delivery, e.g. pulmonary administration, is very active. The UVA/Padova T1DM simulator can be used, beyond AP, also to test new insulin analogs/molecules. In particular, the in silico testing may help an efficient design of clinical trials.

In this section, an example of in silico testing of a recombinant human insulin is presented: the novel ultra-rapid Technosphere® Insulin (TI) inhalation powder (Afrezza®), which has been approved by U.S. FDA to control high blood glucose in adults with type 1 and type 2 diabetes. Afrezza® is a dry powder formulation of recombinant human insulin administered by pulmonary route directly before the meal using a new drug delivery system. It is characterized by fast absorption (with a peak serum concentration achieved in about 15 minutes) and short duration of action (2-3 h) due to a short half-life [6]. This fast absorption of insulin can overcome the delay inherent to the current subcutaneously injected insulin analogs, but opens new challenges to cope with the duration of post-prandial glucose elevation, which might take from 4 to 6 hours to return to pre-meal values. Thus, alternative regimens are being explored, such as post-meal dosing or split doses (e.g. pre- and post-meal), which could achieve optimal post-prandial glucose control.
Here, a system pharmacology modeling approach is used. In particular, a recently proposed version of the T1DM simulator incorporating a pharmacokinetic (PK) model of TI insulin [97] is used to simulate post-prandial glucose response in T1DM patients treated with TI administered at different times (e.g. before the meal or within the meal period), or in different fashions (e.g. split dosage rather than a single bolus).

This study has been conducted in collaboration with Sanofi US, Inc., under the supervision of Dr. Thomas Klabunde.

7.3.1 T1DM simulator incorporating inhaled insulin PK

To describe pharmacokinetic/pharmacodynamic (PK/PD) of Afrezza® observed in T1DM subjects, a PK model of TI insulin has been developed and identified on a T1DM population treated with Afrezza® [9]. In particular, the PK model is a variation of the single-compartment model described by

Potocka in [74]:

$$\dot{I}_{TI}(t) = -k_{aTI} \cdot I_{TI}(t) + F_{TI} \cdot D \quad I_{TI} = 0 \tag{7.3}$$

where I_{TI} is the amount of insulin in the alveolar space, D (pmol/kg/min) is the TI dose, F_{TI} is the fraction of inhaled insulin which actually appears in plasma, and k_{aTI} (min^{-1}) is the rate constant of pulmonary insulin absorption.

The model of T1DM simulator has been properly modified (Figure 7.2): in particular, model equations are the same of those reported in *Chapter* 2.2.1, except for the rate of appearance of external insulin in plasma (Ra_I), which takes into account TI contribution

$$Ra_I(t) = k_{a1} \cdot I_{sc1}(t) + k_{a2} \cdot I_{sc2}(t) + k_{aTI} \cdot I_{TI}(t) \tag{7.4}$$

where k_{aTI} and $I_{TI}(t)$ are those appearing in equation (7.3). Individual PK

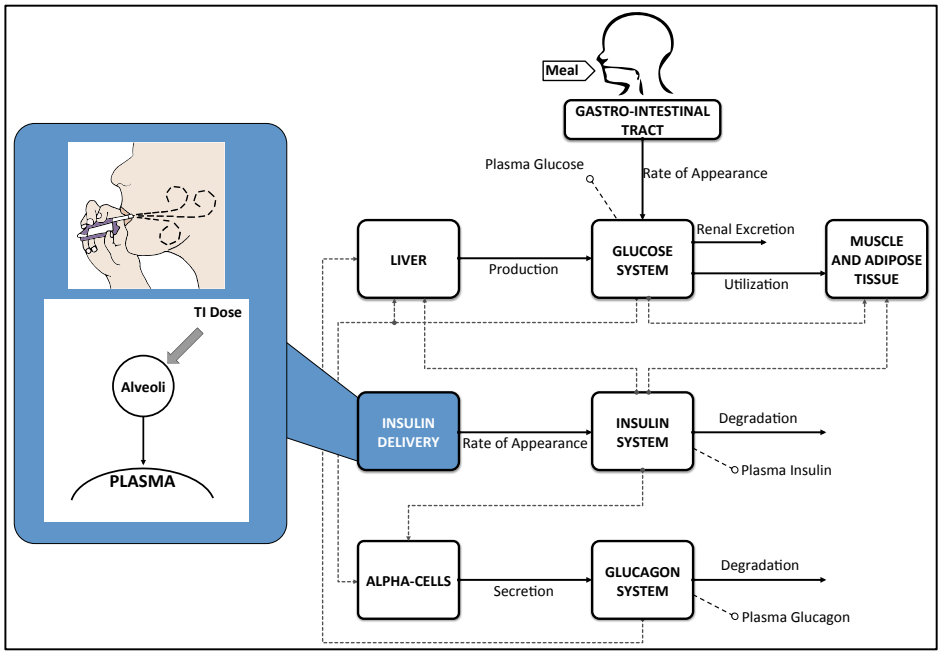


Figure 7.2: Scheme of the T1DM simulator incorporating the PK model of Afrezza®.

parameters of TI insulin have been generated paralleling what is described in

Chapter 2.3.3, i.e. they were randomly extracted from the joint parameter distribution that has been created using the parameter estimates obtained from model identification on insulin data of T1DM subjects [9]. Then, each individual PK has been randomly assigned to each in silico subject. The T1DM simulator incorporating the PK model has been thus tested in terms of ability to predict the meal response observed in clinical data: by replicating in silico the same experimental protocol of [9], it is possible to appreciate how well the simulated glucose dynamics reflected the data (Figure 7.3).

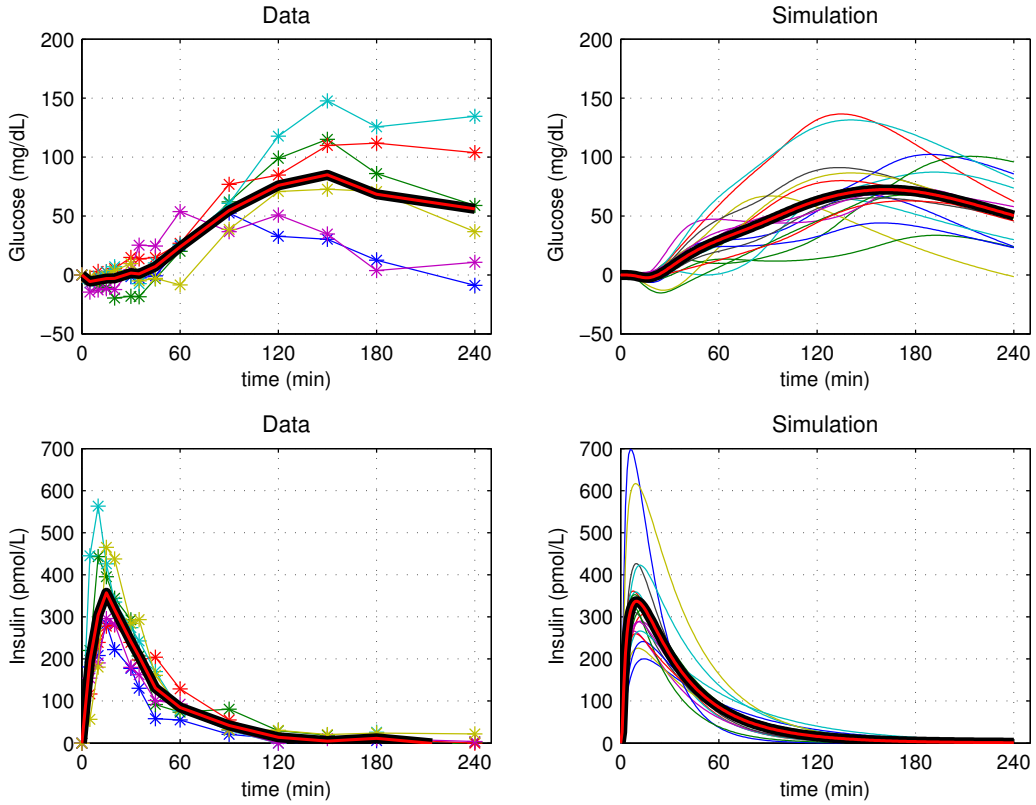


Figure 7.3: Over-basal glucose and insulin data of [9] (*left panels*) and simulations obtained by replicating the same protocol (*right panels*). At $t = 0$, subjects received a meal containing 50g of CHO and an individualized TI dose was given at the same time; in particular, the TI dose was calculated as a function of the ingested CHO and the subject's CR. Individual profiles are shown, with averages plotted as thick red lines.

7.3.2 Simulation scenario

The effect of different dosing regimens of Afrezza® on post-prandial glucose after a meal test has been explored in 100 virtual patients for pre-meal and post-meal dosing as well as for split dosing scenarios. In particular, a meal test with 50 g CHO has been simulated, with Afrezza® doses ranging from 10 to 80 TI Units, with timing ranging from 0 to 120 min after meal, as illustrated in Figure 7.4.

For all the simulations, the expected risk (e.g. number of expected hypoglycemic events) and benefit (e.g. mean plasma glucose of meal test) were evaluated. To select the most suitable dose for each virtual patient, a titration rule has been followed, based on the glucose level at 90 min after meal ingestion ($BG_{90'}$): if $BG_{90'}$ is between $110 - 160$ mg/dL, TI dose is considered as adequate; if $BG_{90'} > 160$ mg/dL, TI dose has to be increased; if $BG_{90'} < 110$ mg/dL, TI dose has to be decreased.

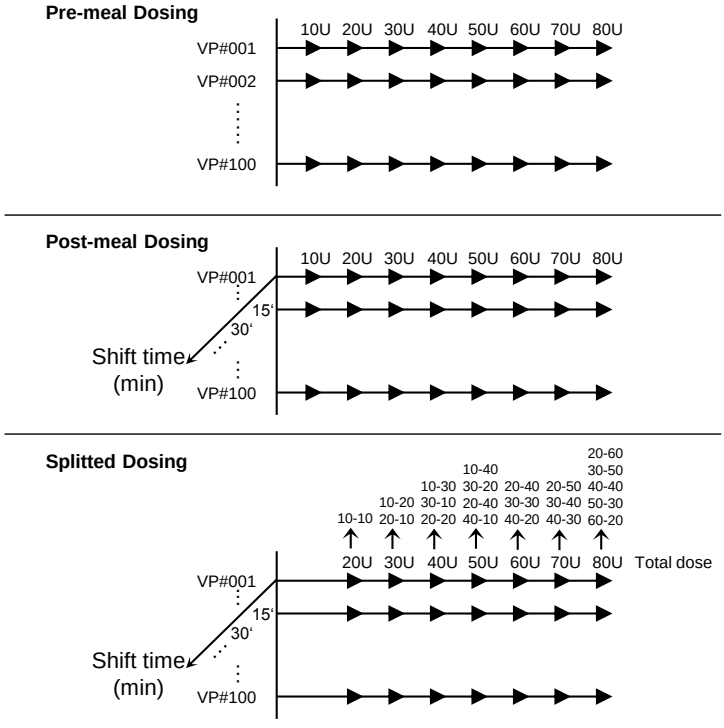


Figure 7.4: Scheme of different dosing regimens explored in simulation: pre-meal dosing (*upper panel*), post-meal dosing (*middle panel*), split dosing (*lower panel*).

7.3.3 Results

Simulations of different dosing regimens are shown in Figure 7.5 for one illustrative in silico subject (VP#004).

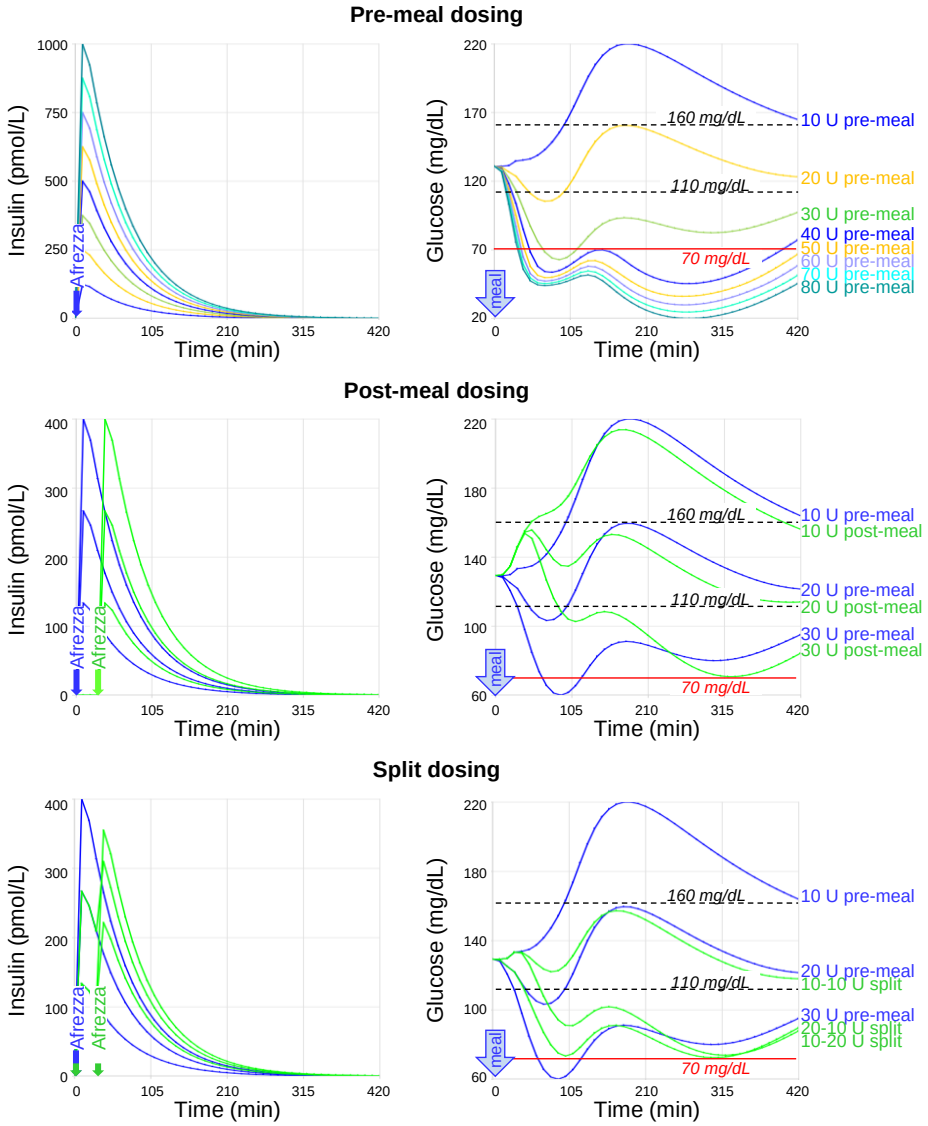


Figure 7.5: Meal experiments in one illustrative in silico subject (VP#004). *Upper panels*: insulin (*left*) and glucose (*right*) in response to pre-meal administration of eight different dosages, indicated with different colors. *Middle panels*: insulin (*left*) and glucose (*right*) in response to post-meal dosing (green lines) compared to pre-meal dosing (blue lines). *Lower panels*: insulin (*left*) and glucose (*right*) in response to split dosing (green lines) compared to pre-meal dosing (blue lines).

The effect of inhaled insulin on post-prandial glucose excursion depends on dose: too low doses lead to suboptimal control of post-prandial glucose; on the other hand, due to fast appearance of Afrezza®, too high pre-meal doses lead to sharp glucose decline and risk of early hypoglycemic events (Figure 7.5, *upper panel*). A flatter profile can be obtained by giving the dose after meal (Figure 7.5, *middle panel*) or by splitting the insulin amount in two doses, one directly before the meal and one after a certain time interval (Figure 7.5, *lower panel*). In these cases, higher total doses may be selected gaining additional efficacy on post-prandial glucose control, without inducing hypoglycemia. Numerical results of this analysis are reported in detail in Table 7.2, which reports, for each dosing pattern, both the optimal insulin dose satisfying the titration rule and the highest dose not leading to hypoglycemia.

Table 7.2: Optimal meal dosing in subject VP#004

	TI dose (U)	BG_{4hr} (mg/dL)	BG<70mg/dL (Y/N)	Optimal dose by titration rule	Highest dose with no BG<70mg/dL
Pre-meal dosing					
	10	177.1	N	§	
	20	133.2	N		†
	30	85.2	Y		
Post-meal dosing					
	10	181.1	N		
	20	143.2	N	§	
	30	113.2	N		†
Split dosing					
	10-10	139.0	N	§	
	10-20	104.0	N		
	20-10	91.9	N		†

For each insulin dose administered (TI dose, *second column*), the glucose value obtained 4 hours after meal (BG_{4hr}, *second column*) and whether hypoglycemia occurs (BG<70 mg/dL, *third column*) are reported. Moreover, for each dosing pattern (i.e. pre-meal, post-meal, split) both the optimal insulin dose satisfying the titration rule ("§" in *fourth column*) and the highest dose not leading to hypoglycemia ("†" in *fifth column*) are indicated.

7.3.4 Conclusions

In this section, the T1DM simulator was used to model prandial response to a novel inhaled insulin powder, i.e. the ultrafast-acting Technosphere® Insulin (TI) inhalation powder (Afrezza®). In particular, a total of more than 20,000 simulations of in silico meal tests have been performed in 100 virtual patients to explore the effect of different doses and dosing regimens of Afrezza®. The in silico clinical trials are being used to inform the design of clinical studies evaluating different proposed treatment regimens of Afrezza® in T1DM subjects.

Acknowledgments

The work presented in this section has been supported by Sanofi U.S., Inc., under the supervision of Dr. Thomas Klabunde.

8

Conclusions

Simulation environments are extremely useful in the type 1 diabetes (T1DM) research field, since they allow to in silico test different T1DM treatments (including AP control algorithms) under several experimental conditions.

The focus of the present work is on the University of Virginia (UVA) and Padova T1DM Simulator [43], a "new generation" simulator based on a rather complex model of glucose dynamics and equipped with an in silico population of 100 adults, 100 adolescents, and 100 children, which is able to reproduce the inter-subject variability of glucose dynamics in a T1DM population. Thanks to this peculiarity, in the 2008 the US Food and Drug Administration accepted the T1DM simulator (S2008) as a substitute for preclinical trials for certain insulin treatments, including closed-loop algorithms. This dramatically accelerated the process for the approval of human trial. The simulator has been updated in 2013 in order to better describe the distribution of glucose concentration observed in clinical trials (S2013). However, at the beginning of this project, the simulator validity was not been assessed against T1DM clinical data: in fact, at the time of simulator conception, its model was identifed on a data set of 204 healthy subjects [3], i.e. the sole including not only plasma glucose and insulin measurements but also estimates of glucose fluxes, required to completely identify all the single processes of the model. Moreover, the domain of validity of the simulator was limited

to a single-meal scenario, making the simulator not fully adequate for the in silico testing of the latest AP algorithms, which aim to glucose control in the long-term.

The aim of this thesis was thus to overcome these limitations. To do so, first the simulator validity has been clinically assessed using T1DM data of the now available clinical trials. Then, the model of the simulator has been validated on a multiple-meal scenario, in order to establish the features required to extend the domain of validity of the simulator. A model of diurnal intra-subject variability of insulin sensitivity has been developed and incorporated into the simulator, thus making it suitable for long-term clinical trials. Finally, the use of the simulator has been presented in two illustrative cases, including setting up a paradigm for in silico testing of pharmacokinetics/pharmacodynamics.

For the clinical assessment of the simulator, a T1DM population has been considered [44], in which 24 T1DM subjects received dinner and breakfast in two occasions, for a total of 96 postprandial glucose traces. Measured plasma glucose profiles have been compared with those obtained with both S2013 and S2008, by replicating in 100 in silico adults the same experimental condition of the data, i.e. the same meal amount, insulin bolus and basal delivery. In particular, a matching criterion has been applied: among the 100 in silico subjects, the one behaving similarly to the real patient from a clinical point of view, i.e. showing similar pattern and lying in the same clinically relevant zones (i.e. hypo-, eu-, hyper-glycemia), has been selected as best match, based on a performance index (FIT) that quantify the agreement between real and virtual glucose trace. Then, the glucose trace of the vitual match has been assessed against the data using the continuous glucose error grid analysis (CG-EGA).
The results have highlighted the improvement achieved with S2013 with respect to S2008, particularly in hypoglycemia, where the percentage of accuracy was more than doubled (from 40.7% to 85.9%), thus well reflecting the frequency of hypoglycemic episodes observed experimentally. In addition, the distribution of the most common outcome metrics obtained with

S2013 was very similar to those obtained experimentally. These results have demonstrated that the in silico adults of the S2013 are representative of the same age T1DM population observed in a clinical trial, thus proving that the S2013 is a valid tool usable to test the robustness of AP closed loop control algorithms. However, this procedure did not provide any information about the goodness of the model or the adequacy of the joint parameter distribution included into the simulator. Moreover, two different in silico subjects have been required to well match the post-dinner and post-breakfast glucose traces of the same real subject, suggesting that intra-subject variability, e.g. due to meals or circadian rithms, should be taken into account to well describe blood glucose in a longer scenario, beyond the single meal.

The T1DM model has been thus validated using data coming from a large multicenter AP clinical trial [58], consisting of 47 T1DM subjects receiving dinner (D), breakfast (B) and lunch (L) in three admissions, for a total of 23 hours per session, for which only plasma glucose and insulin measurements were available. To cope with the lack of glucose fluxes − which are available only if complex experiments with multiple tracers are used − a Bayesian approach has been adopted for the T1DM model identification; in particular, the Maximum a Posteriori (MAP) estimator has been employed, and the model parameter joint distribution included into the simulator has been used as a priori information. Variability of model parameters describing glucose absorption has been allowed among B, L, and D, in order to describe possible differences in meal compositions. Moreover, also variability of parameter describing insulin sensitivity (SI), i.e. the ability of insulin to stimulate glucose disposal and inhibit glucose production, has been permitted, based on the notion that T1DM subjects exhibit, on average, a SI trend lower at B compared to L and D [34].
The results of model identification were satisfactory: the model well fitted the glucose data, providing a very good coefficient of determination ($R^2 = 0.962 \pm 0.027$) and precision of parameter estimates (CV $= 1.3\% \pm 0.2\%$). Absorption parameters at B were significantly different ($P < 0.0001$) from those at L and D, reflecting more rapid dynamics of glucose absorption likely due to the different compositions; on the other hand, SI parameters were

not statistically significant, agreeing with that found in [34]. To evaluate the adequacy of the a priori distribution used in the identification process, an iterative two-stage approach has been employed: in this regard, the relative differences in model parameters were modest, getting lower than 5% already after the second iteration. In addition, the posterior distributions were statistically identical among the iterations, except for some of the absorption parameters: however, this result was in some sense expected, since the meal composition in the employed data (solid meal) differs from that used for generating the simulator prior (Jell-O meal [3]). Thus, with the proposed Bayesian approach, the adequacy of the joint parameter distribution included into the simulator has been demonstrated. Moreover, the differences found in glucose absorption and SI parameters proved that the incorporation of a model of diurnal variability of some parameters is a prerequisite to make the simulator able to describe glucose on a multiple-meal scenario. However, it is worth noting that, due to the small amount of samples collected after lunch, at variance with [34], here SI parameters at L and D were assumed to be equal in each subject. This was necessary to guarantee that model parameters at L were estimated with good precision.

Therefore, to assess intra-day variability of SI parameters a different dataset had to be considered, also to avoid the possible confoundung effect introduced by different compositions and carbs contents of the meal. The assessment of daily variation of insulin sensitivity has been thus tackeled considering data of 20 T1DM subjects undergoing a triple-tracer mixed-meal study protocol [4] during B, L, or D in a randomized Latin square design. Then, meal composition were the same among B, L and D: by doing so, the diurnal pattern of SI (estimated from plasma glucose and insulin data with the oral minimal model [13]) was not affected by any other possible confounding effects. In particular, it was found that, on average, SI was lower at B with respect to L and D, but without showing a significant diurnal pattern, both due to the small population size and the high inter-subject variability. Thus, the idea presented in this work was to classify each subject based on his SI diurnal pattern, and then to incorporate this information into the simulator. Seven possible daily patterns of SI have been identified, and their

probabilities have been estimated from the data. Each in silico subject included into the simulator was then associated to one of the seven variability patterns, and its insulin sensitivity parameters, i.e. V_{mx} and k_{p3}, have been made time-varying, according to the specific SI pattern. Consequently, for each in silico subject, three values of carbohydrate-to-insulin ratio (CR, determining the amount of insulin required to cover a meal) have been calculated for B, L and D, respectively. To test the goodness of the model, the same experimental protocol of the data has been replicated in silico: the comparison of simulated glucose traces against the data was satisfactory, showing the same behavior observed in the data, i.e. higher glucose level at B with respect to L and D, and, furthermore, well capturing the variability of the data in all the post-prandial portions.
Hence, incorporating the model of intra-day insulin sensitivity into the T1DM simulator allowed extending its domain of validity to a multiple-meal scenario, thus allowing a more robust AP testing in the long-term.

Finally, two case studies of application of the simulator have been presented. First, the simulator incorporating the developed variability model has been employed for the testing of a new adative AP controller: a Run-to-Run (R2R) approach has been applied to a model predictive control algorithm, and in silico tested on a monthly scenario. In particular, the intra-/inter-day variations in subjects parameters have permitted to highlight the improvement achievable in glucose control by using the R2R approach. In the second example, the simulator has been used for testing the pharmacological effect of an inaled insulin. Specifically, the simulator have served to evaluate the post-prandial glucose in response to different insulin dosing regimens, thus allowing to determine, for each in silico subject, the best insulin pattern to optimally control post-prandial glucose.

In conclusion, in this thesis, the UVA/Padova T1DM simulator has been assessed against T1DM clinical data, proving to be representative of a T1DM population studied in a clinical trial. Then, the model included into the simulator has been fitted, for the first time, on a large T1DM population studied in an almost 24-hour period. To do this, a Bayesian approach has been adop-

ted, since the availability of glucose and insulin data only does not allow the use of a weighted least squares or maximum likelihood estimator. The chosen prior was the joint parameter distribution included in the T1DM simulator for the generation of the in silico subjects. This implicitly has proved the adequacy of the distribution used in the simulator. In addition, this analysis has pointed out the need to introduce diurnal variation of some key parameters, i.e. those related to meal glucose absorption and insulin sensitivity, to be able to well describe the glucose dynamics in a day. A model of intra-day variability of insulin sensitivity has been developed, based on an appropriate dataset, and incorporated into the simulator in order to make it suitable for the in silico testing of new long-term AP control algorithms. Finally, the new time-varying T1DM simulator has been employed for the preclinical testing phase of an adaptive R2R control algorithm and for the evaluation of the pharmacological effect of a novel inhaled insulin, thus proving to be a useful tool for the in silico testing of T1DM treatments.

Further studies are needed to improve the description of SI variability in long-term (beyond the intra-day variability), and also to develop a model of glucose absorption depending on the meal composition, in order to better cope with the factors that influence the glucose variability in the long-term. Further testing is also needed to apply the Bayesian approach to CGM sensor and insulin pump data available in clinical experiments.

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