Modeling Pharmacokinetics and Pharmacodynamics of Glucagon for Simulation of the Glucoregulatory System in Patients with Type 1 Diabetes.

Wendt, Sabrina Lyngbye; Jørgensen, John Bagterp; Boye Knudsen, Carsten; Madsen, Henrik

Publication date: 2017

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):
Modeling Pharmacokinetics and Pharmacodynamics of Glucagon for Simulation of the Glucoregulatory System in Patients with Type 1 Diabetes

Sabrina Lyngbye Wendt

DTU

Kongens Lyngby 2017
The goal of this thesis was to develop a pharmacokinetics/pharmacodynamics (PK/PD) model for glucagon. The proposed PD model included multiplication of the stimulating glucagon effect and inhibiting insulin effect on the endogenous glucose production (EGP). Moreover, the concentration-response relationship of glucagon and EGP was characterized by a non-linear function, where the response saturated for high concentrations of glucagon. The novel EGP model extended Hovorka’s glucoregulatory model to include the effect of glucagon. The PK/PD model described both regular glucagon and a novel glucagon analogue in healthy dogs. The extended glucoregulatory model translated to the human species and described glucose-insulin-glucagon dynamics in healthy subjects and patients with type 1 diabetes (T1D).

The extended glucoregulatory model was successfully validated by leave-one-out cross-validation in seven T1D patients which justified its use for simulations. The final model parameters were estimated from three to four datasets from each patient.

The validated extended glucoregulatory model was used for in silico studies. The model replicated a clinical study of the effect of glucagon at varying insulin levels. The simulations also suggested new glucagon doses to be tested in a similar in vivo study to provide new insight to the relationship between insulin, glucagon, and EGP. Finally, the model was used to conduct a large original simulation study investigating an insulin-dependent glucagon dosing regimen for treatment of insulin-induced mild hypoglycemia.

PK/PD modellen beskrev både almindelig glukagon samt en ny glukagon analog i raske hunde. Den udvidede model for blodsukkerregulering kunne overføres til mennesker og beskrev glukose-insulin-glukagon dynamikken i raske frivillige og patienter med type 1 diabetes (T1D).

Den udvidede model for blodsukkerregulering blev succesfuldt valideret ved brug af leave-one-out krydsvalidering i syv T1D patienter, hvilket retfærdiggjorde dens brug til simuleringer. De endelige modelparametre for hver patient blev estimeret baseret på tre til fire datasæt.

Den validerede udvidede model for blodsukkerreguleringen blev anvendt til in silico studier. Modellen efterviste et klinisk studie, som undersøgte effekten af glukagon ved forskellige insulin niveauer. Simuleringsstudierne blev også brugt til at foreslå nye glukagon doser, som kunne testes i lignende in vivo forsøg og dermed bidrage med ny viden om forholdet mellem insulin, glukagon og EGP. Endelig blev modellen brugt til at gennemføre et omfattende originalt simuleringssstudie, som undersøgte insulinafhængige disorderings vejledninger for glukagon til behandling af insulininduceret mild hypoglykæmi.
Preface

This thesis was prepared in partial fulfilment of the requirements for acquiring an industrial PhD degree at the Technical University of Denmark (DTU). The project commenced mid March 2014 and the dissertation was handed in mid March 2017. The project was a collaboration between the department of Bioanalysis and Pharmacokinetics at Zealand Pharma A/S and the Scientific Computing section of the department of Applied Mathematics and Computer Science (DTU Compute). Carsten Boye Knudsen provided supervision from the company, and associate professor John Bagterp Jørgensen and professor Henrik Madsen provided supervision from the university.

During June 2015 to October 2015 the project was carried out at the division of Metabolic Diseases at Montreal Clinical Research Institute (IRCM). The external stay was hosted by associate professor Rémi Rabasa-Lhoret and assistant professor Ahmad Haidar.

The PhD project was funded by Zealand Pharma A/S and the Innovation Fund Denmark.

Kgs. Lyngby, 14-March-2017

Sabrina Lyngbye Wendt
Acknowledgements

This industrial PhD project has not only demanded collaboration between Zealand Pharma A/S (Zealand) and the Technical University of Denmark (DTU) but also research groups from Institut de Recherches Cliniques de Montréal (IRCM) and Copenhagen University Hospital Hvidovre have played important roles in several of the subprojects during the past three years.

First of all I would like to express my gratitude for my company supervisor and head of department PhD Carsten Boye Knudsen. Thank you for always respecting me and giving me freedom to do my work with creativity. Thank you for teaching me the importance of keeping deadlines as required by the industry, for feedback to all my publications, and for valuable knowledge sharing in the field of pharmacokinetics and pharmacodynamics. I am looking forward to our continued collaboration at Zealand.

I would also like to thank my main supervisor associate professor John Bagterp Jørgensen. Thanks for believing in me as a PhD student and for recommending me for the industrial PhD programme. Especially thank you for your great feedback and dedication during the thesis writing.

Thanks to my second university supervisor professor Henrik Madsen for wanting to be part of my supervisor team. Thank you for contributing with your inexhaustible knowledge on modeling.

A special thanks to associate professor Jan Kloppenborg Møller for devoting many hours to teaching me the math behind and the application of the software package CTSM for R. Thank you for being available and answering all my
questions.

Although not involved in the project, I would like to thank assistant professor Joseph El Youssef for stimulating discussions on endogenous glucose production both face-to-face and digitally many miles apart.

I would also like to give a sincere thanks to my Montréal family - both the academic and the one who made my four months external stay unforgettable. Thanks to associate professor Rémi Rabasa-Lhoret for welcoming me in his lab at IRCM. Thanks to Master student Ali Emami for valuable discussions on the effect of glucagon on endogenous glucose production. Most importantly, thanks to assistant professor Ahmad Haidar for great scientific discussions, knowledge sharing and our friendship.

Thanks to the team at Copenhagen University Hospital Hvidovre. Thanks to my coauthors PhD Signe Schmidt, clinical associate professor Kirsten Nørgaard, clinical professor Sten Madsbad and professor Jens Juul Holst for contributing with clinical knowledge on diabetes and to the paper writing. A special thanks to my fellow PhD student Ajenthen Ranjan for our collaboration on modeling and simulation. Thanks for making the way to Zealand for our meetings and for the easy and informal communication we have had throughout the project.

Lastly, thanks to all my coworkers at DTU and Zealand for showing interest in my work and encouraging me through the thesis writing. Thanks to family and friends for being understanding and helpful during stressful times.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG</td>
<td>blood glucose concentration</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic AMP</td>
</tr>
<tr>
<td>CGM</td>
<td>continuous glucose monitor</td>
</tr>
<tr>
<td>CTSM</td>
<td>continuous time stochastic modeling</td>
</tr>
<tr>
<td>DM</td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>EGP</td>
<td>endogenous glucose production</td>
</tr>
<tr>
<td>FDA</td>
<td>Food &amp; Drug Administration</td>
</tr>
<tr>
<td>HbA1c</td>
<td>glycated hemoglobin A1c</td>
</tr>
<tr>
<td>IOB</td>
<td>insulin on board</td>
</tr>
<tr>
<td>IIR</td>
<td>insulin infusion rate</td>
</tr>
<tr>
<td>IM</td>
<td>intramuscular</td>
</tr>
<tr>
<td>IV</td>
<td>intravenous</td>
</tr>
<tr>
<td>MAP</td>
<td>maximum a posteriori</td>
</tr>
<tr>
<td>MAPE</td>
<td>mean absolute prediction error</td>
</tr>
<tr>
<td>MCMC</td>
<td>Markov Chain Monte Carlo</td>
</tr>
<tr>
<td>ML</td>
<td>maximum likelihood</td>
</tr>
<tr>
<td>MPE</td>
<td>mean prediction error</td>
</tr>
<tr>
<td>PD</td>
<td>pharmacodynamics</td>
</tr>
<tr>
<td>PID</td>
<td>partial integral derivative</td>
</tr>
<tr>
<td>PK</td>
<td>pharmacokinetics</td>
</tr>
<tr>
<td>SC</td>
<td>subcutaneous</td>
</tr>
<tr>
<td>T1D</td>
<td>type 1 diabetes</td>
</tr>
<tr>
<td>TDD</td>
<td>total daily dose</td>
</tr>
<tr>
<td>Tmax</td>
<td>time to maximum concentration</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
</tbody>
</table>
Contents

Summary (English) i
Summary (Danish) iii
Preface v
Acknowledgements vii
Abbreviations ix

1 Introduction 1
1.1 Hypotheses and Aims 3
1.2 Contributions 4
1.2.1 Summary 6

2 Glucagon 7
2.1 About the Peptide 7
2.2 Pharmacokinetics 8
2.2.1 Intravascular PK 8
2.2.2 Extravascular PK 10
2.2.3 Flip-flop kinetics 13
2.2.4 Assays for Sample Analysis 13
2.3 Pharmacodynamics 14
2.4 Modes of Use 16
2.4.1 Rescue Treatment 16
2.4.2 Treatment of Mild Hypoglycemia 17
2.4.3 The Dual Hormone Artificial Pancreas 18
2.4.4 Glucagon-only Pump 20
2.5 Drugs in Development 20
2.5.1 Nasal Glucagon by Eli Lilly and Company ........................................... 21
2.5.2 XerSol™ Glucagon by Xeris Pharmaceuticals ................................... 21
2.5.3 BioChaperone® Glucagon by Adocia .................................................... 22
2.5.4 Dasiglucagon by Zealand Pharma ........................................................... 22

3 Methods ............................................................................................................. 25
3.1 Model Basics .................................................................................................. 25
   3.1.1 Ordinary Differential Equations ............................................................... 26
   3.1.2 Stochastic Differential Equations ............................................................. 26
   3.1.3 Simulation ................................................................................................ 26
   3.1.4 Prediction ............................................................................................... 27
   3.1.5 Lamperti Transformation ....................................................................... 28
3.2 Likelihood Principles ..................................................................................... 31
   3.2.1 Profile Likelihood .................................................................................. 33
   3.2.2 Bayesian Inference ................................................................................ 35
   3.2.3 Maximum a Posteriori Estimation .......................................................... 35
3.3 Parameter Estimation in CTSM-R ................................................................. 37
   3.3.1 Fitting One Data Set ............................................................................... 37
   3.3.2 Fitting Multiple Data Sets ...................................................................... 38
   3.3.3 Calculating the Parameter Uncertainties ................................................. 38
   3.3.4 Optimization with Genetic Algorithms ................................................. 39

4 Models of Endogenous Glucose Production ....................................................... 43
4.1 Simulation Scenario ....................................................................................... 44
4.2 Extension of the Minimal Model ..................................................................... 46
4.3 UVA/PADOVA Type 1 Diabetes Simulator ................................................... 48
4.4 Multiplicative Model ..................................................................................... 50
4.5 Glucose-Glucagon Model .............................................................................. 52
4.6 Glucagon Evanescent Effect ......................................................................... 53
4.7 Glucagon Rate of Change ............................................................................. 55
4.8 Saturation of Glucose Production .................................................................. 57
4.9 Model Comparison ........................................................................................ 59
   4.9.1 Model Structure ..................................................................................... 59
   4.9.2 Impact of Glucose .................................................................................. 60
   4.9.3 Impact of Insulin .................................................................................... 60
   4.9.4 Glucagon time-response relationship ................................................... 61
   4.9.5 Absolute Glucagon Concentration ......................................................... 63
   4.9.6 Glucagon Concentration-Response Relationship ................................... 64
   4.9.7 Closing Remarks .................................................................................... 66
5 Simulating Glucoregulatory Dynamics

5.1 Simulation Model

5.1.1 Insulin Pharmacokinetics

5.1.2 Glucagon Pharmacokinetics

5.1.3 Glucose Pharmacodynamics

5.1.4 Validation

5.1.5 Parameters

5.1.6 Strengths and Limitations

5.2 In Silico Studies

5.2.1 Replication of an in Vivo Study

5.2.2 Dose-Response Study

5.2.3 Dose Selection Study

5.2.4 In Silico as Supplement to in Vivo

6 Conclusions

6.1 Future Work

A Technical Report 1

PK/PD in Healthy Dogs

B Conference Paper

PD in Healthy Humans

C Journal Paper 1

PK/PD in Diabetes Patients

D Technical Report 2

Simulation Studies

E Journal Paper 2

Simulations for Clinical Recommendations

Bibliography
Chapter 1

Introduction

Diabetes mellitus (DM) or simply diabetes, is a chronic disorder where the body does not have the ability to control the blood glucose concentration (BG). Over time, untreated DM leads to irreversible damage to essential tissues of the body like nerves and blood vessels. The World Health Organisation (WHO) estimated that DM affected 422 million people worldwide in 2014 [1]. The disease is either due to destruction (often autoimmune) or removal of insulin producing cells in the pancreas (type 1, 10%) or due to lifestyle related lowered insulin sensitivity by insulin consuming cells of the body (type 2, 90%).

For almost 100 years, diabetes patients have been treated with insulin which is a hormone that lowers the BG. Traditionally, type 1 diabetes (T1D) was controlled by measuring BG via multiple daily finger pricks and adjusting insulin dosage accordingly to maintain normoglycemic range (70-125 mg/dL or 4-7 mmol/L). The recent introduction of continuous glucose monitors (CGM) has significantly improved BG control in adults with T1D [2]. However, BG regulation using insulin only is a trade-off between achieving closer glycemic control, i.e. minimizing time in hyperglycemia (high BG), and increasing the risk of hypoglycemia (low BG) [3].

The biggest fear to parents of children with diabetes is an unnoticed hypoglycemic event [4]. Hypoglycemic episodes occur when the ratio of glucose to insulin is out of balance: e.g. excessive insulin dosage, lack of glucose intake due to a missed meal or increased use of glucose because of aerobic exercise.
Figure 1.1: Concept of a closed loop artificial pancreas: The CGM measures the glucose concentration, sends it to a control algorithm that determines the hormone dosing, which is then delivered via a pump.

Depending on the severity of hypoglycemia it can lead to seizures, coma or even death. Often, the dangerous events are not associated with warning symptoms and they typically occur during sleep [3, 5]. Large population studies estimate that 6% of deaths among young diabetes patients (< 40 years) can be attributed to dead-in-bed syndrome most likely due to fatal hypoglycemia [6].

In the healthy body, BG is not regulated by insulin alone but by several hormones serving to control BG e.g. glucagon that increases release of glucose from storage as glycogen in the liver. To mimic non-diabetes function and improve BG control, recent studies re-investigated the counterregulatory effect of using glucagon together with insulin [7].

For more than forty years, scientists have worked on developing an artificial pancreas to simplify the life of T1D patients by minimizing the daily treatment decisions and thereby improve quality of life [8]. The artificial or bionic pancreas is an interconnected system of devices including a CGM, a subcutaneous (SC) insulin (and glucagon) infusion pump and a control algorithm determining
1.1 Hypotheses and Aims

The main ambition in the project was to develop high quality PK/PD models of a novel glucagon analogue and regular glucagon. The models should be usable for simulations that could provide new insights into the glucoregulatory dynamics, optimizing study designs and ultimately accelerating the drug development of glucagon analogues. Furthermore, good models could be used for designing and testing dual hormone artificial pancreas control algorithms, which was however beyond the scope of this project.

The project was based on regular glucagon and when possible a novel glucagon analogue developed by Zealand. Previous research related to numerical modeling, simulation, optimization and control for an artificial pancreas provided a foundation for the project [15, 16, 17]. The project was structured in three...
stages with distinct foci. The three stages represented natural progression and extension of previous research in the PhD project.

1. The first stage of the project focused on characterization of PK/PD properties of a novel soluble glucagon analogue and marketed glucagon in animals. Moreover, this stage aimed to develop a model of the glucose PD as a function of insulin and glucagon.

2. The second part of the project focused on applying the developed PK/PD model to human data. Moreover, this stage aimed to cross-validate the new PD model to be used for simulation studies.

3. The third stage of the project focused on using the human PK/PD model for clinical relevant simulation studies to aid in better understanding of the dynamics and design of experiments.

1.2 Contributions

This PhD project has contributed with five publications and five posters presented at conferences in Europe and the USA. A short resume of the publications are provided here in order of publication date.

Wendt et al. published a technical report with the title "PK/PD modelling of glucose-insulin-glucagon dynamics in healthy dogs after a subcutaneous bolus administration of native glucagon or a novel glucagon analogue" through the Technical University of Denmark in April 2016 [18]. The publication is included in Appendix A. The report focused on developing a simulation model of the glucose-insulin-glucagon dynamics using data from healthy dogs. The Hovorka glucoregulatory model was expanded to include a model description of the stimulatory effect of glucagon on endogenous glucose production (EGP). The report explained the applied methods including maximum a posteriori (MAP) parameter estimation and profile likelihood analysis. Moreover, the report compared PK/PD model parameters of regular reconstituted glucagon and a novel stable liquid glucagon analogue invented by Zealand. The PD model described data satisfactorily for both glucagon and the analogue, and the parameter estimates were not significantly different between the two compounds. Some of the applied methods are thoroughly explained in Chapter 3.

To investigate if the glucose-insulin-glucagon model translated to the human species, Wendt et al. published a conference paper with the title "Modelling of
1.2 Contributions

Glucose-Insulin-Glucagon Pharmacodynamics in Man" which was published at the 38th annual international conference of the IEEE Engineering in Medicine and Biology Society (EMBC'16) in Orlando, Florida during August 2016 [19]. The paper is included in Appendix B.

The paper focused on fitting the previously published glucose-insulin-gluca gon model to data from healthy humans. Model parameters were estimated by MAP and parameter identifiability investigated with profile likelihood analysis. The model fitted data well and enabled simulations of the glucose-insulin-gluca gon dynamics within physiologic concentration ranges: glucagon (180-8000 pg/mL), insulin (1.2-81.9 mU/L) and glucose (3.3-11.5 mmol/L).

The EGP model is compared to other glucose production models and discussed in Chapter 4.

A simulation model is rarely useful or trusted without validation, therefore Wendt et al. published a journal paper with the title "Cross-Validation of a Glucose-Insulin-Glucagon Pharmacodynamics Model for Simulation using Data from patients with Type 1 Diabetes" which was accepted for publishing in Journal of Diabetes Science and Technology in January 2017 and published online in February [20]. The published paper is included in Appendix C.

The paper focused on validating the proposed glucose-insulin-gluca gon model in patients with T1D and providing the final model parameters of the virtual patients to be used for simulation studies. Maximum likelihood (ML) and MAP methods were used for parameter estimation. Validation was carried out as a four-fold leave-one-out cross-validation with data from eight patients and assessed using mean predictive error (MPE) and mean absolute predictive error (MAPE). The model was successfully validated in seven of the patients.

The complete glucoregulatory model with equations and parameters are presented in Chapter 5.

Wendt et al. then published a technical report with the title "Simulating Clinical Studies of the Glucoregulatory System: in Vivo Meets in Silico" through the Technical University of Denmark in February 2017 [21]. The report is included in Appendix D.

The report contains results of various simulation studies with the validated glucose-insulin-gluca gon model including replication of an in vivo study. It also contains simulation studies to investigate the glucoregulatory dynamics of discontinuing insulin and glucose infusions prior to gluca gon administration, the delayed effect of insulin, timing of data sampling, and carryover effects from multiple SC doses of gluca gon. Based on simulations, the report discussed two hypotheses of how the interactions between insulin and gluca gon impact the glucose response. Finally, a study design that could potentially explore if the hypotheses are true or false were proposed.

Chapter 5 presents highlights of the report.
The simulation model showed its clinical relevance in a paper with the title "Relationship between Optimum Mini-doses of Glucagon and Insulin Levels when Treating Mild Hypoglycaemia in Patients with Type 1 Diabetes - A Simulation Study" which will be submitted to a high impact journal during Summer 2017 [22]. A draft of the paper is included in Appendix E. The paper presents an original simulation study with the seven validated virtual patients. After insulin-induced mild hypoglycaemia the success of various sized glucagon boluses in restoring plasma glucose in the presence of varying insulin levels was evaluated. Insulin levels were interpreted as serum concentration or effect in the body either uncorrected or normalized to factors describing the individual insulin sensitivities. The success of glucagon in treating mild hypoglycaemia was based on clinically relevant criteria. Chapter 5 presents one of the study results.

1.2.1 Summary

In summary, the main contribution of this thesis is a mathematical model describing the effect of insulin and glucagon on the EGP in patients with T1D. The novel model is built into Hovorka’s glucoregulatory model. The PD model is cross-validated using clinical data in seven T1D patients and the PK/PD model parameters are estimated using ML and MAP methods. The glucose-insulin-glucagon model can be used for simulations that can provide new insights to the glucose dynamics and be a supplement to clinical studies.
Chapter 2

Glucagon

This chapter focuses on the hormone glucagon from a drug perspective. Effects of the body on the drug, PK, and effects of the drug on the body, PD, are explained. The PK for different possible administration routes of glucagon as well as the importance of assay choice for the PK/PD relationship are highlighted. Furthermore, this chapter provides a brief overview of the regulation of EGP. Current and future applications of glucagon include but are not limited to single-use rescue treatment from severe hypoglycemia, multi-use mini-dose for treatment of mild hypoglycemia, and safety feature in a dual hormone artificial pancreas. The chapter concludes with an overview of companies officially working on developing glucagon with increased physical and chemical stability and improved user-friendliness.

2.1 About the Peptide

Glucagon is a 29-amino acid polypeptide hormone secreted by the α cells of the islets of Langerhans in the pancreas. The portion of the pancreatic islets secreting glucagon is 20%. In the liver, glucagon acts by binding to membrane-bound receptors that activates G proteins and increases cyclic AMP (cAMP) synthesis, which ultimately lead to increased glucose output from the pancreas.
High glucagon concentrations cause breakdown of fats (lipolysis) in the adipose tissues whereas the hormone has little effect on skeletal muscle and the nervous system [23]. Natural glucagon is not physically nor chemically stable in an aqueous liquid solution at natural pH. Once dissolved, the peptide starts to aggregate and form fibrils which influences its reactivity. Degraded glucagon can also cause cytotoxic effects and should therefore be avoided [11].

2.2 Pharmacokinetics

Glucagon PK exert linear dose dependency after intravenous (IV) administration of 0.25-2.0 mg in healthy volunteers [24], and after SC administration of 0.11-0.44 mg in patients with T1D [25]. Although doses up to 1.0 mg was administered SC in the study by Blauw et al. [25], the two highest doses did not follow the same linear dose dependency as the smaller doses.

The less than expected concentrations of glucagon after high SC doses could be explained by differences in bioavailability. In general, the bioavailability of glucagon differs between administration routes as listed in Table 2.1. The low bioavailability of glucagon after SC administration was also observed in dogs [18]. Bioavailability following SC or intramuscular (IM) administration can only be determined if studies with IV PK data in same species are available since the PK after IV dosing is defined as reference. Following SC or IM administration, bioavailability can be modelled as elimination directly from the injection site, or by reducing the modelled administered dose with a fraction according to the bioavailability.

Table 2.1: Glucagon bioavailability by administration route calculated from Graf et al. using AUC_{0–inf} [24]. Bioavailability after IV administration is defined as 100%.

<table>
<thead>
<tr>
<th></th>
<th>IV</th>
<th>SC</th>
<th>IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioav</td>
<td>100%</td>
<td>36%</td>
<td>26%</td>
</tr>
</tbody>
</table>
2.2 Pharmacokinetics

Plasma glucagon, pg/mL

Time, minutes

IV
SC
IM

Figure 2.1: Plasma glucagon concentrations in healthy volunteers after injection of 1 mg glucagon at time 0, re-sketched from [26].

When viewed on a logarithmic scale, the PK profile after an IV glucagon bolus reveals to be biphasic. Normally for such an IV PK profile, the first phase represents distribution to peripheral tissues and the second phase represents elimination [27]. The biphasic PK profile and corresponding compartmental model are illustrated in Figure 2.2. The PK model for IV glucagon administration with a peripheral distribution compartment is mathematically described by:

\[
\frac{dQ_C(t)}{dt} = u_{IV}(t) + k_rQ_P(t) - k_dQ_C(t) - k_eQ_C(t) \tag{2.1}
\]

\[
\frac{dQ_P(t)}{dt} = k_dQ_C(t) - k_rQ_P(t) \tag{2.2}
\]

\(Q_C\) is the glucagon mass in the central measured compartment (plasma) and \(Q_P\) is the glucagon mass in the peripheral tissues. \(u_{IV}\) is the IV glucagon bolus. \(k_d\) is the transfer rate constant of distribution, \(k_r\) is the transfer rate constant of redistribution, and \(k_e\) is the elimination rate constant.
Figure 2.2: PK model for IV administration with a peripheral distribution compartment. The PK model is expressed mathematically by (2.1)-(2.2).

### 2.2.2 Extravascular PK

Building on the PK model for IV administration in (2.1)-(2.2), the PK model for SC or IM administration would add a compartment from which the dose is absorbed into the central compartment, see Figure 2.3. Mathematically this can be expressed as:

\[
\begin{align*}
\frac{dQ_I(t)}{dt} &= u_I(t) - k_a Q_I(t) \\
\frac{dQ_C(t)}{dt} &= k_a Q_I(t) + k_r Q_P(t) - k_d Q_C(t) - k_e Q_C(t) \\
\frac{dQ_P(t)}{dt} &= k_d Q_C(t) - k_r Q_P(t)
\end{align*}
\]  

(2.3)  

(2.4)  

(2.5)

\(Q_I\) is the glucagon mass at the extravascular site. \(u_I\) is the glucagon bolus delivered either SC or IM. \(k_a\) is the absorption rate constant.

When the absorption phase is slower than the distribution phase it is not possible to separate the two from one another. Separate phases for absorption and distribution are not visible in glucagon PK data from humans and dogs after SC or IM bolus injection [18, 26], thus the model for extravascular administration of glucagon is for practical purposes reduced to:

\[
\begin{align*}
\frac{dQ_I(t)}{dt} &= u_I(t) - k_a Q_I(t) \\
\frac{dQ_C(t)}{dt} &= k_a Q_I(t) - k_e Q_C(t)
\end{align*}
\]  

(2.6)  

(2.7)

Figure 2.4 visualizes a theoretical example of time-concentration profile and...
Figure 2.3: PK model for SC or IM administration with a peripheral distribution compartment. The PK model is expressed mathematically by (2.3)-(2.5).

A compartmental model describing extravascular glucagon administration. The model was used by Wendt et al. to fit glucagon PK data after SC administration in dogs, healthy volunteers and T1D patients [18, 20]. A simpler version of the model to describe SC glucagon PK was used by Haidar et al. with $k_a$ being equal to $k_e$ [28].

Various other and more complex models describing exogenous glucagon administration have been proposed by Lv et al. [29]. The best model turned out to be the simplest depicted in Figure 2.3 and described mathematically by

\[
\frac{dQ_I(t)}{dt} = u_I(t) - k_a Q_I(t) - k_{e,1} Q_I(t) \tag{2.8}
\]
\[
\frac{dQ_T(t)}{dt} = k_a Q_I(t) - k_t Q_T(t) \tag{2.9}
\]
\[
\frac{dQ_C(t)}{dt} = k_t Q_T(t) - k_e Q_C(t) + k_e Q_{basal} \tag{2.10}
\]

$k_{e,1}$ is the elimination rate constant from the injection site, $Q_T$ is a transit compartment, $k_t$ is the transfer rate constant from the transit compartment to the central compartment, and $Q_{basal}$ is the constant endogenous production. Comparing (2.8)-(2.10) to (2.6)-(2.7) the only differences are an extra transit compartment, bioavailability modelled by elimination directly from the injection site, and basal concentration modelled by a constant endogenous production countered by the elimination in steady state. However, adding an extra
(a) Time-concentration profile, from [27].

(b) Compartmental model.

Figure 2.4: PK model for SC or IM administration. The PK model is expressed mathematically by (2.6)-(2.7).

Figure 2.5: PK model for SC administration described by Lv et al. [29]. The PK model is expressed mathematically by (2.8)-(2.10).

transit compartment and modeling bioavailability as elimination from the injection site would require measurements of both compartments in order for all the parameters to be identifiable. With no figures showing actual data fitting of the complex models, the increased complexity and uncertainty seem unnecessary when simpler models are sufficient to describe glucagon PK after extravascular administration as published by Haidar et al. and Wendt et al. [18, 20, 28]. Usually, both healthy and T1D patients have a basal level of glucagon. The basal concentration can be modelled by adding a constant mass to (2.1) or (2.7) so that it equals 0 in steady state as demonstrated in (2.10). Another option is adding a constant concentration when calculating the glucagon concentration in the central compartment from the mass $Q_C$ [18, 20, 28].
2.2 Pharmacokinetics

2.2.3 Flip-flop kinetics

The PK exerts flip-flop kinetics when elimination rate is faster than absorption rate after extravascular administration [27, 30, 31]. Figure 2.6 illustrates how the interpretation of the time-concentration profile after SC administration changes compared to Figure 2.4a. Flip-flop kinetics can be identified when the half-life is longer following extravascular dose administration compared to intravascular [31]. Reading off the plot by Mühlhauser et al. [26], the half-life of glucagon is around 10 minutes after IV injection and close to 60 minutes after SC administration. Graf et al. found glucagon half-lives of 13, 27 and 23 minutes after IV, SC and IM injections, respectively [24]. Both studies confirm that glucagon exerts absorption limited elimination after extravascular dose administration which was also pointed out by Wendt et al. [18]. Knowledge of flip-flop behaviour is important in order to estimate the correct apparent clearance ($\text{Cl}_F$) and apparent volume of distribution ($V_{d,F}$) which are related as $k_e V_{d,F} = \text{Cl}_F$. Due to the flip-flop phenomenon, the values of $k_a$ and $k_e$ can swap without changing the fit. To avoid this pitfall, $k_e$ can be parametrized so it is always greater than or equal to $k_a$ by $k_a + \Delta k$ with $\Delta k$ being positive or zero et al. [20, 31].

2.2.4 Assays for Sample Analysis

The absolute measured glucagon concentration in clinical samples should be compared between studies with caution since commercially available glucagon assays differ in their detection specificity, precision and accuracy [32]. Because of these differences, glucagon PK model parameters estimated from clinical data analysed by different methods, can not necessarily be compared. Changes in assay performance could influence all PK model parameters (absorption rate, elimination rate, clearance, baseline). Moreover, when glucagon PK is influ-
Figure 2.7: Illustration of the sigmoid $E_{max}$ model for different values of $\gamma$ with a logarithmic x-axis.

enced, parameters describing the response sensitivity to glucagon in the PD model will be influenced, too. Therefore, interpreting parameters of a PD model with glucagon as input can not be isolated from the method used for glucagon sample analysis [33]. This subject will be discussed further in Chapter 4.

2.3 Pharmacodynamics

Glucagon acts through binding to a receptor as mentioned in Section 2.1. Generally, receptor mediated responses can be modelled by the full Hill equation also called the sigmoid $E_{max}$ model [34]

$$E = E_0 + \frac{(E_{max} - E_0)C^\gamma}{EC_{50}^\gamma + C^\gamma}$$  \hspace{1cm} (2.11)

$E$ is the effect, $E_0$ is the baseline effect, $E_{max}$ is the maximal effect, $EC_{50}$ is the concentration producing half maximal effect, $C$ is drug concentration and $\gamma$ translates to number of drug molecules per receptor. When $\gamma$ is equal to 1, (2.11) reduces to the Hill equation. Figure 2.7 displays the graphical interpretation of $\gamma$ in (2.11).

There is a limited number of receptors available for stimulation, thus the re-
2.3 Pharmacodynamics

Figure 2.8: Glucagon action model, modified from [35]. Illustration of how glucagon acts via receptor mechanisms to stimulate EGP by glycogenolysis and gluconeogenesis, and the evanescence effect governing glycogenolysis only.

Response to glucagon must saturate for high concentrations of the hormone. Saturation of dose-response was observed in two previously mentioned studies where healthy volunteers received 1 mg glucagon SC and IM which produced very similar glucose responses, even though the bioavailability between the two administration routes differ as outlined in Table 2.1 [24, 26].

Glucagon primarily affects the liver where it stimulates breakdown of glycogen to glucose through glycogenolysis [36]. The liver is highly sensitive to small changes in the glucagon concentration (<10 pg/mL) and responds immediately to the stimulus; thus the hormone acts as a "fine tuner" of the glucose output. Glucagon has no acute effect on formation of glucose from precursors such as lactate, pyruvate, amino acids, and glycerol known as gluconeogenesis [37]. However, during prolonged hypoglycemia exceeding three hours, glucagon stimulates gluconeogenesis rather than glycogenolysis [38]. The ambient glucose level itself does not influence the immediate response to exogenously dosed glucagon [23]. Figure 2.8 illustrates the glucagon action on the liver.

During continued exposure to glucagon, glycogenolysis wanes over time [36, 39, 40, 41]. This phenomenon has been named the evanescence effect. An explanation for the transitory response to glucagon might be desensitization of the cAMP receptor [42]. The reduced responsiveness to glucagon seem to be fully
expressed after two hours of continuous stimulation both in vivo and in vitro \[36, 39, 40, 41, 42\]. The refractory period needed to restore normal glucagon responsiveness remains unknown in vivo and little is known in vitro \[43\].

Glucagon is not the only hormone influencing the glucose homoeostasis; as opposed to glucagon, insulin inhibits glucose production \[36\]. However, insulin has little if any effect on gluconeogenesis in both healthy volunteers and T1D patients, whereas it efficiently inhibits glycogenolysis \[36, 44, 45\]. A study in healthy volunteers found that the glucose production was completely suppressed when insulin levels exceeded 60 mU/L \[46\]. Another study compared the glucose production during high glucagon and basal insulin, high insulin and basal glucagon, and high concentrations of both insulin and glucagon in dogs \[47\]. The response to glucagon during high insulin infusion was blunted. This indicates that insulin is more powerful at inhibiting glucose production than glucagon is at stimulating it. In line with these findings, a study in T1D patients by El Youssef et al. found that the response to micro-doses of glucagon depends on the ambient insulin level \[48\]. This inability of glucagon to stimulate glucose production sufficiently in the presence of high insulin levels was also observed in a closed-loop study by Russell et al. \[49\], where the average insulin level was highest among the group in which glucagon was ineffective in preventing hypoglycaemia.

### 2.4 Modes of Use

As glucagon raises the blood glucose, the hormone could be used for treatment in all indications having difficulties keeping the blood glucose above hypoglycaemia. The application is not limited to T1D, although that will be the prevailing disease to treat. Within diabetes management glucagon can be used in several different modes with increasing complexity: as a one-time rescue treatment, as multiple daily correcting bolus injections, and in a closed-loop dual hormone artificial pancreas. Glucagon also has the potential to be used in a glucagon-only pump for patients with no glucagon production or increased insulin production.

#### 2.4.1 Rescue Treatment

Patients with T1D have a blunted endogenous glucagon response to hypoglycaemia making it difficult to recover from low blood sugars \[50\]. If the patient experiences severe hypoglycaemia and is unconscious there is two options to increase glucose levels: glucose IV, or glucagon SC or IM. A person without a
medical background or training is not able to insert an IV catheter, but most people can give a SC or IM injection. The currently marketed rescue kits in Figure 2.9 with 1 mg glucagon involve several difficult and crucial preparation steps and the drug is therefore often not administered as intended by the manufacturer. In a study examining the usability of GlucaGen HypoKit, an average of 20-30% of the intended dose was not administered and 69% of parents experienced handling difficulties [51].

2.4.2 Treatment of Mild Hypoglycemia

More often than severe hypoglycemia, patients with T1D experience mild hypoglycemia while still conscious. To increase their blood glucose levels, patients have to ingest carbohydrates like dextrose which are rapidly absorbed. This snacking behaviour unintentionally but inevitably increases the total daily calorie intake of the patient. Another means of increasing glucose levels slightly, is through mini-doses of glucagon [54]. Administering glucagon rather than oral carbohydrates when patients experience mild hypoglycemia will metabolize already ingested calories thereby increasing the energy expenditure of the patient [55].

A study by Ranjan et al. in T1D patients showed that boluses of 100-300 µg glucagon was sufficient to increase the blood glucose levels after insulin-induced mild hypoglycemia [56]. Strategies for mini-doses of glucagon in combination with a closed-loop single hormone artificial pancreas have only started to emerge [57].

There are a few caveats to treating mild hypoglycemia with glucagon. As highlighted in Section 2.3, the ambient insulin level inhibits the glucose stimulating effect of glucagon and must therefore be considered. Moreover, Ranjan et al.
found that a low carbohydrate diet impairs the effect of glucagon in treating mild insulin-induced hypoglycemia \cite{58}. This impairment is speculated to be due to decreased storage of glycogen for glycogenolysis. On the contrary, Castle \textit{et al.} found that eight mini-doses of glucagon within 18 hours did not deplete the glycogen stores in the liver nor did they influence the response of subsequent boluses \cite{59}.

2.4.3 The Dual Hormone Artificial Pancreas

Although substitution of oral carbohydrates with glucagon as a treatment option for mild hypoglycemia is sought, it will inevitably introduce another decision point for T1D patients. Instead, automating the decision making of both insulin and glucagon administration will alleviate the burden of diabetes care and is the ultimate treatment goal for T1D as illustrated in Figure 2.10. Less than a decade after the first feasibility trials of dual hormone closed-loop glucose control in pigs \cite{9,60}, several closed-loop solutions both single and dual hormone are being developed worldwide \cite{61}. The American Food & Drug Administration (FDA) has encouraged the development of artificial pancreas systems and approved the first single hormone closed-loop device in Autumn 2016, the Medtronic Minimed 670G \cite{62,63}. However, a community of patients and care-
givers called OpenAPS have long been desperately waiting for a commercial solution and in the meantime they built their own artificial pancreas devices, willing to put their glucose regulation in the "hands" of algorithms and devices not rigorously tested nor approved by the authorities [64]. These individuals are probably the ones having most experience with the use of closed-loop systems in real life without restrictions other than personal ones. They found that using a self-built system rather than controlling their glucose levels themselves, their HbA1c dropped, time in range increased and most subjects experienced increased sleep quality.

Undoubtedly, in T1D patients dual hormone closed loop systems increase time in target and reduce the risk of hypoglycemia compared to conventional therapy [65, 66, 67, 68, 69, 70]. Researchers argue whether a single hormone closed-loop system is sufficient for treatment of T1D or if a dual hormone artificial pancreas with glucagon adds benefits outweighing the increased complexity and costs associated with a second hormone [8, 7, 10, 71, 72, 73, 74]. With the ongoing debate, it is surprising that only few studies demonstrate head-to-head comparisons of single and dual hormone artificial pancreas systems [10]. The first study to make direct comparisons found that dual hormone closed-loop reduced hypoglycemia and the need for oral carbohydrates compared to single hormone closed-loop [75]. Subsequent studies from the group located in Montréal, Quebec have showed varying benefits of dual hormone versus single hormone, although the risk of hypoglycemia seems reduced in the dual hormone setting [76, 77, 78, 79].

Advocates of including glucagon in a dual hormone artificial pancreas argue that glucagon can prevent hypoglycemia induced from exercise. A study in T1D patients found that dual hormone closed-loop outperformed single hormone closed-loop during announced continuous and interval exercise [80], whereas another study did not find further benefits of glucagon compared to adjustment of insulin infusion before exercise onset [81]. In the latter study it should be noted that patients had glucose levels in the higher end of the normoglycemic range borderline hyperglycemia before exercise onset. Patients would instinctively refrain from exercise due to the fear of hypoglycemia when the blood glucose is in the lower normoglycemic range or borderline hypoglycemia [82]. In those situations, glucagon would add a major benefit to a closed-loop system. Moreover, a study in T1D patients found that including glucagon in an artificial pancreas can alleviate the cumbersome burden of counting carbohydrates before meal ingestion [83].

The dual hormone artificial pancreas might not only benefit T1D patients, but potentially also type 2 diabetes patients [84]. Patients becoming diabetic from one day to the other e.g. after removal of the pancreas due to cancer or chronic pancreatitis, will benefit from an automatic closed-loop device that safely and efficiently controls the blood glucose, too. Moreover, a dual hormone closed-loop system could be applied to critically ill patients not necessarily having diabetes but admitted to the intensive care unit. These patients are prone to experience
stress-induced hyperglycaemia, and hypoglycaemia following septic shock and thus a safe and automated device for blood glucose regulation would reduce costs and increase survival rate \[85\].

### 2.4.4 Glucagon-only Pump

Glucagon is not only relevant for treatment of hypoglycaemia in T1D patients. An orphan disease named congenital hyperinsulinism belongs to the category of hyperinsulinemic hypoglycaemia \[86\]. Patients simply produce too much insulin leading to frequent episodes of hypoglycaemia. A glucagon-only closed-loop device could be used to continuously monitor the blood glucose and provide correcting boluses of glucagon throughout the day to prevent and treat hypoglycaemia.

### 2.5 Drugs in Development

The development of new drugs takes several years, and the time from a drug is discovered till it reaches the consumer is increasing. The increasing time and cost associated with drug development is mainly due to regulatory authorities demanding larger and longer clinical trials. Traditionally, drug development can be divided in distinct phases:

- **pre-clinical**: Studies in animals
- **clinical**: Trials in humans
  - phase I: Trials in healthy volunteers - focus on safety
  - phase II: Trials in small number of patients - focus on efficacy
  - phase III: Trials in large number of patients - confirmation of safety and efficacy

Pre-clinical and clinical trials are conducted to evaluate drug safety, efficacy and dosing. Computer simulations with reliable population models can aid drug-development by allowing *in silico* trials exploring dosing size and regimen further, and investigate the effects of potential changes in clearance or metabolism \[87\].

The extent of clinical trials required to obtain approval from the authorities for a new drug depend on the novelty of the drug compared to previously approved
and marketed drugs. Thus getting approval of an analogue to a hormone, rather than an optimized pharmaceutical formulation of an already approved hormone, requires substantially more work and increased costs [88].

As described in Section 2.1, glucagon is not stable in an aqueous liquid solution and must be reconstituted immediately before use through numerous steps making it prone to errors. Various pharmaceutical companies across the globe are or were therefore focusing on creating a ready-to-use product. Some approaches have failed including immobilizing glucagon in a micelle [89, 90] and developing a glucagon analogue [91, 92]. Officially, there is currently four companies competing in accessing the market with an improved solution to treating hypoglycemia with glucagon. Eli Lilly and Company is altering the delivery route circumventing the need for stability in liquid solution [93]. The remaining three companies focus on developing liquid stable glucagon: Xeris Pharmaceuticals by optimizing the formulation [94], Adocia by adding excipients [95], and Zealand Pharma by altering the sequence of the original peptide thereby creating an analogue [14].

2.5.1 Nasal Glucagon by Eli Lilly and Company

In 2015 Eli Lilly and Company acquired a glucagon nasal powder from Locemia Solutions for intranasal administration [93]. The product consists of ten percent glucagon in dry powder and is intended for absorption through the nasal mucosa [96].

The absorption of nasal glucagon appear to be delayed compared to IM administration [97]. Moreover, the bioavailability after intranasal administration is lower than after IM injection, since a three times higher dose is required through the intranasal route to produce a similar response [96]. The intranasal administration of glucagon was associated with more adverse events related to head, nose, eyes, and throat than IM administration [97].

As of beginning 2017, the product is in phase III [93].

2.5.2 XeriSol™ Glucagon by Xeris Pharmaceuticals

The American based company Xeris Pharmaceuticals is developing an improved formulation for native human glucagon named XeriSol™ Glucagon (XeriSol) [94]. The native peptide is stabilized in a non-aqueous solution containing dimethyl sulfoxide (DMSO) [98]. The drug is stable for up to six days in a pump and intended for SC administration. Figure 2.11 visualizes product examples. The PK/PD in pigs were similar to results obtained with GlucaGen (Novo Nordisk, Bagsværd, Denmark) [98, 99]. Similarity with GlucaGen was also
observed in diabetes patients [100]. However, with increasing doses, patients experienced more irritation at the injection site using XeriSol than Novo’s product which could be related to the DMSO in the XeriSol formulation. Similarly to marketed glucagon, the response to mini-doses of XeriSol was dampened by the presence of insulin [101].

As of end 2016, the product for rescue treatment is in preparation for phase III [94]. The product for pen mini-dose treatment and a dual hormone artificial pancreas device is in phase II, whereas it is in preparation for phase I for treatment of congenital hyperinsulinism and for use in a glucagon-only pump.

2.5.3 BioChaperone Glucagon by Adocia

The French company Adocia is developing a stable aqueous solution of recombinant human glucagon named BioChaperone (BC) Glucagon [95]. The native peptide is stabilized by forming a physical complex with an excipient. The drug is stable at neutral pH and intended for injection.

Based on the few preclinical results published online [95], the BC Glucagon appears to have similar effects on the glucose response as GlucaGen. The results are reported without standard errors and therefore it is not possible to conclude if the response to BC Glucagon wanes faster than the response to GlucaGen. The PK profile of BC glucagon is not published.

Currently, the product is in preclinical development. Adocia is planning to initiate a phase I trial before the end of 2017 [95].

2.5.4 Dasiglucagon by Zealand Pharma

The Danish company Zealand Pharma is developing a liquid stable glucagon analogue in aqueous solution named Dasiglucagon (proposed International Non-
Figure 2.12: Example of the look of a ready-to-use rescue pen for treatment of severe hypoglycemia with Dasiglucagon.

proprietary Name) or ZP4207 [14]. The drug is intended for SC administration. Figure 2.12 illustrates a product example.

Data from the clinical trials have not been published. However, the results confirm that Dasiglucagon is safe, well tolerated, and can raise the blood glucose level after insulin-induced hypoglycemia in T1D patients [14]. Results from another phase I trial confirm that Dasiglucagon can be used for multiple daily injections making it suitable for use in a pump or a dual hormone artificial pancreas device [102].

At the end of 2016, the product for rescue treatment and for use in a dual hormone artificial pancreas device is in phase II.
Glucagon
This chapter focuses on describing the theory of the methods for model fitting applied in this thesis. Likelihood principles and their applications in parameter estimation are discussed along with parameter sensitivity analysis using profile likelihood. The chapter presents practical and advanced usage of the software package CTSM for R [103], but also illustrates workarounds to problems not yet handled directly by the package.

3.1 Model Basics

This section defines the model structure for representation of a physical system. It also provides the math associated with simulation and prediction of the future states of a system. Finally, a useful variable transformation is explained.
3.1.1 Ordinary Differential Equations

Deterministic ordinary differential equations (ODEs) can be used to model a physical system with known structure. Generally, an ODE with discrete-time observations corrupted by measurement noise is defined as

\[ \text{d}x_t = f(x_t, u_t, t, \theta) \cdot \text{d}t \]
\[ y_k = h(x_k, u_k, t_k, \theta) + e_k \quad e_k \sim N_{iid}(0, S_k) \]

\( x_t \) is the state of the system, \( f(\cdot) \) is the model, \( u_t \) is the input, \( t \) is time, \( \theta \) is the parameter set, \( y_k \) is the discrete observations, and \( e_k \) is the measured errors, i.e. observation noise, assumed to be independent and identically distributed (i.i.d.) following a Gaussian distribution with mean zero and variance \( S_k \).

Equation (3.1) is the continuous ODE and (3.2) is the discrete observation equation.

3.1.2 Stochastic Differential Equations

One does not always know the true underlying system generating observations. This is particularly true for physiological systems. In such cases, the discrepancies between the deterministic model and data from the physical system is composed of noise from two sources: measurement noise and systemic noise. The system noise covers structural model deficiencies either from effects described incorrectly by the model or from effects not accounted for by the model. The magnitude of the systemic noise can be identified using stochastic differential equations (SDEs) with discrete-time noise corrupted measurements as

\[ \text{d}x_t = f(x_t, u_t, t, \theta) \cdot \text{d}t + \sigma(x_t, u_t, t, \theta) \cdot \text{d}w_t \quad \text{d}w_t \sim N_{iid}(0, I \cdot \text{d}t) \]
\[ y_k = h(x_k, u_k, t_k, \theta) + e_k \quad e_k \sim N_{iid}(0, S_k) \]

The only difference between the ODE formulation in (3.1) and the SDE formulation in (3.3) is the stochastic system noise \( \sigma(x_t, u_t, t, \theta) \cdot \text{d}w_t \). Thus, solving an SDE with a very small value of \( \sigma \) is approximating solving an ODE. The term \( f(x_t, u_t, t, \theta) \cdot \text{d}t \) is called the drift and is the main process driving the system when \( \sigma \) is smaller than one. The diffusion term is denoted by \( \text{d}w_t \). Together, the drift and the diffusion describes the physical state of the system.

3.1.3 Simulation

In a simulation, the system does not change over time. Moreover, the initial conditions are the only known measurements of the systems and therefore de-
determines the entire time course of the simulation. This can mathematically be expressed as

$$\hat{y}_{n|0} \quad \text{given} \quad x(t_0) = x_0$$  \hspace{1cm} (3.5)$$

An ODE as defined in (3.1) can be simulated through Euler’s forward method

$$x_{n+1} = x_n + \Delta t \cdot f(x_n, u_n, \theta)$$  \hspace{1cm} (3.6)$$

One realization of an SDE can be simulated in the same manner by drawing one value of $\Delta w_n$ from the distribution and keeping it constant throughout the simulation using the Euler-Maruyama method:

$$x_{n+1} = x_n + \Delta t \cdot f(x_n, u_n, \theta) + \sigma(x_n, u_n, t_n, \theta) \cdot \Delta w_n \quad \Delta w_n \sim N_{iid}(0, I\Delta t)$$  \hspace{1cm} (3.7)$$

The mean realization of an SDE, thus when $\Delta w_n$ is zero, corresponds to the ODE solution.

### 3.1.4 Prediction

In the case of ODEs, prediction and simulation of a system are identical because the state is exact given the state at a previous time point. The mean prediction of a system of SDEs corresponds to simulation of an ODE. But as opposed to ODEs, prediction and simulation are not identical when considering SDEs because of the stochastic system noise which changes the system from each realization to the next.

The following illustrates prediction of the system defined in (3.3)-(3.4). The one-step prediction is defined as

$$\hat{y}_{n+1|n} \quad \text{given} \quad x(t_n) = x_n$$  \hspace{1cm} (3.8)$$

More generally, the k-step prediction is defined as

$$\hat{y}_{n+k|n} \quad \text{given} \quad x(t_n) = x_n$$  \hspace{1cm} (3.9)$$

Due to $dw_t$ being a random process, the uncertainty of $\hat{x}_{n+k|n}$ and thereby $\hat{y}_{n+k|n}$ increases with increasing number of k.

Predictions are initialized with an estimate of the current state based on the previous state and the current covariance based on the previous covariance

$$\hat{x}_{k|k-1} \quad P_{k|k-1}$$  \hspace{1cm} (3.10)$$
The observation equation is then linearised
\[ \hat{y}_{k|k-1} = h(\hat{x}_{k|k-1}, u_k, t_k, \theta) \] (3.11)
\[ C_{k|k-1} = \frac{\partial h(\hat{x}_{k|k-1})}{\partial x} \] (3.12)

\( C_{k|k-1} \) is the derivative of the observation equation \( h(\cdot) \) with respect to \( x \) evaluated at \( \hat{x}_{k|k-1} \). The errors are then calculated using the extended Kalman filter as
\[ \epsilon_k = y_k - \hat{y}_{k|k-1} \] (3.13)
\[ R_{k|k-1} = C_{k|k-1} P_{k|k-1} C_{k|k-1}^T + S_k \] (3.14)
\[ K_k = P_{k|k-1} C_{k|k-1}^T (R_{k|k-1})^{-1} \] (3.15)

\( \epsilon_k \) is the observation error vector, \( R_{k|k-1} \) is the observation covariance matrix, \( S_k \) is the variance of the observation noise, and \( K_k \) is the Kalman gain. The filtered values of the state, \( \hat{x}_{k|k} \), and covariance, \( P_{k|k} \), are then calculated as
\[ \hat{x}_{k|k} = \hat{x}_{k|k-1} + K_k \epsilon_k \] (3.16)
\[ P_{k|k} = P_{k|k-1} - K_k R_{k|k-1} K_k^T \] (3.17)

The new 1-step predictions are found by solving the following system of differential equations
\[ \frac{d\hat{x}_k(t)}{dt} = f(\hat{x}_k(t)) \quad \hat{x}(t_k) = \hat{x}_{k|k} \] (3.18)
\[ \frac{dP_k(t)}{dt} = \frac{\partial f(x)}{\partial x} P_k(t) + P_k(t) \left( \frac{\partial f(x)}{\partial x} \right)^T + \sigma \sigma^T \quad P_k(t_k) = P_{k|k} \] (3.19)
\[ \hat{x}_{k+1|k} = \hat{x}_k(t_{k+1}) \] (3.20)
\[ P_{k+1|k} = P_k(t_{k+1}) \] (3.21)

Table 3.1 shows a direct comparison of the steps involved in predictions of ODEs and SDEs using the same notation.

### 3.1.5 Lamperti Transformation

Some implementations for solving SDEs do not allow the system noise to depend directly on the state of the system as defined in (3.3) [104]. Thus, the system equations must be written in a form satisfying
\[ dz_t = f(z_t, u_t, t, \theta) \cdot dt + \sigma(u_t, t, \theta) \cdot dw_t \] (3.22)
Table 3.1: Direct comparison of 1-step prediction in ODEs and SDEs. A) model structure. B) initial condition(s). C) linearisation of the observations. D) filtering. E) filtered state. F) updated system. G) one step prediction.

<table>
<thead>
<tr>
<th>A</th>
<th>ODE</th>
<th>SDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>dx_t = f(x_t, u_t, t, \theta) \cdot dt</td>
<td>dx_t = f(x_t, u_t, t, \theta) \cdot dt</td>
<td>dw_t \sim N_{idd}(0,1\cdot dt)</td>
</tr>
<tr>
<td>y_k = h(x_k, u_k, t_k, \theta) + e_k \sim N_{idd}(0, S_k)</td>
<td>y_k = h(x_k, u_k, t_k, \theta) + e_k \sim N_{idd}(0, S_k)</td>
<td>e_k</td>
</tr>
</tbody>
</table>

| B | \hat{x}_{k|k-1} | \hat{x}_{k|k-1} |
|---|---|---|
| \hat{y}_{k|k-1} = h(\hat{x}_{k|k-1}, u_k, t_k, \theta) | \hat{y}_{k|k-1} = h(\hat{x}_{k|k-1}, u_k, t_k, \theta) |

| C | \epsilon_k = y_k - \hat{y}_{k|k-1} | \epsilon_k = y_k - \hat{y}_{k|k-1} |
|---|---|---|
| R_{k|k-1} = S_k | R_{k|k-1} = C_{k|k-1} P_{k|k-1} C_{k|k-1}^T + S_k |

| D | \hat{x}_{k|k} = 0 \hat{x}_{k|k-1} + K_k \epsilon_k | \hat{x}_{k|k} = 0 \hat{x}_{k|k-1} - K_k R_{k|k-1} K_k^T |
|---|---|---|
| P_{k|k} = P_{k|k-1} - K_k R_{k|k-1} K_k^T | |

| E | \frac{d\hat{x}_{k|k}(t)}{dt} = f(\hat{x}_{k|k}) | \frac{d\hat{x}_{k|k}(t)}{dt} = f(\hat{x}_{k|k}) |
|---|---|---|
| \hat{x}(t_k) = \hat{x}_{k|k} | \hat{x}(t_k) = \hat{x}_{k|k} |

| F | \hat{x}_{k+1|k} = \hat{x}_{k|k}(t_{k+1}) | \hat{x}_{k+1|k} = \hat{x}_{k|k}(t_{k+1}) |
|---|---|---|
| P_{k+1|k} = P_k(t_{k+1}) | P_{k+1|k} = P_k(t_{k+1}) |
To maintain the property of state dependent diffusion without explicitly writing it in the state equations, the SDE may be transformed using the Lamperti transformation \[104, 105, 106\]. Generally, the Lamperti transformation is expressed as

\[
\Psi = \int \frac{1}{\sigma(\xi)} d\xi|_{\xi=x_t}
\]

(3.23)

In the special case where the system noise is directly proportional to the state, i.e \(\sigma(x_t) = x_t\), the Lamperti transformation uses the following change of variables according to (3.23)

\[
\Psi(x_t) = \log(x_t) = z_t \iff x_t = e^{z_t}
\]

(3.24)

\[
\Psi'(x_t) = \frac{1}{x_t}
\]

(3.25)

\[
\Psi''(x_t) = -\frac{1}{x_t^2}
\]

(3.26)

(3.24)-(3.26) are inserted into Itô’s lemma \[107\]

\[
dz_t = \Psi'(x_t) \cdot dx_t + \frac{1}{2} \Psi''(x_t) \cdot \sigma^2(x_t) \cdot dt
\]

(3.27)

\[
= \frac{1}{x_t} dx_t - \frac{1}{2} \frac{1}{x_t^2} \sigma^2(x_t) \cdot dt
\]

(3.28)

The transformation is best illustrated with an example. Consider a simple two states model often used to describe PK of SC administered drugs distributing in one plasma compartment with state dependent diffusion

\[
dx_1(t) = (u(t) - k_1x_1(t)) \cdot dt + \sigma_1x_1(t)dw_1
\]

(3.29)

\[
dx_2(t) = (k_1x_1(t) - k_2x_2(t)) \cdot dt + \sigma_2x_2(t)dw_2
\]

(3.30)
Using (3.28) the system in (3.29)-(3.30) is transformed to
\[
dz_1(t) = \frac{1}{x_1(t)} \left((u(t) - k_1 x_1(t)) \cdot dt + \sigma_1 x_1(t) dw_1\right) - \frac{1}{2} \frac{1}{x_1(t)} \sigma_1^2 x_1^2(t) dt
\]
(3.31)
\[
= \left(\frac{u(t)}{x_1(t)} - k_1\right) \cdot dt + \sigma_1 dw_1 - \frac{1}{2} \sigma_1^2 dt
\]
(3.32)
\[
= \left(u(t) \cdot e^{-z_1(t)} - k_1 - \frac{\sigma_1^2}{2}\right) \cdot dt + \sigma_1 dw_1
\]
(3.33)
\[
dz_2(t) = \frac{1}{x_2(t)} \left((k_1 x_1(t) - k_2 x_2(t)) \cdot dt + \sigma_2 x_2(t) dw_2\right) - \frac{1}{2} \frac{1}{x_2(t)} \sigma_2^2 x_2^2(t) dt
\]
(3.34)
\[
= \left(k_1 \frac{x_1(t)}{x_2(t)} - k_2\right) \cdot dt + \sigma_2 dw_2 - \frac{1}{2} \sigma_2^2 dt
\]
(3.35)
\[
= \left(k_1 \cdot e^{z_1(t)-z_2(t)} - k_2 - \frac{\sigma_2^2}{2}\right) \cdot dt + \sigma_2 dw_2
\]
(3.36)

Equation (3.24) is used to transform the solution back to the units of the original system.

### 3.2 Likelihood Principles

The likelihood measures how likely a set of parameters are given a model and data. Different parameters of the model will give different values of the likelihood function. Finding the parameter set that maximizes the likelihood function for given data and model gives the ML.

The likelihood is equal to the probability density considered as a function of the parameter set, \( \theta \), and a time series, \( Y_N \), of \( N \) observations exemplified in Figure 3.1

\[
L(\theta, Y_N) = p(Y_N | \theta) = \left(\prod_{k=1}^{N} p(y_k | Y_{k-1}, \theta)\right) p(y_0 | \theta)
\]
(3.37)

where

\[
Y_k = [y_k, y_{k-1}, \ldots, y_1, y_0]
\]
(3.38)

Thus the likelihood is a product of the initial probability density, \( p(y_0 | \theta) \), and all subsequent conditional probability densities. Practically, this approach is computationally infeasible as it would involve solving the Fokker-Planck equation.
and instead Kalman filtering can be used. When the model structure is linear
the Kalman filter is exact. However, when the model structure is nonlinear
the extended Kalman filter must be used which only provides approximations
of the observation error and covariance. By using (3.13)-(3.14) equation (3.37)
becomes
\[
p(Y_N | \theta) = \left( \prod_{k=1}^{N} \frac{\exp \left( -\frac{1}{2} \epsilon_k^T R_{k|k-1}^{-1} \epsilon_k \right) }{\sqrt{\det(R_{k|k-1}) (\sqrt{2\pi})^l}} \right) p(y_0 | \theta) \quad (3.39)
\]
In practice, one rarely maximizes the likelihood function, instead the negative
log-likelihood is used for minimization. Conditioning the posterior probability
on $y_0$ and taking the negative logarithm on both sides we obtain the negative
log-likelihood function
\[
- \log(p(Y_N | \theta, y_0)) = \frac{1}{2} \sum_{k=1}^{N} \left( \log(\det(R_{k|k-1})) + \epsilon_k^T R_{k|k-1}^{-1} \epsilon_k \right) + \frac{1}{2} \log(2\pi) \sum_{k=1}^{N} l \quad (3.40)
\]
Consequently, the ML-parameters are determined by computing

$$
\hat{\theta}_{ML} = \arg \min_{\theta \in \Theta} \left\{ \sum_{k=1}^{N} \left( \log(\det(R_{k|k-1})) + \epsilon_k^T R_{k|k-1}^{-1} \epsilon_k \right) \right\} \tag{3.41}
$$

$\epsilon_k$ is the one-step prediction error defined in (3.13) and $R_{k|k-1}$ is the covariance defined in (3.14) computed using the extended Kalman filter. Kalman filter approximations can similarly be used during k-step predictions as during 1-step predictions. For the Kalman filter to be a good approximation it requires a sufficiently fast sampling frequency and accuracy of observations. Further details on Kalman filtering can be found in [108].

### 3.2.1 Profile Likelihood

Visualization of the likelihood function becomes difficult when a parameter set includes more than three parameters. Cross sections of the likelihood function are called likelihood profiles. The simplest likelihood profile of parameter $\theta_i$ is found by keeping all other parameters of $\theta$ constant and then plotting the likelihood as a function of $\theta_i$. With this approach, the value of $\theta_i$ that maximizes the likelihood function depends on the choice of fixed parameters in $\theta$. However, this approach largely underestimates the uncertainties of the profiled parameter. A better approach to likelihood profiling is for fixed values of the parameter $\theta_i$ to optimize the likelihood for all other parameters of $\theta$, and then plot the optimal likelihood as a function of $\theta_i$ [109, 110]. Mathematically this is defined as

$$
L_p(\theta_i, Y_N) = \max_{\theta \neq \theta_i} L(\theta, Y_N) \tag{3.42}
$$

The value of $\theta_i$ that maximizes $L_p(\theta_i, Y_N)$ is the global maximizer of the likelihood function.

The profile likelihood of a parameter can be used to evaluate whether the parameter in the model is identifiable. Identifiability of parameters are determined by model structure (structural identifiability) and the input dynamics (practical identifiability) [109].

Structural identifiability is related to having a unique representation of the model for each parameter set. Profile likelihood analysis provides a method for investigating the parameter identifiability even in large complex systems, where it can be difficult to evaluate the model uniqueness directly.

Practical identifiability is related to the dynamics of the input. Thus, a model can only identify parameters describing dynamics present in data used for model fitting. Also in this case, profile likelihood analysis is a powerful tool.

A parameter is identifiable only if the maximum of the profile likelihood is well defined [109]. Whether the maximum of the profile likelihood of $\theta_i$ is well defined
Figure 3.2: Theoretical examples of three types of profile likelihood plots; from
left to right: highly peaked (identifiable), flat (structural non-
identifiable), and asymmetric (practical non-identifiable). The
95% confidence limit (CL) is blue. The x-axis shows the 95%
lower limit (LL) and upper limit (UL) of the parameter value.
Graph from [18].

In words, the profile likelihood is log-transformed yielding the profile log-
likelihood. The maximum value of the profile log-likelihood is subtracted from
the profile log-likelihood so that the maximum function value is zero. The
limit of the confidence interval is determined by the 100(1-\(\alpha\)) percentile of the
chi-squared distribution with one degree of freedom. As an example, a 95%
confidence interval of a model parameter is bound by the log-likelihood ratio
exceeding approximately \(-1.92\). A profile likelihood confidence interval could
be asymmetric, whereas e.g. the Wald statistic applies a quadratic and thus
symmetric approximation of the confidence interval [110]. Figure 3.2 provides
examples of symmetric and asymmetric profile likelihoods that are either iden-
tifiable or non-identifiable.
3.2.2 Bayesian Inference

Bayesian estimates refer to parameters of a model being treated as random variables belonging to some distribution. To fit a parameter in a Bayesian framework, a prior distribution of the parameter is needed. The prior distribution of a parameter describes the population, whereas a single realization of the parameter from the distribution describes an individual.

The parameters of the prior distribution are called hyper-parameters i.e. if a prior follows a normal distribution, two hyper-parameters define it: mean ($\mu$) and standard deviation ($\sigma$). A normally distributed prior for parameter $\theta_i$ is thus defined as

$$\theta_i \sim N(\mu_{\theta_i}, \sigma^2_{\theta_i})$$  \hspace{1cm} (3.44)

The estimated parameter will then be a summary of the posterior probability density function conditioned on the data and the prior. The posterior distribution of a parameter, $\theta$, given the data, $Y_N$, is identified using Bayes’ theorem.

$$p(\theta|Y_N) \propto p(Y_N|\theta)p(\theta)$$  \hspace{1cm} (3.45)

$p(\theta)$ is the prior distribution of $\theta$, $p(Y_N)$ is the marginal distribution, and $p(Y_N|\theta)$ is the likelihood of $Y_N$ given $\theta$ as defined in (3.37). Finding the set of parameters given data, a model and prior distributions of parameters yielding the maximum of the posterior distribution is called MAP.

3.2.3 Maximum a Posteriori Estimation

MAP estimation is an optimization approach seeking the parameter estimate that maximizes the posterior distribution [111]. Maximizing (3.45) then reduces to optimizing:

$$p(\theta|Y_N) \propto p(Y_N|\theta)p(\theta)$$  \hspace{1cm} (3.46)

MAP estimation reduces to maximizing the likelihood function when the prior is a uniform distribution, i.e. $p(\theta)$ is constant, see (3.37) and (3.46). This indicates that ML is a special case of MAP. Also, the weaker a prior is, i.e. having a large standard deviation, the less difference there is between MAP estimation and ML. In general, one distinguishes between informative (highly peaked) and non-informative (not peaked) priors. Thus, the likelihood profile of a parameter estimated by MAP is composed of the likelihood of the parameter itself conditioned on the data and the prior parameter distribution.
Introducing the following notation where $\sigma_\theta$ is a matrix with the prior standard deviations in the diagonal and $R_\theta$ is the prior correlation matrix:

$$\mu_\theta = E\{\theta\}$$

(3.47)

$$\Sigma_\theta = \sigma_\theta R_\theta \sigma_\theta = V\{\theta\}$$

(3.48)

$$\epsilon_\theta = \theta - \mu_\theta$$

(3.49)

Assuming that the prior parameters follow Gaussian distributions as defined in (3.44) and using equation (3.39), the posterior distribution in (3.46) can be rewritten as

$$p(\theta|Y_N) \propto \left( \prod_{k=1}^{N} \frac{\exp\left(-\frac{1}{2} \epsilon_k^T R_{k|k-1}^{-1} \epsilon_k\right)}{\sqrt{\det(R_{k|k-1}) (2\pi)^l}} \right) p(y_0|\theta) \frac{\exp\left(-\frac{1}{2} \epsilon_\theta^T \Sigma_\theta^{-1} \epsilon_\theta\right)}{\sqrt{\det(\Sigma_\theta) (2\pi)^p}}$$

(3.50)

Conditioning the posterior probability on $y_0$ and taking the negative logarithm gives:

$$-\log(p(\theta|Y_N, y_0)) \propto \frac{1}{2} \sum_{k=1}^{N} \left( \log(\det(R_{k|k-1})) + \epsilon_k^T R_{k|k-1}^{-1} \epsilon_k \right) + \frac{1}{2} \left( \sum_{k=1}^{N} l \right) + p \log(2\pi) + \frac{1}{2} \log(\det(\Sigma_\theta)) + \frac{1}{2} \epsilon_\theta^T \Sigma_\theta^{-1} \epsilon_\theta$$

(3.51)

The MAP solution is found by solving the nonlinear optimization problem:

$$\hat{\theta}_{MAP} = \arg\min_{\theta \in \Theta} \{-\log(p(\theta|Y_N, y_0))\}$$

(3.52)

$$= \arg\min_{\theta \in \Theta} \left\{ \sum_{k=1}^{N} \left( \log(\det(R_{k|k-1})) + \epsilon_k^T R_{k|k-1}^{-1} \epsilon_k \right) + \log(\det(\Sigma_\theta)) + \epsilon_\theta^T \Sigma_\theta^{-1} \epsilon_\theta \right\}$$

This nonlinear optimization can be non-trivial to solve analytically through gradient-methods, but can be approximated with a finite difference method [112]. Another method for finding the MAP solution is by using Markov Chain Monte Carlo (MCMC) simulations. MCMC is a brute force method that samples from the posterior distribution to create a rough shape of the posterior distribution and thereby estimates the MAP solution as implemented in WinBUGS [113, 114]. It is computationally time consuming because it can require thousands of samples before reaching convergence. On the contrary, gradient methods converge faster, but suffer great difficulties if the objective function is noisy with local gradients not leading to a smaller value of the objective function.
3.3 Parameter Estimation in CTSM-R

Continuous time stochastic modeling (CTSM) is also known as grey-box modeling with one or more system equations being stochastic [111]. One tool to handle CTSM is the CTSM-R package for R [103]. The software package uses likelihood principles as described in Section 3.2 and a finite-difference approximation method to gradient-based optimization method to find the most likely set of model parameters for a series of observations. The commands of the following sections will refer to R functions and the R environment thereby providing some practical information for use of the CTSM-R package, which might not be straightforward.

**Algorithm 1** Defining CTSM-R model and estimating model parameters.

**Require:** ctsmr

- Define CTSM-R model object: model = ctsmr$new()
- Define system equations:
  - model$addSystem(dx1 ∼ (Dose-x1/tmax)*dt+sigma1*dw1)
  - model$addSystem(dx2 ∼ (x1/tmax-x2/tmax)*dt+sigma2*dw2)
- Define observation equation:
  - model$addObs(conclog ∼ log(1/tmax*x2/(BW*ClF)*1e6+Ib))
- Define observation variance: model$setVariance(conclog ∼ exp(ls))
- Define input: model$addInput(Dose)
- Set options e.g.: model$options$InitialVarianceScaledIdentity = TRUE and model$options$inputinterpolation = FALSE
- Build CTSM-R model: ctsmr:::Compile(model)
- Set initial values for states and parameters (only a subset shown):
  - model$setParameter(x10=0, Ib=c(µ_Ib,min_Ib,max_Ib)),
  - tmax=c(µ_tmax,min_tmax,max_tmax,psd=σ_tmax)
- Fit ← model$estimate(data)

3.3.1 Fitting One Data Set

To fit a single data set using CTSM-R, one must first define the model, set the initial guesses for model states and parameters and then estimate the model parameters as in Algorithm 1. Various options can be set depending on the problem at hand. Model parameters and initial values of states can be estimated in the CTSM-R environment as indicated in Algorithm 1, where x10 is the initial value of state x1 which is fixed, Ib is a parameter estimated by ML, and tmax is a parameter estimated by MAP. Further practical information can be found in [103].
3.3.2 Fitting Multiple Data Sets

CTSM-R also allows parameter estimation in multiple independent data sets. However, the package requires that initial values are equal in all data sets. As this is rarely the case in biological data sets, the objective function must be defined manually and an appropriate optimizer chosen [103]. To implement MAP estimation for multiple data sets, first the negative log-likelihood as a function of a data set and a parameter set was defined as in Algorithm 2. The joint likelihood function for multiple data sets was calculated as in Algorithm 3. The optimal parameter set across multiple datasets was then found by optimizing Algorithm 3 using constrained optimization with \texttt{nlminb}().

It should be noted, that Algorithm 3 has a minor error leading to the priors having unchanged influence despite multiple datasets. The MAP solution for J datasets should have been implemented as

$$
\hat{\theta}_{MAP,J} = \arg \min_{\theta \in \Theta} \left\{ \sum_{j=1}^{J} \left( \sum_{k=1}^{N} \left( \log(\det(R_{k|k-1})) + \epsilon_k^T R_{k|k-1}^{-1} \epsilon_k \right) + \log(\det(\Sigma_\theta)) + \epsilon_\theta^T \Sigma_\theta^{-1} \epsilon_\theta \right) \right\}
$$

(3.53)

Thus, the more datasets available, the proportionally less impact of the priors on the MAP solution.

**Algorithm 2** Manual implementation of the negative log-likelihood.

```plaintext
function -log-likelihood(parameters, data)
    (data\_predict, SD\_predict) ← predict(parameters, data)
    y ← data
    ŷ ← data\_predict
    ySD ← SD\_predict
    log-likelihood ← \(-\frac{1}{2} \sum_{n=1}^{N} \frac{(y-ŷ)^2}{ySD^2} - \sum_{n=1}^{N} \log ySD - (N - 1)\frac{1}{2} \log 2\pi \)
    return -log-likelihood
end function
```

3.3.3 Calculating the Parameter Uncertainties

When CTSM-R is not used for optimization of the model parameters, the associated uncertainties are not estimated directly. The parameter uncertainties can
3.3 Parameter Estimation in CTSM-R

Algorithm 3 Manual implementation of MAP estimate of multiple data sets.

**Require:** Set prior distribution of parameter \( i \sim N(\mu_i, \sigma_i) \)

**function** \(-\text{MultiData MAP}(\text{parameter}_{i=1..I}, \text{data}_{j=1..J})\)

\[
V_{\text{MAP}} \leftarrow 0 \\
\text{for } j = 1..J \text{ do} \\
\quad \text{Set initial values for data}_j \\
\quad V_{\text{MAP}} \leftarrow V_{\text{MAP}} + -\log\text{-Likelihood}(\text{parameter}_{i=1..I}, \text{data}_j) ... \\
\quad - \sum_{i=1}^{I} \text{dnorm(\text{parameter}_i, \text{mean}=\mu_i, \text{sd}=\sigma_i, \text{log}=\text{TRUE})} \\
\text{end for} \\
\text{return } -V_{\text{MAP}} \\
\text{end function}
\]

then be calculated using an approximation of the inverse Hessian, which provides the curvature of the log-likelihood function [110]. Calculations of parameter uncertainties using R are explained in Algorithm 4. We used the function `hessian` from the R package `numDeriv` to approximate the Hessian. The parameter "r" controls the precision of the numerically derived Hessian with a default value of 4. In some cases it was necessary to increase the precision at the cost of more evaluations in order to get non-negative diagonal elements of the inverse Hessian.

Algorithm 4 Estimation of model parameter uncertainties using the Hessian.

**Require:** `numDeriv`

Calculate the Hessian as `hessian(object function, optimized parameters, method="Richardson", method.args=list(r=6), extra parameters to object function)`

Calculate Hessian\(^{-1}\) by `solve(Hessian)`

Calculate the SD of parameters by \(\sqrt{\text{diag}(\text{Hessian}^{-1})}\)

3.3.4 Optimization with Genetic Algorithms

Object functions defined outside the CTSM-R environment, but using optimizations from the package for parts of the calculations can sometimes be noisy. This noise can stem from numerical approximations. As an example, if one wishes to optimize the distribution of population parameters, i.e. the priors for MAP estimation, based on multiple individual datasets, the object function for the problem can be noisy. When the object function is non-smooth, gradient based optimizers have difficulties converging to the global maximum and are likely to
stop in a local maximum instead [115]. Although they are computationally fast, if one uses a gradient based optimizer on the noisy object function exemplified in Figure 3.3, the solution will highly depend on the starting guess. To avoid this, a non-gradient based optimizer like genetic algorithms (GA) could be used for optimizations of non-smooth object functions [116].

GA can be slow and computationally heavy. However, with some tuning of the optimizer settings, GA often reach fast convergence. Moreover, the optimization can be speeded up by allowing parallel computations. The tuning is a compromise between getting to the solution fast and not stopping the iterations sooner than the parameter space has been explored sufficiently. Although GA have global convergence one can not be completely certain that the optimizer finds the global maximum - there might still exist a solution with an even better fit that has yet to be discovered.

The principles of GA are inspired by biological evolution. A population of possible parameter sets are created at the beginning of each iteration. During an iteration the value of the object function is determined for each parameter set. To find the population of the next iteration the best parameter sets of the current iteration are used to build new parameter sets by combining different parts of the best parameter sets with each other (cross-over) and occasionally introduce ran-
dom parameter values (mutations). The optimization is stopped when the best solution does not improve after a certain number of consecutive populations. Tuning of GA involves setting the population size, cross-over rate, mutation rate, number of best solutions to keep for next iteration (elitism), maximum number of iterations, and maximum number of runs without improvement.
This chapter focuses on presenting and discussing models of a small part of the gluco-regulatory system - the glucose production of the liver. Large gluco-regulatory models including many different effects like meals and exercise are not described, but can be found in the literature \cite{117, 118, 119}. The here presented models describe how the EGP is stimulated by glucagon and some models describe the inhibitory effects of insulin, too. Models of EGP without glucagon action are not considered \cite{117, 120}.

Simulations provide a direct comparison of the responses of the different models to a glucagon bolus and a glucagon infusion. Furthermore, models including the effect of insulin on EGP are compared using simulation of a glucagon bolus preceded by an insulin bolus. The models in sections 4.6-4.8 each contain one unique element: the evanescence effect of glycogenolysis, the glucagon rate of change, and saturation of glycogenolysis. All proposed models are discussed at the end of the chapter and related to the physiology described in Chapter 2.
4.1 Simulation Scenario

The EGP models in this chapter were implemented in MATLAB along with glucagon and insulin PK models. The glucagon PK model was described by (4.1)-(4.3). Similarly, the insulin PK model was described by (4.4)-(4.6).

\[
\frac{dZ_1(t)}{dt} = u_C(t) - k_1 Z_1(t) \quad (4.1)
\]
\[
\frac{dZ_2(t)}{dt} = k_1 Z_1(t) - k_2 Z_2(t) \quad (4.2)
\]
\[
C(t) = \frac{k_2 Z_2(t)}{W \cdot Cl_{F,C}} + C_b \quad (4.3)
\]
\[
\frac{dX_1(t)}{dt} = u_I(t) - \frac{X_1(t)}{t_{max}} \quad (4.4)
\]
\[
\frac{dX_2(t)}{dt} = \frac{X_1(t) X_2(t)}{t_{max}} - \frac{X_2(t)}{t_{max}} \quad (4.5)
\]
\[
I(t) = \frac{1}{t_{max}} \frac{X_2(t)}{W \cdot Cl_{F,I}} \cdot 10^6 + I_b \quad (4.6)
\]

The glucagon and insulin PK models are explained in details in Section 5.1. For the current purpose of comparable EGP simulations, the PK model parameters were based on a single virtual patient with parameters listed in Table 4.1. The EGP models were implemented in MATLAB according to the original scientific publications to the best of the author’s knowledge.

**Table 4.1:** Specific model parameter values used for simulation of the glucagon and insulin PK models. Occasionally, some of these parameters are also included in the EGP models.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$W$</td>
<td>69</td>
<td>kg</td>
</tr>
<tr>
<td>$k_1$</td>
<td>0.058</td>
<td>min$^{-1}$</td>
</tr>
<tr>
<td>$k_2$</td>
<td>0.058</td>
<td>min$^{-1}$</td>
</tr>
<tr>
<td>$Cl_{F,C}$</td>
<td>159</td>
<td>mL/kg/min</td>
</tr>
<tr>
<td>$C_b$</td>
<td>10.9</td>
<td>pg/mL</td>
</tr>
<tr>
<td>$t_{max}$</td>
<td>67.9</td>
<td>min</td>
</tr>
<tr>
<td>$Cl_{F,I}$</td>
<td>17.4</td>
<td>mL/kg/min</td>
</tr>
<tr>
<td>$I_b$</td>
<td>8.7</td>
<td>mU/L</td>
</tr>
</tbody>
</table>
4.1 Simulation Scenario

The PK models were used to perform three simulation scenarios:

- Euglycemic clamp (5 mmol/L), constant basal insulin ($I_b$), SC glucagon bolus of 0.3 mg at $t=0$
- Euglycemic clamp (5 mmol/L), constant basal insulin ($I_b$), SC glucagon infusion of 1 $\mu$g/minute starting at $t=0$
- Euglycemic clamp (5 mmol/L), SC insulin bolus of 4 U at $t=0$, SC glucagon bolus of 0.3 mg at $t=150$ minutes

The simulated glucagon and insulin concentrations of each of these scenarios are displayed graphically in Figure 4.1 and were used as inputs to the EGP models to compare their strengths and weaknesses, and to investigate how certain parameters influence the model responses to glucagon.

**Figure 4.1:** Simulated plasma glucagon and serum insulin concentrations after a SC glucagon bolus of 0.3 mg (left), SC continuous glucagon infusion of 1 $\mu$g/minute (middle), and SC insulin bolus of 4 U at $t=0$ and SC glucagon bolus of 0.3 mg at $t=150$ (right).
4.2 Extension of the Minimal Model

One of the oldest glucoregulatory models including the effects of insulin on glucose was published by Bergman in 1979 and is known as the minimal model \[121\]. In 2013, Herrero et al. suggested an extension to the model including the effects of glucagon on the net EGP \[122\]. Mathematically the glucagon-extended minimal model is:

\[ EGP(t) = [C_E(t) - I_E(t)]G(t)V_{d,G} + S_G(G_b - G(t)) \]  
\[ \frac{dC_E(t)}{dt} = k_C S_C [C(t) - C_b] - k_C C_E(t) \]  
\[ \frac{dI_E(t)}{dt} = k_I S_I [I(t) - I_b] - k_I I_E(t) \]

\( G, C \) and \( I \) are the plasma glucose, glucagon, and insulin concentrations and \( G_b, C_b, \) and \( I_b \) denotes the basal values thereof. \( I_E \) and \( C_E \) are the actions of the effect compartments of insulin and glucagon on the net EGP. \( S_G \) is the glucose sensitivity on promoting or inhibiting the EGP. \( S_C \) is the glucagon sensitivity and \( k_C \) is the transfer rate constant of glucagon from plasma to the effect compartment. \( S_I \) is the insulin sensitivity and \( k_I \) is the transfer rate constant of insulin from plasma to the effect compartment. \( V_{d,G} \) is the glucose volume of distribution per mass.

Compared to the publication, (4.7) is rewritten to emphasize that the effects of insulin and glucagon are additive and that the absolute difference in glucagon and insulin actions interact with the plasma glucose concentration to yield the EGP. Moreover, with this formulation the contribution to the EGP from the deviation in glucose concentration from basal becomes obvious.

Table 4.2: Specific model parameter values used for simulation of the Herrero et al. EGP model \[122\].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S_G )</td>
<td>NA</td>
<td>( \mu \text{mol/kg/min per mmol/L} )</td>
</tr>
<tr>
<td>( k_C )</td>
<td>0.210</td>
<td>min(^{-1} )</td>
</tr>
<tr>
<td>( S_C )</td>
<td>( 1.25 \times 10^{-4} )</td>
<td>min(^{-1} ) per pg/mL</td>
</tr>
<tr>
<td>( k_I )</td>
<td>0.002</td>
<td>min(^{-1} )</td>
</tr>
<tr>
<td>( S_I )</td>
<td>( 1.16 \times 10^{-3} )</td>
<td>min(^{-1} ) per mU/L</td>
</tr>
<tr>
<td>( V_{d,G} )</td>
<td>160</td>
<td>mL/kg</td>
</tr>
</tbody>
</table>

Data used for the model building originated from a clinical study of eleven subjects with T1D undergoing a dual-hormone closed-loop study for 27 hours \[67\]. Thus neither glucose, glucagon or insulin were constant during the entire time of the study.
4.2 Extension of the Minimal Model

Figure 4.2: EGP simulations of the model proposed by Herrero et al. [122] after a SC glucagon bolus (left), SC glucagon infusion (middle), and SC insulin bolus followed 150 minutes after by a SC glucagon bolus (right), see Figure 4.1 for the corresponding PK profiles. The solid curves (left and middle) show the responses using a published parameter value of $S_C$ [122], and the dashed curves show responses when $S_C$ is three times as large. The solid curve (right) show the impact of insulin above basal. The dotted curve is equal to the solid curve of the left graph with 150 minutes delay.

Table 4.2 lists the model parameter values of (4.7)-(4.9) used for the simulations displayed in Figure 4.2. As the glucose level is clamped at basal, 5 mmol/L, the last part of (4.7) equals zero and thus the value of $S_G$ is non-relevant for the present simulations and omitted from the table. The value of $C_b$ was more than four times greater reported by Herrero et al. than the value of the glucagon PK simulation model, which could be attributed to assay differences [32]. The difference in basal glucagon levels could lead to a mismatch in glucagon sensitivity. Figure 4.2 displays the EGP responses using the published glucagon sensitivity compared to a sensitivity increased by a factor of three following a SC bolus injection and initiation of a SC glucagon infusion. As the glucagon sensitivity is increased, the EGP responses to both glucagon disturbances are also increased. Overall, the EGP responses to glucagon follow the dynamics of the plasma glucagon concentrations with a slight delay representing the transfer of glucagon to the effect compartment.

Figure 4.2 also compares the EGP response in the presence or absence of a preceding insulin bolus. The dotted line of the right graph equals the solid line of the left graph although with a 150 minutes delay. Increasing insulin above basal but maintaining basal glucagon, the EGP becomes negative. The response to the glucagon bolus measured by peak EGP is somewhat dampened in the presence of the insulin bolus, even when correcting for the negative EGP.
4.3 UVA/PADOVA Type 1 Diabetes Simulator

The only FDA approved simulator of the glucoregulatory system was built in a collaboration between University of Virginia and University of Padova [118]. The model was extended by Dalla Man et al. to include the effects of glucagon on EGP [123]. The EGP is described by the following equations:

\[\begin{align*}
\text{EGP}(t) &= G_{GNG} - S_G G(t) - S_I I_E(t) + S_C C_E(t) \\
\frac{dC_E(t)}{dt} &= k_C \cdot \max(C(t) - C_b, 0) - k_C C_E(t) \\
\frac{dI_E(t)}{dt} &= -k_I[I_E(t) - I_{rem}(t)] \\
\frac{dI_{rem}(t)}{dt} &= -k_I[I_{rem}(t) - I(t)]
\end{align*}\]

\[\text{Table 4.3: Specific model parameter values used for simulation of the Dalla Man et al. EGP model [123].}\]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(G_{GNG})</td>
<td>6</td>
<td>(\mu\text{mol/kg/min})</td>
</tr>
<tr>
<td>(S_G)</td>
<td>0.678</td>
<td>(\mu\text{mol/kg/min per mmol/L})</td>
</tr>
<tr>
<td>(S_I)</td>
<td>0.3</td>
<td>(\mu\text{mol/kg/min per mU/L})</td>
</tr>
<tr>
<td>(S_C)</td>
<td>0.25</td>
<td>(\mu\text{mol/kg/min per pg/mL})</td>
</tr>
<tr>
<td>(k_C)</td>
<td>0.2</td>
<td>min(^{-1})</td>
</tr>
<tr>
<td>(k_I)</td>
<td>0.033</td>
<td>min(^{-1})</td>
</tr>
</tbody>
</table>

Table 4.3 lists the model parameter values of (4.10)-(4.13) used for the simulations displayed in Figure 4.3. The parameter values were guessed by the author since the model has undisclosed parameter values.
Generally, the EGP responses to glucagon follow the dynamics of the plasma glucagon concentrations with a delay representing the transfer of glucagon to the effect compartment. The parameter $k_C$ controls the delay of glucagon action on EGP as exemplified in the left and middle graphs of Figure 4.3. The more delayed glucagon action on EGP is, the later will EGP reach maximum concentration after a bolus and steady state after SC infusion start. Moreover, when the time to maximum EGP response increases, the peak response decreases.

In this model, insulin is delayed twice as described by (4.12)-(4.13). The delayed effect of insulin on EGP is visible in the right graph of Figure 4.3, where the solid line represents the impact of insulin on EGP compared to the dotted line with insulin remaining constant at the basal level. With insulin above basal and glucagon at basal, the EGP becomes negative after the insulin action delay. The insulin level has very little influence on the EGP response to glucagon as the peaks of the two curves are almost identical.
4.4 Multiplicative Model

A semi-mechanistic integrated glucose-insulin-glucagon model was proposed by Schneck et al. [124]. The model includes multiplicative effects of glucose, insulin and glucagon and is formulated as:

\[
E_{GP}(t) = EGP_b \cdot \left( \frac{G_E(t)}{G_b} \cdot \frac{I_E(t)}{I_b} \cdot \frac{C_b}{C_E(t)} \right)^{-E_{GIC}}
\] (4.14)

\[
\frac{dC_E(t)}{dt} = k_C[C(t) - C_E(t)]
\] (4.15)

\[
\frac{dI_E(t)}{dt} = k_I[I(t) - I_E(t)]
\] (4.16)

\(G_b, I_b, \) and \(C_b\) are the basal concentrations of glucose, insulin and glucagon, respectively. Similarly, \(G_E, I_E, \) and \(C_E\) are the effect compartment concentrations of glucose, insulin, and glucagon. \(E_{GP_b}\) is the basal \(E_{GP}.\) \(E_{GIC}\) determines the stimulatory effect of glucagon and inhibitory effects of glucose and insulin on \(E_{GP}.\) \(C\) is the plasma glucagon concentration and \(k_C\) is the transfer rate constant of glucagon from plasma to the effect compartment. Likewise, \(I\) is the plasma insulin concentration and \(k_I\) is the transfer rate constant from plasma to the effect compartment.

Equation (4.14) describes how \(E_{GP}\) above basal depends on a multiplicative form of glucose, insulin and glucagon. When glucose and insulin exceed their basal levels, \(E_{GP}\) is inhibited, whereas \(E_{GP}\) is stimulated when glucose and insulin are below basal levels. On the contrary, \(E_{GP}\) is stimulated when glucagon exceeds the basal level, and inhibited when glucagon is lower than basal.

**Table 4.4:** Specific model parameter values used for simulation of the Schneck et al. \(E_{GP}\) model [124].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E_{GP_b})</td>
<td>10.2</td>
<td>(\mu)mol/kg/min</td>
</tr>
<tr>
<td>(E_{GIC})</td>
<td>0.79</td>
<td>-</td>
</tr>
<tr>
<td>(k_C)</td>
<td>0.118</td>
<td>min(^{-1})</td>
</tr>
<tr>
<td>(k_I)</td>
<td>0.005685</td>
<td>min(^{-1})</td>
</tr>
</tbody>
</table>

Table 4.4 lists the model parameter values of (4.14)-(4.16) used for the simulations displayed in Figure 4.4. The parameter of \(E_{GP_b}\) is calculated based on the weight of the virtual subject whereas the other parameters are from [124]. As the glucose level is clamped at basal, 5 mmol/L, the fraction of the glucose contribution to \(E_{GP}\) equals 1.

Generally, the \(E_{GP}\) responses to glucagon during a euglycemic clamp with constant basal insulin follow the dynamics of the plasma glucagon concentrations.
4.4 Multiplicative Model

Figure 4.4: EGP simulations of the model proposed by Schneck et al. [124] after a SC glucagon bolus (left), SC glucagon infusion (middle), and SC insulin bolus followed 150 minutes after by a SC glucagon bolus (right), see Figure 4.1 for the corresponding PK profiles. The solid curves (left and middle) show the responses using a published parameter value of $E_{GIC}$ [124], and the dashed curves show responses when $E_{GIC}$ is half as large. The solid curve (right) show the impact of insulin above basal. The dotted curve is equal to the solid curve of the left graph with 150 minutes delay.

with a delay representing the transfer of glucagon to the effect compartment. The parameter $E_{GIC}$ determines the size of the EGP response to the combined stimulatory and inhibitory effects of glucose, insulin and glucagon as exemplified in the left and middle graphs of Figure 4.4. When the parameter is reduced, the response is reduced as well.

The delayed effect of insulin on EGP is visible in the right graph of Figure 4.4 where the solid line represents the influence of insulin on EGP compared to the dotted line with insulin remaining constant at the basal level. With insulin above basal and glucagon at basal, the EGP decreases from its basal level of $EGP_b$. The insulin level has quite some influence on the EGP response to glucagon as the peak is noticeably lower in the presence of insulin.
4.5 Glucose-Glucagon Model

Inspired by the same model as in Section 4.4, Peng et al. proposed a different model [125]. The model includes additive effects of glucose and glucagon on EGP described by the following equation:

\[
EGP(t) = EGP_b \cdot 0.5 \cdot \left[ \left( \frac{G_E(t)}{G_b} \right)^{-EG} + \left( \frac{C(t)}{C_b} \right)^{EC} \right] \tag{4.17}
\]

*\(EGP_b\) is the basal EGP. \(G_E\) is the glucose concentration in the effect compartment, \(G_b\) is the basal glucose concentration, and \(EG\) is the inhibitory effect on EGP of glucose exceeding basal levels. \(C\) is the plasma glucagon concentration, \(C_b\) is the basal glucagon concentration, and \(EC\) is the stimulatory effect on EGP of glucagon exceeding basal levels.*

Equation (4.17) describes how the basal EGP is stimulated by glucagon concentrations exceeding basal and glucose concentrations below basal. Similarly, EGP is inhibited when glucagon is below basal and glucose is above basal. The effects of glucose and glucagon levels different from basal are determined independently and the overall contribution to EGP is found by addition.

Table 4.5: Specific model parameter values used for simulation of the Peng et al. EGP model [125]. *25% of the original value.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(EGP_b)</td>
<td>7.53</td>
<td>µmol/kg/min</td>
</tr>
<tr>
<td>(EC)</td>
<td>1.013*</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.5 lists the model parameter values of equation (4.17) used for the simulations displayed in Figure 4.5. As the glucose level is clamped at basal, 5 mmol/L, the fraction of the glucose contribution to EGP equals 1 and the value of \(EG\) becomes irrelevant. Notice in this model, that the effect of glucagon is directly proportional to the concentration in the plasma compartment without delay. The parameter of \(EC\) used for simulations is one-fourth of the published value in order to have a response in the same range as the other simulations. With an even lower value of \(EC\) the response to glucagon is further reduced as exemplified in Figure 4.5.

The influence of insulin on EGP is not captured in the model equations, and thus the third scenario with an insulin bolus preceding a glucagon bolus is not simulated.
4.6 Glucagon Evanescence Effect

Based on data from a study with glucose clamp and constant insulin and IV glucagon infusions Hinshaw et al. proposed a new model of EGP. The model is the first to describe the evanescence effect of glycogenolysis:

\[ EGP(t) = k_{G6P}G_6P(t) \]  
\[ \frac{dG_6P(t)}{dt} = -k_{G6P}G_6P(t) + G_{GG}(t) + G_{GNG,b} \]  
\[ G_{GG}(t) = \left[ G_{GG,b} + S_C \cdot \max(0, C(t) - C_{thres}) \right] \cdot E(t) \]  
\[ E(t) = \frac{1}{2} \left[ 1 - \tanh \left( \frac{t - t_D}{\tau} \right) \right] \]

\( G_6P \) is glucose 6-phosphate and \( k_{G6P} \) is the rate of dephosphorylation. \( G_{GG} \) is the EGP contribution from glycogenolysis. \( G_{GG,b} \) and \( G_{GNG,b} \) are the basal glycogenolysis and gluconeogenesis, respectively. \( S_C \) is the sensitivity of glycogenolysis to glucagon concentration, \( C \), exceeding a threshold concentration, \( C_{thres} \). \( E \) is the evanescence effect of glycogenolysis. \( t_D \) is the delay of the evanescence effect at which it reduces the glycogenolysis by 50% and \( \tau \) is the time constant of the evanescence phenomenon.

Equations (4.18)-(4.21) describe how EGP depends on glucagon. EGP also depends on glucose indirectly as it affects gluconeogenesis with some delay. However, in this simulation with a euglycemic clamp the glucose dependency is neglected. The relationship between glucagon concentration and glycogenolysis...
Figure 4.6: EGP simulations of the model proposed by Hinshaw et al. [35] after a SC glucagon bolus (left), and SC glucagon infusion (right), see Figure 4.1 for the corresponding PK profiles. The solid curves show the responses when including the evanescence effect as published [35], and the dashed curves show responses when neglecting the evanescence effect of the model. The effect of insulin is not shown as it is not included in the model.

is linear when glucagon exceeds a threshold, although it is dampened and made non-linear by the evanescence effect.

Table 4.6: Specific model parameter values used for simulation of the Hinshaw et al. EGP model [35].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{GEP}$</td>
<td>0.032</td>
<td>min$^{-1}$</td>
</tr>
<tr>
<td>$G_{GNG,b}$</td>
<td>4.4</td>
<td>µmol/kg/min</td>
</tr>
<tr>
<td>$G_{GG,b}$</td>
<td>6.6</td>
<td>µmol/kg/min</td>
</tr>
<tr>
<td>$S_C$</td>
<td>0.272</td>
<td>µmol/kg/min per pg/mL</td>
</tr>
<tr>
<td>$C_{thres}$</td>
<td>80</td>
<td>pg/mL</td>
</tr>
<tr>
<td>$t_D$</td>
<td>74.77</td>
<td>min</td>
</tr>
<tr>
<td>$\tau$</td>
<td>15.99</td>
<td>min</td>
</tr>
</tbody>
</table>

Table 4.6 lists the model parameter values of (4.18)-(4.21) used for the simulations displayed in Figure 4.6. The values of $G_{GG,b}$ and $G_{GNG,b}$ are guessed by the author based on Cherrington stating that basal EGP is approximately 11 µmol/kg/min with gluconeogenesis contributing 40% [36]. The value of $S_C$ might be off by a factor of $10^3$ because the unit is reported as per ng/mL in [35]. If assuming the unit is correct, the response to changes in glucagon from the threshold becomes very small and in fact the EGP response to a bolus and an infusion becomes practically identical. In this model, the effect of glucagon on EGP is not delayed but suppressed by the evanescence effect after $t_D$ minutes.
4.7 Glucagon Rate of Change

The difference in the response with and without the evanescence effect is exemplified in Figure 4.6. Note, that the model was based on IV glucagon infusions which would reach steady state much faster than SC infusion of glucagon. Also, because neither the SC bolus or SC infusion yield a steep increase in glucagon concentration like an IV infusion would, the parameter accounting for a rapid increase in glucagon is ignored.

The influence of insulin on EGP is not captured in the model equations, and thus the third scenario with an insulin bolus preceding a glucagon bolus is omitted.

4.7 Glucagon Rate of Change

Based on a model comparison of nine published or new EGP models, Emami et al. found that a multiplicative relationship between insulin and glucagon was needed to describe the interaction between the two hormones [114]. Moreover, the glucagon rate of change was included in the final model description achieving the best fit:

\[
EGP(t) = \max(1 - S_I I_E(t), 0) \cdot \max(G_{\Delta C}(t) + S_C C(t), 0) + G_{GNG} \tag{4.22}
\]

\[
\frac{dG_{\Delta C}(t)}{dt} = -k_{GdC} G_{\Delta C}(t) + S_{\Delta C} \Delta C(t) \tag{4.23}
\]

\[
\frac{dI_E(t)}{dt} = k_I [I(t) - I_E(t)] \tag{4.24}
\]

\(I\) and \(C\) are the plasma insulin and glucagon concentrations. \(I_E\) is the insulin concentration in the effect compartment. \(G_{\Delta C}\) is an effect compartment depending on the glucagon rate of change. \(S_I\) and \(S_C\) are insulin and glucagon sensitivities. \(S_{\Delta C}\) is the sensitivity to glucagon rate of change. \(k_{GdC}\) and \(k_I\) are transfer rate constants. \(G_{GNG}\) is a constant EGP contribution independent of insulin and glucagon corresponding to gluconeogenesis.

Table 4.7: Specific model parameter values used for simulation of the Emami et al. EGP model [114].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(G_{GNG})</td>
<td>5.54</td>
<td>(\mu)mol/kg/min</td>
</tr>
<tr>
<td>(S_I)</td>
<td>0.02</td>
<td>per mU/L</td>
</tr>
<tr>
<td>(S_C)</td>
<td>0.063</td>
<td>(\mu)mol/kg/min per pg/mL</td>
</tr>
<tr>
<td>(S_{\Delta C})</td>
<td>0.0015</td>
<td>(\mu)mol/kg/min per pg/mL</td>
</tr>
<tr>
<td>(k_{GdC})</td>
<td>0.022</td>
<td>min(^{-1})</td>
</tr>
<tr>
<td>(k_I)</td>
<td>0.068</td>
<td>min(^{-1})</td>
</tr>
</tbody>
</table>

Equations (4.22)-(4.24) describe how insulin and glucagon pull the EGP in op-
Figure 4.7: EGP simulations of the model proposed by Emami et al. [114] after a SC glucagon bolus (left), SC glucagon infusion (middle), and SC insulin bolus followed 150 minutes after by a SC glucagon bolus (right), see Figure 4.1 for the corresponding PK profiles. The solid curves (left and middle) show the responses using a published parameter value of $S_{AC}$ [114], and the dashed curves show responses when $S_{AC}$ is 35 (bolus) or 200 (infusion) times as large. The solid curve (right) show the impact of insulin above basal. The dotted curve is equal to the solid curve of the left graph with 150 minutes delay.

Data used for model building originated from a clinical study of 11 subjects with T1D undergoing a euglycemic glucose clamp with constant insulin infusions at various levels and multiple glucagon boluses [48]. Both glucose and insulin were constant throughout a study day.

Table 4.7 lists the model parameter values of (4.22)-(4.24) used for the simulations displayed in Figure 4.7. Overall, the EGP responses to glucagon follow the dynamics of the plasma glucagon concentrations. The rise and fall of EGP becomes steeper with an increased sensitivity to the glucagon rate of change as illustrated in the left and middle graphs of Figure 4.7. The increased steepness of EGP also shortens time to peak EGP with a few minutes. With a very high sensitivity to the glucagon rate of change, the EGP response to glucagon infusion start overshoots and returns to a baseline above basal. The effect of insulin is visible in the right graph of Figure 4.7 where the solid line represents the influence of insulin on EGP compared to the dotted line with insulin remaining constant at the basal level. After the insulin bolus but with
57

4.8 Saturation of Glucose Production

Inspired by the model presented in section 4.7, Wendt et al. proposed yet another model of EGP [18]. The model was developed based on data from dogs who received very high glucagon boluses making it necessary to introduce a saturation effect of the response to glucagon. Furthermore, the model was tested in healthy volunteers [19], and validated using leave-one-out cross-validation in seven diabetes patients [20]. Using the same terminology as in the previous sections the model is described by the following equations:

\[
\text{EGP}(t) = G_{GG}(t) + G_{GNG}
\]

\[
G_{GG}(t) = \frac{\max(1 - S_I I_E(t), 0)}{1 - S_I I_b} \left[ E_{max} - G_{GNG} \right] \frac{C(t)}{C_{E50} + C(t)}
\]

\[
\frac{dI_E(t)}{dt} = k_I [I(t) - I_E(t)]
\]

\[4.25\]

\[4.26\]

\[4.27\]

\(G_{GG}\) and \(G_{GNG}\) are the glycogenolysis and gluconeogenesis, respectively. \(S_I\) is the insulin sensitivity, \(I_E\) is the insulin concentration in the effect compartment, and \(I_b\) is the basal insulin concentration. \(C\) is the glucagon concentration, \(C_{E50}\) is the glucagon concentration at which the response is half maximum, and \(E_{max}\) is the maximum response at basal insulin. \(k_I\) is the transfer rate constant of insulin from plasma to the effect compartment.

Equations \[4.25\]-\[4.27\] describe how the EGP depends on insulin and glucagon. Insulin reduces the response to glucagon and completely suppresses the response when the remote insulin concentration exceeds \(S_I^{-1}\). The glycogenolytic response to glucagon follows Michaelis-Menten kinetics and saturates for high concentrations of glucagon.

**Table 4.8:** Specific model parameter values used for simulation of the Wendt et al. EGP model [20].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(G_{GNG})</td>
<td>6</td>
<td>(\mu\text{mol/kg/min})</td>
</tr>
<tr>
<td>(S_I)</td>
<td>0.0415</td>
<td>(\text{per mU/L})</td>
</tr>
<tr>
<td>(E_{max})</td>
<td>84.4</td>
<td>(\mu\text{mol/kg/min})</td>
</tr>
<tr>
<td>(C_{E50})</td>
<td>285</td>
<td>(\text{pg/mL})</td>
</tr>
<tr>
<td>(k_I)</td>
<td>0.0068</td>
<td>(\text{min}^{-1})</td>
</tr>
</tbody>
</table>
Figure 4.8: EGP simulations of the model proposed by Wendt et al. [20] after a SC glucagon bolus (left), SC glucagon infusion (middle), and SC insulin bolus followed 150 minutes after by a SC glucagon bolus (right), see Figure 4.1 for the corresponding PK profiles. The solid curves (left and middle) show the responses using a published parameter value of $C_{E50}$ [20], and the dashed curves show responses when $C_{E50}$ is reduced by a factor of 2.85. The solid curve (right) show the impact of insulin above basal. The dotted curve is equal to the solid curve of the left graph with 150 minutes delay.

Data used for model validation originated from a clinical study of 8 subjects with T1D undergoing four study days with an insulin bolus followed by a glucagon or saline bolus [56]. Neither glucose, insulin or glucagon were constant during an entire study day.

Table 4.8 lists the model parameter values of (4.25)-(4.27) used for the simulations displayed in Figure 4.8. The EGP responses to glucagon boluses are not as peaked as previous simulations which might be explained by the high glucagon concentrations leading to saturation of the EGP response during parts of the simulation. The value of $C_{E50}$ influences the glucagon concentration at which the response saturates - the smaller the value, the lower glucagon concentration is required to reach maximum response. Changes in $C_{E50}$ influences the overall magnitude and the peakedness of the EGP response as illustrated in the left and middle graphs of Figure 4.8. The effect of insulin is visible in the right graph of Figure 4.8 where the solid line represents the influence of insulin on EGP compared to the dotted line with insulin remaining constant at the basal level. After the insulin bolus but with glucagon remaining constant and basal, the EGP drops slightly from its basal level. The insulin level has a pronounced influence on the EGP response to glucagon as the peak is noticeably lower in the presence of insulin above basal.
4.9 Model Comparison

The seven presented models of glucagon’s effect on EGP have some similarities but also numerous differences. This section compares the models to each other, and discusses the models in relation to the known physiology explained in Section 2.3. Model comparison is based on the general structure, the dynamic responses to a glucagon bolus or infusion, the absolute responses to glucagon at basal insulin and following an insulin bolus, and the steady state glucagon concentration-response relationship.

4.9.1 Model Structure

The models in the previous sections seek to describe the EGP response to glucagon. Three of the models depend on glucagon delayed by an effect compartment [122, 123, 124], whereas the four other models depend directly on the plasma glucagon concentration [20, 35, 114, 125]. Four of the seven models depend on insulin delayed by a single effect compartment [20, 114, 122, 124], and one model delays insulin by two effect compartments [123]. Thus two of the models do not describe the effect of insulin on EGP [35, 125].

Table 4.9 provides an overview of some of the most important structural model features: glucose dependency, interaction (multiplicative) or independence (additive) relationship of glucagon and insulin effects, constant production from independent mechanism, evanescence effect of glycoegenolysis, glucagon rate of change dependency, and saturation of glucose response to glucagon.

Table 4.9: Comparison of structural model elements in the seven presented EGP models: glucose level (G), insulin level (I), glucagon level (C), gluconeogenesis (G_{GNG}), evanescence effect (E), glucagon rate of change (\Delta C), saturation of EGP response (EGP_{max}).

<table>
<thead>
<tr>
<th>Model</th>
<th>G</th>
<th>I + C</th>
<th>I - C</th>
<th>G_{GNG}</th>
<th>E(t)</th>
<th>\Delta C</th>
<th>EGP_{max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herrero [122]</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Dalla Man [123]</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Schnick [124]</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Peng [125]</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hinshaw [35]</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Emami [114]</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Wendt [20]</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>
4.9.2 Impact of Glucose

Four of the presented models explicitly include the effects of glucose on EGP. Two models describe an additive effect of glucose and glucagon, whereas two other models present a multiplicative relationship between glucose and glucagon. The model by Hinshaw et al. includes the effect of glucose indirectly by increasing gluconeogenesis after prolonged hypoglycemia. The remaining two models are not influenced by the glucose level. As outlined in Section 2.3, the ambient glucose level does not influence the immediate effect of glucagon on EGP. Therefore, the models with multiplicative relationships of glucagon and glucose seem least physiologic. Moreover, glucagon stimulates gluconeogenesis rather than glycogenolysis after three hours of hypoglycemia. Though the model by Hinshaw et al. describes this phenomenon, it only describes this for a specific blood glucose level of 3.3 mmol/L and is thus not generalizable to other hypoglycemic glucose levels. Furthermore, the number of delay compartments determining the delay of the hypoglycemia stimulating effect on gluconeogenesis are not public available.

4.9.3 Impact of Insulin

The EGP models behave very similar after a glucagon bolus during euglycemia and constant basal insulin concentration. Though, in the models including the effects of insulin, the EGP response to glucagon changes in the presence of a preceding insulin bolus and the model differences become more obvious. Due to the extra remote insulin compartment, the insulin action is delayed the longest in the model by Dalla Man et al., see Figure 4.9. Comparing the EGP responses in Figures 4.2-4.4 and 4.7-4.8, we observe that insulin has little influence on the EGP response to glucagon when the model structure has an additive relationship of insulin and glucagon. On the contrary, when the insulin-glucagon relationship is multiplicative, the response to a glucagon bolus in the presence of insulin is noticeably reduced. Figure 4.9 shows that the absolute EGP responses using the Emami and Wendt models in the presence of insulin are very similar. As outlined in Section 2.3, high insulin levels suppress the EGP response to glucagon, and thus the models with multiplicative descriptions of insulin and glucagon seem most physiologic as they can describe this phenomenon. Another issue with the additive insulin-glucagon models is visible in Figure 4.9, where the EGP becomes negative when insulin is above basal and glucagon remains basal because the basal EGP is zero. The EGP being zero at basal and becoming negative during the simulation reveal that an additive insulin-glucagon relationship is non-physiologic.
Figure 4.9: Direct comparison of EGP response to glucagon in the presence of insulin above basal. At time=0 a bolus of 4 U insulin was given followed by a glucagon bolus of 300 µg at time=150.

4.9.4 Glucagon time-response relationship

The EGP response generally follows the glucagon PK when insulin is basal, except when the evanescence effect is present [35], when the sensitivity to glucagon rate of change dominates [174], or when the glucagon response saturates for large parts of the simulated glucagon levels [20]. Figure 4.10 directly compares the seven EGP models at basal insulin using nominal parameter values. After an identical SC glucagon bolus, all models simulate a rise in EGP although with varying steepness and time of maximum response as seen in the left graph. After 100 to 150 minutes all models simulate that the EGP has returned to baseline which is zero in two models [122, 123] and around 10 µmol/kg/min according to the other models [20, 35, 114, 124, 125]. In Figure 4.11a the EGP response to a glucagon bolus peaks after approximately 20 minutes and returns to baseline after 120 minutes. Comparing the left graph of Figure 4.10 to Figure 4.11a the return to baseline is well captured by the EGP models, but several of them simulate a much slower time to maximum response.

Following initiation of an identical SC glucagon infusion, six of the models simulate with nominal parameter values that the EGP immediately increases and reach a steady state plateau within 100 minutes although at levels ranging from less than 10 to 60 µmol/kg/min as seen in Figure 4.10. The model by Hinshaw et al. stands out as the increase in EGP is delayed and the effect transient
which is due to the evanescence effect of glucagon included in the model. In Figure 4.11b, the EGP response to initiation of a glucagon infusion rapidly increases and then returns to a new elevated baseline after 120 minutes despite the continued infusion of glucagon. Although the Hinshaw model includes the evanescence effect, it still does not seem to capture the true time course of EGP after glucagon infusion. However, remembering the middle graph of Figure 4.7, the response mimics the evanescence effect described in literature when the sensitivity to glucagon rate of change is 200 times the nominal value.

The literature in Section 2.3 confirms that the EGP response to a continuous glucagon stimulation wanes over time. Due to the mathematical formulation in (4.21), the evanescence effect is irreversible due to the tangent hyperbolic function. It is unclear what happens to the evanescence effect when multiple doses are injected. Physiologically, when glucagon stimulation is discontinued the EGP system might be desensitized for some time, but should then return to normal. Having a refractory period where further stimulation does not produce a response is well known from neurons essentially also acting through receptor mechanisms although in such cases the refractory period only lasts for milliseconds. However, the reversal of the evanescence effect of glucagon is not studied thoroughly and can therefore not be modelled until investigated in a clinical
4.9 Model Comparison

(a) EGP response to SC glucagon boluses, from [48].

(b) EGP response to an IV glucagon infusion, from [36].

Figure 4.11: Time course of EGP response to glucagon.

setting. To have a reversible situation the evanescence effect could perhaps be modelled through a chain of compartments rather than a tangent hyperbolic function.

4.9.5 Absolute Glucagon Concentration

The left graph of Figure 4.11 displays the glucagon PK profile after a bolus of 300 µg based on the model and parameters summarized in Section 4.1. According to the model parameter, $C_b$, the baseline glucagon concentration for this virtual subject is 11 pg/mL. The glucagon PK model was based on data measured by a novel assay technique with high accuracy. Comparison of this assay to commercially available assays showed that they tend to overestimate the glucagon concentration by as much as 100% due to lack of specificity and precision [32]. This difference in assay performance could explain the variations in baseline glucagon concentration reported together with the models: $\sim$48 pg/mL [122], $\sim$130 pg/mL [124], $\sim$60 pg/mL [125], $\sim$35 pg/mL [35], and $\sim$100 pg/mL [114].

Data used for model building by both Herrero et al. and Emami et al. were measured by an immunoassay from Millipore, and the data used by Hinshaw et al. was measured by a double antibody radio-immunoassay from Linco Research. Neither Schneek et al. nor Peng et al. provide information on which assay was used for sample analysis of glucagon. Thus, in all of these EGP models based on glucagon data with higher baseline than the virtual subject, the EGP responsiveness to glucagon i.e. the sensitivity, might be underestimated. Moreover, the model by Hinshaw et al. uses a specific threshold of 80 pg/mL to describe the concentration above which glucagon affects EGP. Applying this threshold to the virtual subject, the required glucagon concentration increase
Figure 4.12: Comparison of the concentration-response relationship between glucagon and EGP for the presented EGP models assuming a steady state for discrete glucagon concentrations.

from baseline to yield an effect is close to 70 pg/mL, which conflicts with literature stating that even small glucagon increments of 10 pg/mL are effective in stimulating EGP [36].

These issues emphasize what was already pointed out in Section 2.2.4. Thus applying a PK model based on data from one method of sample analysis could lead to over or under estimation of the concentration-response sensitivity in the PD model if the PD parameters are based on glucagon data obtained by an assay with different accuracy.

4.9.6 Glucagon Concentration-Response Relationship

Although most of the EGP models are time-dependent, their behaviour in steady state for various levels of glucagon can be compared. Figure 4.12 presents the steady state EGP response to glucagon of the seven EGP models with nominal parameter values. Six of the models describe the glucagon-EGP relationship with a linear or piece-wise linear function. The model by Wendt et al. stands out as the only model describing a saturation of the EGP response to high concentrations of glucagon. As described in Section 2.3, glucagon stimulates EGP through a receptor mediated mechanism and therefore it makes physio-
Figure 4.13: EGP as a function of glucagon concentration with data from dogs (black dots) and humans (white dots), from [36]. To convert from mg/kg/min to μmol/kg/min the value is multiplied by 5.55, thus 10 mg/kg/min = 55.5 μmol/kg/min.

logically sense to have a model with a maximum EGP response. Figure 4.13 provides another evidence that the EGP response to glucagon approaches saturation for high glucagon concentrations. Comparing Figure 4.12 and 4.13 the concentration-response relationship of the Wendt model compares well to the clinical results in terms of maximum response and concentration yielding half maximum effect. For low concentrations of glucagon not exceeding 400 pg/mL a linear approximation of the glucagon-EGP relationship might be sufficient, but for higher concentrations of glucagon, the discrepancies between the model with saturation and the linear models become large. In the presented simulation example with a bolus of 300 μg glucagon the plasma concentration reached 600 pg/mL and even at this concentration we observe large variations in the size of the steady state EGP response. Notice, that the models by Herrero et al. and Emami et al. having used the same assay for glucagon analysis, both underestimate the EGP response to low concentrations of glucagon compared to the Wendt model. The remaining four models seem to follow the Wendt model for low concentrations of glucagon but greatly overestimates the EGP response for glucagon concentrations exceeding ~200 pg/mL.

It is interesting to observe how the models describe the EGP response to glucagon for concentrations approaching zero. Figure 4.12 visualizes that both the models by Dalla Man et al. and Hinshaw et al. require glucagon to exceed a threshold before having a stimulatory effect on EGP. The model by Herrero et al. reveals to be even more non-physiologic as the EGP becomes negative when the glucagon concentration is zero.
4.9.7 Closing Remarks

Calculation of EGP from clinical data is not straightforward. A major limitation in development of EGP models is the data used for model building. The golden standard for measuring EGP is a triple tracer dilution technique followed by application of a two compartment model [126]. However, none of the above models have been developed based on data from a study using the triple tracer technique. Two studies used a single glucose tracer technique to estimate EGP [35, 114], and the remaining models were developed based on datasets with measurements of insulin, glucagon and glucose. Using a single tracer technique rather than a triple tracer technique, the calculations highly depend on the associated compartment model [127]. When EGP is not estimated, but included as part of a larger glucoregulatory model, the parameter values associated with the EGP model will depend even more on the encompassing model.

The present model comparison and discussion of seven published EGP models serves to highlight advantages and disadvantages of each model. Unfortunately, a single model does not seem to capture the full complexity of the EGP response to glucagon. This overview might inspire creation of more exhaustive glucagon-EGP models. Although, creating more complex models will also require more complex data preferably obtained using triple tracer technique and demonstrating the physiologic phenomenons sought to be described mathematically.
The first part of this chapter provides the model equations and parameters for a simulation model describing the glucoregulatory system in patients with T1D. The glucose PD model accounts for the effects of both insulin and glucagon on EGP. The equations and parameters of the associated insulin and glucagon PK models are provided for completeness. The model validation is briefly described. A discussion of the model strengths and weaknesses when applying it for simulations concludes the model presentation.

The second half of this chapter provides examples of what the glucose-insulin-glucagon simulation model have been used for. The selected in silico studies include replication of an in vivo study showing the effect of micro-boluses of glucagon at varying insulin levels. The in vivo study was also recreated by administering a wider range of glucagon boluses to further investigate the dose-response relationship of glucagon and EGP. Finally, the simulation model was used to propose glucagon dosing regimens for treatment of insulin-induced mild hypoglycemia depending on the ambient insulin level.
5.1 Simulation Model

The PK and PD simulation models in this section including equations and parameter values were published by Wendt et al. in Journal of Diabetes Science and Technology February 2017 [20]. This section serves as a summary of the glucoregulatory model providing the information necessary to discuss it and use it for simulations. For details on the model validation and parameter estimation not stated in this section, the reader is referred to [20], which is included in Appendix C.

5.1.1 Insulin Pharmacokinetics

The insulin PK model is adopted from Haidar et al. [28] and describes how insulin is transferred from the SC tissue to the central compartment using a simple two-state model with identical time constants for the absorption rate and the elimination rate.

\[
\frac{dX_1(t)}{dt} = u_I(t) - \frac{X_1(t)}{t_{\text{max}}} \quad X_{1,SS} = u_{I,SS} \cdot t_{\text{max}} \tag{5.1}
\]

\[
\frac{dX_2(t)}{dt} = \frac{X_1(t)}{t_{\text{max}}} - \frac{X_2(t)}{t_{\text{max}}} \quad X_{2,SS} = u_{I,SS} \cdot t_{\text{max}} \tag{5.2}
\]

\[
I(t) = \frac{1}{t_{\text{max}}} \frac{X_2(t)}{W \cdot Cl_{F,I}} 10^6 + I_b \quad I_{SS} = \frac{u_{I,SS}}{W \cdot Cl_{F,I}} 10^6 + I_b \tag{5.3}
\]

Table 5.1: Interpretation of insulin PK model states, input, output and parameters.

<table>
<thead>
<tr>
<th>Class</th>
<th>Variable</th>
<th>Unit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>States</td>
<td>$X_1(t)$</td>
<td>U</td>
<td>mass due to SC dosing, in SC tissue</td>
</tr>
<tr>
<td></td>
<td>$X_2(t)$</td>
<td>U</td>
<td>mass due to SC dosing, in serum</td>
</tr>
<tr>
<td>Input</td>
<td>$u_I(t)$</td>
<td>U/min</td>
<td>dose</td>
</tr>
<tr>
<td>Output</td>
<td>$I(t)$</td>
<td>mU/L</td>
<td>concentration in serum</td>
</tr>
<tr>
<td>Parameters</td>
<td>$I_b$</td>
<td>mU/L</td>
<td>basal concentration</td>
</tr>
<tr>
<td></td>
<td>$t_{\text{max}}$</td>
<td>min</td>
<td>time to maximum serum concentration</td>
</tr>
<tr>
<td></td>
<td>$W$</td>
<td>kg</td>
<td>body weight</td>
</tr>
<tr>
<td></td>
<td>$Cl_{F,I}$</td>
<td>mL/kg/min</td>
<td>apparent clearance</td>
</tr>
</tbody>
</table>
Figure 5.1: Examples of insulin PK profiles after one bolus administration of 2 U in eight virtual patients (left), and in one randomly selected virtual patient (no. 2) following various insulin boluses (right). The left orange curve is identical to the right purple curve.

Table 5.1 lists the interpretations of the insulin PK model variables. The steady state conditions are listed together with the model equations in (5.1)-(5.3). When the system is undisturbed, thus $u_I$ being zero, both states are equal to zero and the serum concentration equals the basal concentration $I_b$, which is maintained by a constant basal insulin infusion. The basal concentration was modelled as a parameter due to the basal infusion rate being unknown in the data used for model fitting. However, rearranging the steady state equation in (5.3), the required insulin infusion rate to yield a specific basal steady state concentration can be obtained when the model parameters are known. The parameter, $I_b$, can then be omitted and replaced by an input describing the constant basal infusion accordingly.

The model could be expanded to handle IV insulin infusions by addition of an input variable to the second state equation in (5.2). Caution must be taken if the bioavailability after SC and IV administration are not identical as this will influence the model parameters. Moreover, as regular insulin exerts flip-flop kinetics [31], the elimination of drug estimated after SC administration will underestimate the true elimination of drug after IV administration due to the slow SC absorption. The slow SC absorption might also mask the existence of a distribution compartment that is clearly identified after IV administration and the model in (5.1)-(5.2) might therefore not be adequate to describe PK after IV administration after all. Chapter 2 explained the PK after intravascular and
extravascular administration in details and can be consulted for further clarification. Section 5.1.5 presents individual insulin PK model parameter values and when they apply for eight virtual T1D patients. Figure 5.1 visualizes the differences in insulin PK after administration of an identical bolus to each patient of the virtual population, and the dose-concentration relationship in one of the patients.

5.1.2 Glucagon Pharmacokinetics

The glucagon PK model is adopted from Wendt et al. [18] and describes how glucagon is transferred from the SC tissue to the central compartment using a simple two-state model with different absorption rate and elimination rate constants, where the elimination rate is greater than or equal to the absorption rate.

\[
\frac{dZ_1(t)}{dt} = u_C(t) - k_1 Z_1(t) \quad Z_{1,SS} = \frac{u_{C,SS}}{k_1} \\
\frac{dZ_2(t)}{dt} = k_1 Z_1(t) - k_2 Z_2(t) \quad Z_{2,SS} = \frac{u_{C,SS}}{k_2} \\
C(t) = \frac{k_2 Z_2(t)}{W \cdot Cl_{F,C}} + C_b \quad C_{SS} = \frac{u_{C,SS}}{W \cdot Cl_{F,C}} + C_b
\]

The steady state conditions are listed together with the model equations in (5.4)-(5.6). When the system is undisturbed thus no SC glucagon bolus or infusion, both states are equal to zero and the plasma concentration equals the basal concentration maintained by a constant endogenous production. Similar to the insulin PK model described in Section 5.1.1 the glucagon PK model can also be modified to include IV dosing of glucagon, although the same cautions apply regarding bioavailability, and underestimation of elimination of drug and applicability due to flip-flop kinetics. The PK of glucagon is explained in details in Section 2.2.

Table 5.2 lists the interpretations of glucagon PK model variables. Individual glucagon PK model parameter values of eight virtual patients with T1D are presented in Section 5.1.5. Figure 5.2 visualizes the differences in plasma glucagon concentration after an identical bolus administered to each patient of the virtual population, and the dose-concentration relationship in one of the patients.
Table 5.2: Interpretation of glucagon PK model states, input, output and parameters.

<table>
<thead>
<tr>
<th>Class</th>
<th>Variable</th>
<th>Unit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>States</td>
<td>$Z_1(t)$</td>
<td>pg</td>
<td>mass due to SC dosing, in SC tissue</td>
</tr>
<tr>
<td></td>
<td>$Z_2(t)$</td>
<td>pg</td>
<td>mass due to SC dosing, in plasma</td>
</tr>
<tr>
<td>Input</td>
<td>$u_C(t)$</td>
<td>pg/min</td>
<td>dose</td>
</tr>
<tr>
<td>Output</td>
<td>$C(t)$</td>
<td>pg/mL</td>
<td>concentration in plasma</td>
</tr>
<tr>
<td>Parameters</td>
<td>$C_b$</td>
<td>pg/mL</td>
<td>basal concentration</td>
</tr>
<tr>
<td></td>
<td>$k_1$</td>
<td>min$^{-1}$</td>
<td>absorption rate constant</td>
</tr>
<tr>
<td></td>
<td>$k_2$</td>
<td>min$^{-1}$</td>
<td>elimination rate constant</td>
</tr>
<tr>
<td></td>
<td>$W$</td>
<td>kg</td>
<td>body weight</td>
</tr>
<tr>
<td></td>
<td>$Cl_{F,C}$</td>
<td>mL/kg/min</td>
<td>apparent clearance</td>
</tr>
</tbody>
</table>

Figure 5.2: Examples of glucagon PK profiles after one bolus administration of 300 µg in eight virtual patients (left), and in one randomly selected virtual patient (no. 6) following various glucagon boluses (right). The left turquoise curve is identical to the right yellow curve.
5.1.3 Glucose Pharmacodynamics

The majority of the glucose PD model explained in the following was originally published by Hovorka et al. [17], and then extended with an EGP model including the effects of both insulin and glucagon by Wendt et al. [18]. The glucoregulatory model by Hovorka was chosen as the model foundation since it was validated using a multi tracer technique and the publication provided parameter values for six subjects, which served as prior information during parameter estimation.

The model extension was developed using preclinical data from healthy dogs [18], included in Appendix A. It was then tested with clinical data from healthy humans to confirm that the model extension translated to the human species [19], included in Appendix B. Finally, the PD model was cross-validated successfully in seven T1D patients [20], included in Appendix C The complete model structure is described by (5.7)-(5.13).

\[
\frac{dQ_1(t)}{dt} = -F_0 - F_R - S_T x_1(t)Q_1(t) + k_{12}Q_2(t) + G_{GG}(t) + G_{GNG} 
\]

(5.7)

\[
\frac{dQ_2(t)}{dt} = S_T x_1(t)Q_1(t) - [k_{12} + S_D x_2(t)]Q_2(t) 
\]

(5.8)

\[
G_{GG}(t) = \max(1 - S_E x_3(t), 0) \frac{E_{max} - G_{GNG}}{1 - S_E I_b} C(t) \frac{C(t)}{C_{E50} + C(t)} 
\]

(5.9)

\[
G(t) = \frac{Q_1(t)}{V} 
\]

(5.10)

\[
\frac{dx_1(t)}{dt} = k_{a1}[I(t) - x_1(t)] 
\]

(5.11)

\[
\frac{dx_2(t)}{dt} = k_{a2}[I(t) - x_2(t)] 
\]

(5.12)

\[
\frac{dx_3(t)}{dt} = k_{a3}[I(t) - x_3(t)] 
\]

(5.13)

Table 5.3 lists the interpretations of PD model states, inputs, outputs and parameters. In this model formulation, the EGP is the sum of $G_{GG}$ and $G_{GNG}$ as explained in detail in Section 4.8. Equations (5.14)-(5.18) list the steady state conditions of the model.
Figure 5.3: Examples of glucose PD profiles after an insulin bolus of 2 U followed by a glucagon bolus of 200 µg when the blood glucose decreased below 3.9 mmol/L in eight virtual patients.

\[ Q_{1,SS} = G_{SS} \cdot V \]  
\[ Q_{2,SS} = Q_{1,SS} \cdot \frac{x_{1,SS}}{x_{2,SS} + k_{12}} \]  
\[ x_{1,SS} = I_b \]  
\[ x_{2,SS} = I_b \]  
\[ x_{3,SS} = I_b \]

Section 5.1.5 presents subject specific glucose PD model parameters. Figure 5.3 visualizes the differences in plasma glucose concentration after an identical SC insulin bolus followed by an identical SC glucagon bolus when plasma glucose decreased below 3.9 mmol/L in each patient of the virtual population.
### Table 5.3: Interpretation of Glucose PD model states, input, output and parameters.

<table>
<thead>
<tr>
<th>Class</th>
<th>Variable</th>
<th>Unit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>States</strong></td>
<td>glucose concentration in plasma</td>
<td>mmol/L</td>
<td>insulin sensitivity of glucose transport</td>
</tr>
<tr>
<td></td>
<td>insulin concentration in plasma</td>
<td>mmol/L</td>
<td>remote effects of insulin on glycogenolysis</td>
</tr>
<tr>
<td></td>
<td>glucose concentration in non-accessible compartiment</td>
<td>mmol/L</td>
<td>remote effects of insulin on glucose transporter</td>
</tr>
<tr>
<td></td>
<td>glucose mass M1 in the non-accessible compartiment</td>
<td>mmol/kg</td>
<td>insulin sensitivity of glucose disposed</td>
</tr>
<tr>
<td></td>
<td>glucose mass M1 in the accessible compartiment</td>
<td>mmol/kg</td>
<td>insulin sensitivity of glucose disposed</td>
</tr>
<tr>
<td><strong>Inputs</strong></td>
<td>glucose production due to glycogenolysis</td>
<td>mmol/kg/min</td>
<td>insulin independent glucose flux</td>
</tr>
<tr>
<td></td>
<td>glucose production due to gluconeogenesis</td>
<td>mmol/kg/min</td>
<td>maximum EGP at basal insulin concentration</td>
</tr>
<tr>
<td></td>
<td>glucagon concentration yielding half of maximum EGP</td>
<td>pg/mL</td>
<td>glucagon concentration yielding half of maximum EGP</td>
</tr>
<tr>
<td><strong>Outputs</strong></td>
<td>glucose concentration in plasma</td>
<td>mmol/L</td>
<td>renal glucose clearance</td>
</tr>
<tr>
<td></td>
<td>glucose production due to glycogenolysis</td>
<td>mmol/kg/min</td>
<td>insulin independent glucose flux</td>
</tr>
<tr>
<td></td>
<td>glucose production due to gluconeogenesis</td>
<td>mmol/kg/min</td>
<td>maximum EGP at basal insulin concentration</td>
</tr>
</tbody>
</table>

**Parameters**

- \( A \)
- \( S^G \)
- \( S^D \)
- \( \frac{k_1}{min} \cdot 10^{-4} \)
- \( A \)
- \( S^G \)
- \( S^D \)
- \( \frac{k_a}{min} \cdot 10^{-4} \)
- \( \frac{k_a}{min} \cdot 10^{-4} \)
- \( \frac{k_a}{min} \cdot 10^{-4} \)
- \( \frac{S_D}{min/L} \cdot 10^{-4} \)
- \( \frac{S_T}{min/L} \cdot 10^{-4} \)
- \( V_{glucose} \)
5.1 Simulation Model

5.1.4 Validation

The PD model validation used data from a glucagon dose-finding study in eight well-controlled patients with T1D [56]. The patients completed four similar study days in random order. On each study day, patients received a SC insulin bolus aiming to lower plasma glucose to 3 mmol/L. When plasma glucose decreased below 3.9 mmol/L, a single SC bolus of either 100 µg (visit B), 200 µg (visit C), 300 µg (visit D) glucagon, or saline (visit A) was administered. Serum insulin, plasma glucagon and plasma glucose was measured during the study. Model validation was carried out as a 4-fold leave-one-out cross-validation leaving all data from one visit out per fold. As each subject participated in four visits, each subject had four training datasets comprised of data from three visits and four corresponding test datasets with data from one visit:

- Training: B-C-D, Test: A
- Training: A-C-D, Test: B
- Training: A-B-D, Test: C
- Training: A-B-C, Test: D

Thus, all four visits were used for testing once without being used for optimization during that fold. To validate the PD model in a subject, at least one PD model test-fit of a dataset from a glucagon visit (B, C or D) should be accepted. The simulation accuracy of the model on datasets not used for parameter optimization was assessed by the bias calculated as the mean prediction error (MPE) and the precision calculated by the mean absolute prediction error (MAPE). A model test-fit was accepted if the MPE was less than ±15% and the MAPE was less than 20%. Datasets with MAPE of test-fits exceeding 50% was considered outliers and removed from further analysis. Therefore, the final individual model parameters were based on data from either three or four visits. Further details on study design, model validation and the results thereof can be found in [20].
5.1.5 Parameters

The majority of PK and PD model parameters are subject specific and listed in Table 5.4. The insulin PK parameters describe insulin aspart (NovoRapid®, Novo Nordisk A/S, Bagsværd, Denmark) concentration in serum after SC bolus administration measured by a Merodia assay (Merodia AB, Uppsala, Sweden). The glucagon PK parameters describe glucagon (GlucaGen®, Novo Nordisk A/S, Bagsværd, Denmark) concentration in plasma after SC bolus administration measured by a novel assay technique with high accuracy [32]. Due to the flip-flop kinetics of both insulin and glucagon, where absorption rate is slower than elimination rate following SC administration, the elimination of drug will be underestimated compared to data following IV administration. Other insulin and glucagon types might be described by the same PK models as defined in Sections 5.1.1 and 5.1.2, however their parameters will differ depending on assay accuracy and the formulation of drug-product - some agents will increase others decrease the absorption rate. Moreover, analogues of insulin and glucagon might have different potencies that would influence the sensitivities of the PD model.

It should be noted that both insulin and glucagon were sampled suboptimal in the clinical study. Insulin samples were missing around the time of expected maximum concentration (Tmax) and the exact dosing time of glucagon was uncertain. Due to the lack of dense sampling around Tmax, the insulin PK model could only be estimated by inferring prior knowledge on the parameters describing the kinetics, which could have introduced a bias. Given the short time to maximum concentration for glucagon, identification of the correct dosing time was critical. The most likely dosing time of glucagon was identified before estimation of the final glucagon PK parameters, which could have led to errors in Tmax of up to ±4 minutes.

The glucose PD model defined in Section 5.1.3 was validated using leave-one-out cross-validation in seven out of the eight T1D patients. The model parameters of patient 8 are reported although the model could not be validated in this subject. Therefore, simulations in the following sections include only subjects 1-7. The population of T1D patients were well-controlled and had no endogenous insulin production. The publication of the clinical study provides detailed patient characteristics [56].

A few parameters of the PD model were fixed for all subjects including the rate of gluconeogenesis, $G_{NG}$, at 6 $\mu$mol/kg/min [37], and the glucose volume of distribution, $V$, at 160 mL/kg [117]. The renal clearance of glucose was zero unless the plasma glucose concentration exceeded 9 mmol/L in which case it was calculated as $0.003 \cdot (G - 9) \cdot V$ [128]. Similarly, the insulin independent glucose flux was calculated as $F_{01} \cdot G/4.5$ when the plasma glucose concentration was
## 5.1 Simulation Model

### Table 5.4: Subject-specific PK/PD model parameters used for simulations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
<th>Patient 7</th>
<th>Patient 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>( W ) [kg]</td>
<td>54</td>
<td>30</td>
<td>81</td>
<td>69</td>
<td>87</td>
<td>72</td>
<td>73</td>
<td>59</td>
</tr>
<tr>
<td>( \text{Ib} ) [mU/L]</td>
<td>7.3</td>
<td>10.6</td>
<td>11.7</td>
<td>8.7</td>
<td>6.6</td>
<td>5.5</td>
<td>19.7</td>
<td>7.4</td>
</tr>
<tr>
<td>( t_{\text{max}} ) [min]</td>
<td>57.6</td>
<td>57.3</td>
<td>40.8</td>
<td>67.9</td>
<td>48.5</td>
<td>68.5</td>
<td>55.4</td>
<td>26.8</td>
</tr>
<tr>
<td>( C_{\text{F,I}} ) [mL/kg/min]</td>
<td>18.9</td>
<td>18.5</td>
<td>14.8</td>
<td>17.4</td>
<td>17.3</td>
<td>24.6</td>
<td>23.7</td>
<td>18.0</td>
</tr>
<tr>
<td>( C_{\text{b}} ) [pg/mL]</td>
<td>10.7</td>
<td>7.6</td>
<td>7.6</td>
<td>10.9</td>
<td>8.7</td>
<td>8.9</td>
<td>11.6</td>
<td>7.4</td>
</tr>
<tr>
<td>( k_1 ) [min^{-1}]</td>
<td>0.042</td>
<td>0.056</td>
<td>0.022</td>
<td>0.058</td>
<td>0.035</td>
<td>0.035</td>
<td>0.052</td>
<td>0.035</td>
</tr>
<tr>
<td>( k_2 ) [min^{-1}]</td>
<td>0.14</td>
<td>0.10</td>
<td>0.10</td>
<td>0.19</td>
<td>0.19</td>
<td>0.28</td>
<td>0.35</td>
<td>0.25</td>
</tr>
<tr>
<td>( C_{\text{F,C}} ) [mL/kg/min]</td>
<td>94</td>
<td>106</td>
<td>114</td>
<td>159</td>
<td>200</td>
<td>136</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>( E_{\text{max}} ) [µmol/kg/min]</td>
<td>37</td>
<td>38</td>
<td>39</td>
<td>40</td>
<td>41</td>
<td>42</td>
<td>43</td>
<td>44</td>
</tr>
<tr>
<td>( F_{01} ) [mol/kg/min]</td>
<td>14.2</td>
<td>13.8</td>
<td>15.5</td>
<td>12.8</td>
<td>12.0</td>
<td>13.1</td>
<td>14.2</td>
<td>13.4</td>
</tr>
<tr>
<td>( k_{12} \cdot 10^{-4} ) [min^{-1}]</td>
<td>244</td>
<td>285</td>
<td>307</td>
<td>213</td>
<td>281</td>
<td>238</td>
<td>358</td>
<td>289</td>
</tr>
<tr>
<td>( k_{13} \cdot 10^{-4} ) [min^{-1}]</td>
<td>244</td>
<td>285</td>
<td>307</td>
<td>213</td>
<td>281</td>
<td>238</td>
<td>358</td>
<td>289</td>
</tr>
<tr>
<td>( S_{\text{D}} \cdot 10^{-4} ) [min^{-1}]</td>
<td>1.5</td>
<td>1.2</td>
<td>1.4</td>
<td>2.0</td>
<td>1.1</td>
<td>2.6</td>
<td>4.4</td>
<td>4.2</td>
</tr>
<tr>
<td>( S_{\text{E}} \cdot 10^{-4} ) [min^{-1}]</td>
<td>1.5</td>
<td>1.2</td>
<td>1.4</td>
<td>2.0</td>
<td>1.1</td>
<td>2.6</td>
<td>4.4</td>
<td>4.2</td>
</tr>
<tr>
<td>( S_{\text{T}} \cdot 10^{-4} ) [min^{-1}]</td>
<td>1.5</td>
<td>1.2</td>
<td>1.4</td>
<td>2.0</td>
<td>1.1</td>
<td>2.6</td>
<td>4.4</td>
<td>4.2</td>
</tr>
</tbody>
</table>
below 4.5 mmol/L [28].

5.1.6 Strengths and Limitations

Although the presented simulation model of the glucoregulatory system is detailed and accounts for many glucose-insulin-glucagon dynamics, it is still not perfect. This section serves to discuss pros and cons of the model. Moreover, it provides guidelines for when the model is appropriate to use and which dynamics it can not be expected to capture.

A model can only be expected to describe dynamics present in the data used for model development and parameter estimation, if data is sampled sufficiently often and at the right times. As an example, the PD model failed to identify the correct steady state glucose level [21]. This limitation arose because the data used for parameter estimation contained very little information about the insulin, glucagon and glucose levels before the system was disturbed with an insulin bolus. On the contrary, in the data used for parameter estimation, a steady state seemed to appear towards the end of the experiment when the glucose concentration was approaching hypoglycaemia and both glucagon and insulin had returned to their baseline levels. This data sampling explains why the model assumes glucose steady state lower than one would expect at baseline levels of insulin and glucagon.

Unlike other datasets used for model development, this dataset was dynamic and included an insulin-only phase and an insulin-glucagon phase. Thus, the model is best used to describe glucose dynamics when insulin and glucagon levels are changing. As the effect of glucagon is governed by the evanescence effect during continuous exposure, the simulation model can not be used to simulate the glucose response to continued glucagon infusion. Datasets with constant glucose, insulin and/or glucagon levels should be used for investigating isolated dynamics like the evanescence effect.

During the experiment, the patients received relatively small correcting insulin boluses aiming to lower the blood glucose to 3 mmol/L. The dataset did not contain information about how the glucose dynamics i.e. the fall rate might change in the case of an over-bolus and can therefore not be expected to simulate this reliably.

Factors influencing the model parameters can not be included in the description of the parameters if the factors do not vary in the training dataset. As an example, exercise and stress are long known to alter the insulin sensitivity [82], and recently Ranjan et al. found that diet might influence the response to glucagon [58]. None of these factors are accounted for in the glucose PD model used for simulations in the following sections.
The model does not include a feedback mechanism of the glucose levels to the endogenous production of insulin and glucagon. Typically, patients with T1D do not have endogenous insulin production and therefore it is fair not to include this mechanism in a model describing the glyco-regulatory system of this population. Although blunted, the glucagon production is existing in patients with T1D. However, the endogenous glucagon response to hypoglycemia corresponds to plasma increases observed after SC boluses of 1-10 µg [20]. With SC glucagon doses significantly larger than 10 µg, it is fair to neglect the possible endogenous glucagon response to hypoglycemia as the endogenous contribution will be minor.

Despite all the limitations, the model definitely also has some advantages. The model equations together with parameters describing seven T1D patients are public accessible [20]. The model describes the glucose dynamics of patients rather than healthy subjects and features both insulin and glucagon. The model describes a population for which it is relevant to have a simulation model so that researchers can develop improved treatment regimens for patients with T1D. Although the population is limited to seven patients, the model is validated using leave-one-out cross-validation, which is rarely executed when new models are proposed.

5.2 In Silico Studies

Having a reliable simulation model of the glyco-regulatory system can aid in the planning of clinical trials and investigation of dose selection. In silico studies benefit over in vivo trials in not being restricted by the number of studies per subject or in total, the number of samples during same day experiments, and the amount of drug administrated. Moreover, nuisance factors disturbing clinical study outcomes are not present in simulation studies. The following sections present examples of applications for the simulation model just presented in Section 5.1.

5.2.1 Replication of an in Vivo Study

The simulation model was used to replicate a clinical study by El Youssef et al. with the title "Quantification of the Glycemic Response to Microdoses of Subcutaneous Glucagon at Varying Insulin Levels" [48]. The simulation study was published as a technical report with in silico replications of all in vivo results [21], which is included in Appendix D. This section presents highlights of
the technical report focusing on comparison of three \textit{in silico} to \textit{in vivo} results: time course of EGP after four different glucagon boluses, the EGP over 60 minutes separated by insulin infusion rate (IIR) and glucagon bolus size, and finally dose-response curves of glucagon and EGP separated by IIR.

The study was a cross-over where each patient participated in three study days of ten hours duration. Patients received IV IIRs of either low, medium or high. Glucose infusion rates were controlled using a proportional integral derivative (PID) controller aiming at a blood glucose concentration of 85 mg/dL. When blood glucose read below 60 mg/dL the controller regulated the glucose infusion rate every five minutes, otherwise every ten minutes. After an initial two hours run-in period the subjects received the first glucagon bolus. They received the second glucagon bolus after another two hours until a total of four glucagon boluses were delivered and observed for the following two hours. The glucagon boluses were delivered in a pseudo-random order by varying the initial dose, but keeping the order: 25 µg, 75 µg, 125 µg, and 175 µg (e.g. 125-175-25-75). Each subject received the same pseudo-random order of glucagon boluses during each study day. The \textit{in vivo} study included 11 patients while the \textit{in silico} study was based on the seven virtual patients. However, one virtual subject (no. 7) was excluded due to almost similar IIRs thereby almost no difference in EGP response at various insulin levels. The T1D patients in the \textit{in vivo} study had slightly higher body mass indexes and were less well-controlled with higher HbA1c values than the virtual patients. However, we consider these population differences of minor importance. The \textit{in vivo} study used regular human insulin (Humulin R, Eli Lilly and Company) and glucagon (GlucaGen, Novo Nordisk). The simulation model is based on GlucaGen too, but insulin is described by insulin aspart (NovoRapid, Novo Nordisk). Since the insulin infusion was constant during a study day the potential PK differences are negligible, but the PD effect of the two insulins could be different. The EGP was calculated using a single tracer technique \textit{in vivo}, and obtained directly from the model \textit{in silico}.

Figure 5.4b replicates Figure 5.4a with many similarities but also some differences showing the time course of EGP after four different glucagon boluses. Most importantly, the magnitudes of average peak EGP to the four glucagon doses were similar. The EGP increase appeared to be more rapid \textit{in silico} than \textit{in vivo} yielding a faster T\text{max}, which could be partly explained by a faster glucagon T\text{max} observed \textit{in silico} than \textit{in vivo}. However, with sampling of only every ten minutes the observed T\text{max} could be anywhere between 10-30 minutes and the simulated T\text{max} could be between 0-20 minutes (the average is in fact 12 minutes). If the true T\text{max} of EGP in response to glucagon was between 10-20 minutes, this agreed with both the observed and simulated results.

The \textit{in vivo} estimated EGP returned fast to baseline and after 60 minutes it was below the production before injection of the preceding glucagon bolus. The simulated EGP had slower return to baseline and only slightly negative values after the lowest glucagon dose.
Figure 5.5a presents one of the most interesting results which is replicated by simulation in Figure 5.5b showing the EGP over 60 minutes separated by IIR and glucagon bolus size. The averages of the simulated data were different from the observed averages. However, considering the standard error of measurement of both datasets, the simulated data was not different from the observed data. The EGP responses to doses of glucagon during medium IIR were very similar to the EGP responses during low IIR in the measured data, whereas they were different between the responses during the two IIRs when simulating the experiments. The simulated data showed small increases in response to increasing glucagon boluses even at high IIR, which was not pronounced in the original observed data. In general, the standard error of measurements were smaller in silico than in vivo.

Figure 5.6b replicates Figure 5.6a but without extrapolation of the dose-response curves of glucagon and EGP separated by IIR. The original graph shows the actual data in the dose-range of 25-175 $\mu$g glucagon and extrapolates the presumed trends down to 1 $\mu$g and up to 10 mg. Note, this is a wild extrapolation with no data to support it. Within the data-range the simulated results matched the observed results although the simulated EGP at low IIR tended to be higher than the observed. Having only four points very closely spaced on a log-scale, a single point can largely influence the overall interpretation of the curves.

In conclusion, the simulated results are similar to the results obtained in vivo. The simulation also emphasized some limitations of the clinical study which then inspired the simulation study in the following section.
Figure 5.4: Time profiles of calculated EGP by glucagon dose, baseline corrected for EGP at the time of dose.
5.2 *In Silico* Studies

Figure 5.5: Mean EGP AUC separated by glucagon dose and insulin infusion rate.

(a) *In vivo* from [48].

(b) *In silico* (n=6) from [21].

Figure 5.6: Dose-response curve across all doses, and for low and high insulin infusion rate experiments, estimated from simulated data.

(a) *In vivo* from [48].

(b) *In silico* (n=6 or 18) from [21].
Figure 5.7: Simulated averages of seven T1D patients’ dose-response curves of glucagon boluses ranging from 1 µg to 10 mg at various insulin levels expressed as multiples of the basal IIR, modified from [21].

5.2.2 Dose-Response Study

Inspired by the replicated in vivo study in Section 5.2.1, the simulation model in Section 5.1 was used to further investigate the dose-response relationship of glucagon and EGP at various constant insulin levels normalized to the basal insulin level. This section presents the most important results and learnings from the simulation study reported in [21]. The technical report is provided in Appendix D.

The seven virtual patients were included in an in silico cross-over study that comprised 115 study days per subject. At each study day, the IIR was constant at one to five times the basal rate and the glucose infusion rate was controlled every five minutes by a PID controller to maintain a glucose clamp of 5 mmol/L. A glucagon bolus was administered at steady state and followed up till five hours after the bolus. The effect of glucagon boluses in the range from 1 µg to 10 mg was assessed by calculating the area under the EGP curve for the first 60 minutes following the bolus.

Figure 5.7 plots the results of the simulation study for each glucagon dose stratified by IIR. The response to glucagon doses below approximately 25 µg are very similar independent of IIR. However, with increasing glucagon doses the curves for each IIR separate. The higher the IIR, the lower response to a glucagon
5.2 In Silico Studies

Figure 5.8: Fitted dose response curves when using doses of 25, 75, 125, and 175 µg as in the study by El Youssef et al. [48] assuming independence of insulin for either ED$_{50}$ or R$_{max}$, from [21].

bolus. Small increases in glucagon dose during low IIR increase the response significantly although it seems to saturate for some glucagon dose. The individual curves could be described by classic Michaelis-Menten kinetics as defined in (2.11) in Chapter 2 with similar values for the concentration yielding half maximum response and distinct values for maximum response depending on the insulin level. This observation was expected, as the model used for simulations describes how insulin modulates the maximum achievable EGP response to glucagon, but does not influence the concentration yielding half-maximum response.

Whether the simulated dose-response curves in Figure 5.7 reflect the reality is currently unknown. Discussion with peers have led to two hypotheses of how the ambient insulin level might affect the EGP response to glucagon:

- insulin level influences the maximum response to glucagon (R$_{max}$)
- insulin level influences the glucagon dose at which half-maximum response is achieved (ED$_{50}$)

The hypotheses could be examined by carrying out a smaller in vivo study. However, the glucagon doses must be carefully chosen to make sure to capture the essential parts of the dose response curve. If all tested doses are below the true ED$_{50}$ both hypotheses would describe the data equally well. According to a small simulation study, the validity of the above hypotheses cannot be investigated using the glucagon doses from the in vivo study in Section
Simulating Gluco regulato ry Dynamics

Figure 5.9: Fitted dose response curves when using SC glucagon doses of 25, 100, and 1000 µg assuming independence of insulin for either \( ED_{50} \) or \( R_{max} \), from [21].

5.2.1 thus 25, 75, 125, and 175 µg as displayed with simulations in Figure 5.8.

In the left graph, the \( ED_{50} \) is constant across all insulin levels and in the right graph the \( R_{max} \) is constant across all insulin levels. Because the four doses are within a narrow dose range and all doses are below the simulated \( ED_{50} \), both hypotheses fit the simulated data equally well.

If the glucagon doses had been distributed across a larger dose range encompassing the \( ED_{50} \), the difference between the hypotheses would be clearer according to a simulation study similar to the one just described. Figure 5.9 shows the results when using realistic glucagon doses spanning a larger dose range, thus 25 µg, 100 µg, and 1 mg. The graphs visualize a clear difference in the fitness of the two hypotheses making one more plausible than the other; that \( ED_{50} \) does not depend on ambient insulin levels, but that \( R_{max} \) does.

This simulation study has helped to identify which glucagon doses could be relevant to test in an in vivo study to investigate how the ambient insulin level influences dose-response of glucagon and EGP, and specifically test the hypotheses that insulin impacts maximum EGP response or the glucagon dose at which half maximum EGP response occurs.
5.2 In Silico Studies

5.2.3 Dose Selection Study

Both of the previous simulation studies were designed to investigate the EGP response to glucagon at various insulin levels and how this interaction can be explored further. The simulation model can also be used for more practical purposes with direct relevance for clinicians and patients. We used the model in Section 5.1 to conduct in silico studies exploring insulin-dependent optimal glucagon dosing regimens for treatment of insulin-induced mild hypoglycemia based on clinically relevant criteria [22]. A draft of the paper is included in Appendix E.

As explained in Section 2.3, the EGP responsiveness to glucagon depends on the insulin level. Thus, the insulin level must influence the glucagon dose needed to recover from hypoglycemia. Currently, it is not possible to measure serum insulin concentration in real time, but research is being done to develop a continuous insulin monitor [129]. However, pumps with bolus calculators offer as standard calculation of insulin on board (IOB), which is provided as information to the user to avoid insulin stacking due to the slow insulin absorption and onset of action. The IOB approximates the remaining effect of insulin in the body after an insulin bolus.

The in silico study evaluated the effect of glucagon at various insulin levels interpreted as: serum insulin concentration uncorrected or normalized to basal insulin concentration, and IOB uncorrected or normalized to individual total daily dose (TDD) of insulin. The serum insulin concentration was directly obtained from the PK model and the IOB was calculated as a linear decay based on individual insulin action times. The insulin level interpreted as normalized serum concentration to basal was included in the paper, and the results of the experiments are presented in this section.

At the beginning of each experiment in a study the patients’ blood glucose were normoglycemic. The patients then received an individualized insulin bolus that would yield a predefined insulin level when the plasma glucose concentration decreased beyond the hypoglycemia threshold. At hypoglycemia, a glucagon bolus in the range of 25 µg to 2.5 mg was administered. The glucose response after the glucagon bolus was evaluated in terms of average success among the virtual patient population in raising the glucose concentration above 5 mmol/L, keeping glucose concentration less than 10 mmol/L and maintaining the glucose concentration above the threshold of hypoglycemia for at least two hours. The individual success criteria of recovering from hypoglycemia was summarized in a single variable describing the overall success of hypoglycemia treatment calculated as a weighted harmonic mean. The optimal glucagon bolus was chosen as the lowest dose yielding maximum overall success.

Figure 5.10 presents the optimal glucagon dose as a function of the insulin level
interpreted as serum insulin concentration normalized to basal insulin concentration. The graph showed that the higher ambient insulin concentration compared to basal, the larger glucagon bolus was required to best treat the population for hypoglycemia. At concentrations of insulin less than two and a half times the basal value, glucagon boluses from 125 to 500 µg were ideal to treat the majority of the virtual population for hypoglycemia. Notice, that an optimal glucagon bolus might be suboptimal for an individual, but viewing the seven virtual patients as a population, the optimal doses could be given to any subject in the population and still yield satisfying recovery from hypoglycemia in most cases. With serum insulin concentrations exceeding two and a half times the basal level it would be more desirable for the patient to consume a carbohydrate snack since the optimal glucagon bolus to recover from mild hypoglycemia would be close to the bolus of 1 mg used to rescue patients from severe hypoglycemia.

The normalized concentration was chosen over the absolute concentration, because one patient had very high basal concentration making the obtainable range of absolute concentrations for all virtual patients very narrow and less meaningful than the range obtained by normalizing the concentration.

The studies interpreting insulin level by IOB had similar relationships between insulin level and optimal glucagon dose. Mild hypoglycemia was treatable with less than 0.5 mg glucagon when IOB was less than 2 U or IOB corresponded to less than 6% of TDD.
This simulation study showed that unlike the fixed rescue bolus of 1 mg glucagon for treatment of severe hypoglycemia, there might not be a one-size-fits-all-solution to treatment of mild hypoglycemia because of the impeding insulin level highly influencing the effect of glucagon. The treatment success of mild hypoglycemia greatly depended on the ambient insulin level independent of if the level was assessed as a normalized concentration to basal, IOB or IOB normalized to TDD.

5.2.4 *In Silico* as Supplement to *in Vivo*

This section presented examples of application of a glucose-insulin-glucagon simulation model spanning replication of an independent clinical study, dose optimization for a clinical study, and a large original simulation study. The model was used to show the effect of glucagon on EGP at various insulin levels and also suggested new glucagon doses that should be applied clinically in a similar study design to further investigate the dose-response relationship of glucagon and EGP. Finally, the model was used to generate glucagon mini-bolus dosing regimens for patients with T1D to recover from insulin-induced mild hypoglycemia depending on the ambient insulin level.

The simulation model could be expanded to include models of e.g. meal absorption, exercise, and other hormones. Such a simulation model could be used in optimization of diabetes treatment and specifically in developing and testing control strategies for a dual hormone closed-loop device (artificial pancreas). The simulation model can however not replace clinical studies. It should be regarded as a supplement that can aid in study design to maximize the yield of clinical studies and investigate glucoregulatory dynamics qualitatively.
This thesis demonstrated methods for PK/PD modeling of glucagon, insulin, and glucose in biological data. It showed how likelihood principles and Bayesian methods can be used for parameter estimation in datasets not initially intended for modeling and thus not optimally sampled. The CTSM package for R proved to be useful for ML and MAP parameter estimation.

The project succeeded in developing a SC PK model of glucagon and the glucagon analogue ZP-GA-1 in healthy dogs as documented in Appendix A. The same dataset was used to design a model describing the EGP as a function of both glucagon or ZP-GA-1 and insulin. A sigmoid $E_{max}$ model described the effect of glucagon and ZP-GA-1 on EGP. A multiplicative relationship between the effects of insulin and glucagon or ZP-GA-1 on EGP was needed to capture that EGP is suppressed at high insulin concentrations. The EGP model was embedded in Hovorka’s glucoregulatory model and fitted 20 datasets from five dogs well.

The novel EGP model of glucagon translated from healthy dogs to healthy humans. Only the PK/PD models of marketed glucagon was fitted to human data due to unavailability of data describing ZP-GA-1 in humans. The glucoregulatory model fitted ten datasets from ten healthy subjects well as documented in Appendix B.

In a model comparison, the novel EGP model showed both strengths and weak-
nesses compared to other published models. Among the strengths, it was not possible for the model to yield non-physiologic negative glucose production as the lowest output corresponded to the constant gluconeogenesis. The model included the effect of insulin and glucagon and could describe insulin’s suppressing effect even at high glucagon concentrations. The immediate effect of glucose on EGP was not included in the model. The PK/PD model parameter values were published for seven validated T1D patients. Moreover, the novel model was the only model among the reviewed ones, that included physiologic saturation of response at high glucagon concentrations and matched the concentration-response relationship reported in literature. However, the model failed to identify the correct glucose steady state and it did not account for the evanescence effect of glucagon.

Model parameters of insulin and glucagon PK models were estimated in a population of eight diabetes patients. The glucoregulatory model including the novel description of EGP was successfully validated by leave-one-out cross-validation in seven of the eight T1D patients. The model thus proved its validity for simulations of seven virtual subjects with T1D using the parameter sets of the seven validated patients. The final patient specific model parameter sets described three to four study days in each patient with SC glucagon boluses ranging 0 to 300 \( \mu \text{g} \).

The simulation model replicated a clinical study within the uncertainty limits. Simulations were also used to recommend glucagon doses for a theoretical repetition of the \textit{in vivo} study to further investigate the relationship between EGP and glucagon at various insulin levels. Finally, the simulation model was used to conduct a large original \textit{in silico} study to determine the optimal glucagon mini-bolus to treat mild insulin-induced hypoglycemia at various insulin levels assessed as concentration or IOB based on clinically relevant criteria. Although validated, the simulation model can not replace clinical studies, but it has proved to be a useful supplement and tool in the planning of studies.

### 6.1 Future Work

The novel EGP model has some limitations that could be subject for future studies and would justify collection of new physiologic data. More data describing the glucose concentration during steady state should be collected to identify the correct level thereof. The evanescence effect of glucagon also needs to be studied further in order to include it in an EGP model. Especially the refractory period indicating when normal glucagon responsiveness is restored after desensitization, should be investigated.
The simulation model could be used to conduct other \textit{in silico} studies than the ones here presented. The simulation environment can also aid in the design of clinical studies to avoid common pitfalls and in the planning of relevant nominal sampling times. It would be interesting to carry out the proposed dose-response study with a wide range of glucagon boluses to investigate how the effects of insulin and glucagon interact and influence the EGP. Furthermore, the proposed insulin-dependent glucagon dosing regimens would be highly relevant to study further \textit{in vivo}.
Conclusions
This appendix presents the technical report with the title "PK/PD modelling of glucose-insulin-glucagon dynamics in healthy dogs after a subcutaneous bolus administration of native glucagon or a novel glucagon analogue" published by the Technical University of Denmark in April 2016 [18].
PK/PD modelling of glucose-insulin-glucagon dynamics in healthy dogs after a subcutaneous bolus administration of native glucagon or a novel glucagon analogue

Sabrina Lyngbye Wendt∗1,2,3, Jan Kloppenborg Møller2, Carsten Boye Knudsen1, Henrik Madsen2, Ahmad Haidar3,4, and John Bagterp Jørgensen2

1Department of Bioanalysis and Pharmacokinetics, Zealand Pharma A/S, Glostrup, Denmark
2Department of applied mathematics and computer science, Technical University of Denmark, Kongens Lyngby, Denmark
3Institut de Recherches Cliniques de Montréal, Montreal, Quebec, Canada
4Division of Experimental Medicine, McGill University, Montreal, Quebec, Canada

Version 1
April, 2016

DTU Compute Technical Report-2016-2. ISSN: 1601-2321.
∗ Email: slw@zealandpharma.com or slwe@dtu.dk

Objective We aim to develop a simulation model of the complex glucose-insulin-glucagon dynamics based on physiology and data. Furthermore, we compare pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of marketed reconstituted glucagon with a stable liquid glucagon analogue invented by Zealand Pharma A/S.

Research Design and Methods We expanded a physiological model of endogenous glucose production with multiplicative effects of insulin and glucagon and combined it with the Hovorka glucoregulatory model. We used a Bayesian framework to perform multidimensional MAP estimation of model parameters given priors reported in the literature. We used profile likelihood analysis to investigate parameter identifiability and reduce the number of model variables. We estimated model parameters in pre-clinical data from one cross-over study with a total of 20 experiments in five dogs. The dogs received two subcutaneous (SC) bolus injections of low and high doses of glucagon and ZP-GA-1 (20 and 120 nmol/kg).

Results We report posterior probability distributions and correlations for all identifiable model parameters. Based on visual inspection and residual analysis, the PD model described data satisfactorily for both glucagon and the analogue. Parameter estimates of the PD model were not significantly different between the two compounds.

Conclusions The new PK/PD model enables simulations of the glucose-insulin-glucagon dynamics after a SC bolus of glucagon or glucagon analogue. The novel glucagon analogue by Zealand Pharma A/S shows PK and PD characteristics similar to marketed glucagon.

Keywords: Pharmacokinetics, PK, Pharmacodynamics, PD, modeling, modelling, glucagon, glucagon analogue, glucose, insulin, glucoregulatory, ODE, SDE, MAP, simulation, profile likelihood
# Contents

1 Introduction ........................................... 4

2 Data .................................................. 8
   2.1 Data collection ..................................... 8
   2.1.1 Study 1 ......................................... 8
   2.2 Bioavailability .................................... 8
   2.3 Unit conversion ................................... 8
       2.3.1 Glucagon ..................................... 9
       2.3.2 Insulin ................................ ...... 9
   2.4 Basal concentrations ................................ 10
   2.5 Data cleaning ..................................... 10

3 Models ............................................... 11
   3.1 PK model ....................................... 11
   3.2 PD model ....................................... 12
       3.2.1 Version 1.0 - Integrating Emami in Hovorka .... 12
       3.2.2 Version 1.1 - Saturation of EGP ................. 14
       3.2.3 Version 1.2 - Basal insulin ...................... 14
       3.2.4 Version 2.0 - Sigmoid $E_{max}$ model ........... 15
       3.2.5 Final PD model ................................ 17

4 Methods .............................................. 19
   4.1 Mathematical concepts ............................... 19
       4.1.1 Maximum likelihood .............................. 19
       4.1.2 Profile likelihood .............................. 19
       4.1.3 Bayesian inference .............................. 20
       4.1.4 Maximum a posteriori estimation ............... 20
       4.1.5 Stochastic differential equations ............... 21
   4.2 Application in CTSM-R ............................... 21
       4.2.1 Model structure ............................... 21
       4.2.2 Initial values .................................. 22
       4.2.3 Prior information .............................. 22
       4.2.4 Parameter identifiability ....................... 25
       4.2.5 Model fitting and validation .................... 26

5 Results .............................................. 26
   5.1 PK .............................................. 27
       5.1.1 Parameter estimates ............................ 27
       5.1.2 PK Model fits .................................. 27
   5.2 PD .............................................. 27
       5.2.1 Reducing variables ............................. 27
       5.2.2 Model validity .................................. 30
       5.2.3 Residual Analysis ............................. 30
       5.2.4 PD Model fits .................................. 31
       5.2.5 Parameter estimates ............................ 33
       5.2.6 Native glucagon versus glucagon analogue ...... 35
# CONTENTS

6 Discussion .............................................. 35

References ............................................. 39

Appendix .................................................. 43

A Raw data .............................................. 43

B PK Model fits ......................................... 46

C PD model fits ........................................ 49
1 Introduction

Conventionally, diabetes type 1 is treated with multiple daily injections of insulin or continuous infusion of insulin using a pump. The stress of calculating the needed amount of insulin based on food intake, exercise and insulin sensitivity has led to research in creating an artificial pancreas (AP). A basic AP is a closed-loop (CL) system consisting of an insulin pump, a continuous glucose monitor (CGM) and a control algorithm to adjust insulin dosage through the pump based on CGM sensor readings.

Until recently, researchers and developers of the AP have mainly focused on a single hormone approach [1]. However, research in the field of dual hormone AP systems is growing substantially and clinical studies are being conducted by research groups in Boston [2–4], Montreal [5–8], Portland [9,10], and Amsterdam [11–14]. In multiple studies of single hormone open-loop (OL) versus single hormone CL and/or dual hormone CL systems these groups have demonstrated that time in range increases when using a CL system compared to an OL system. Moreover, comparative studies show significant reduction of time spent in hypoglycaemia and number of hypoglycaemic events using a dual hormone CL system versus a single hormone CL system [6, 7, 9].

The unstable nature of native glucagon in liquid formulation challenges the development of a dual hormone AP. The hormone is currently marketed in dry form and needs reconstitution daily [16, 17]. Immediately after reconstitution glucagon starts degrading and forming fibrils. The fibrillation can cause the pump tubing to occlude and the degradation reduces the efficacy of the compound. Currently, only reconstituted glucagon is available for dual hormone AP studies which frequently experience glucagon pump occlusions [3, 13, 14].

At least two pharmaceutical companies are developing glucagon stable in liquid solution suitable for pump use. Xeris Pharmaceuticals Inc. is developing native glucagon stabilized in dimethyl sulfoxide (DMSO) [18], whereas Zealand Pharma A/S is developing a glucagon analogue in aqueous solution [19]. With this ongoing development of liquid stable glucagon suitable for pump use, the realization of a dual hormone AP is becoming practically possible.

In silico experiments are useful during the development of a control algorithm for a dual hormone AP.
before applying the algorithm in a clinical trial. Simulations in silico can also provide new insights in physiological system. A model describing the dynamics between glucose and insulin is validated by tracer data [20], and widely used in the literature for simulations of the endocrine regulatory system [21–23]. Recent proposed extensions include the effect of glucagon on endogenous glucose production (EGP) [24–26]. It is important to understand the glucose dynamics of the body to evaluate if these glucagon-glucose models are capturing the complexity of the reality.

The quantitative dynamics of insulin and glucagon on EGP are complex and not completely understood [15]. Two processes contribute to EGP: gluconeogenesis (GN) and glycogenolysis (GG). GN is the formation of glucose from non-carbohydrate substrates like glucogenic amino acids, glycerol, pyruvate and lactate. GG is the breakdown of stored glycogen in the liver to glucose. Studies show that glucagon and insulin have very little effect on GN as opposed to GG [15, 29, 30], see Figures 1 and 2. Thus the hormones influence mainly the EGP by regulating GG; glucagon stimulates it whereas insulin inhibits it. Increasing the glucagon concentration stimulates GG until a certain point where-after the response saturates. Saturation of response is typical for receptor mediated processes due to the limited number of receptors in a physiological system [31]. As opposed to glucagon, insulin inhibits GG and completely suppresses the breakdown of glycogen at insulin concentrations exceeding approximately 45 mIU/L [15]. A recent study by El Youssef et al. showed that at high insulin concentrations (46.0±12.5 mIU/L) the EGP is greatly reduced independent of the glucagon dose [28], see Figure 3. Moreover, the rates of EGP

![Figure 2: The effect of increased glucagon on glycogenolysis and gluconeogenesis in the dog. Somato-statin (SRIF) was given along with basal (B) replacement amounts of insulin and glucagon intraportally. At time zero the glucagon infusion rate was increased. The data is from Wada et al. [27].](image-url)
Figure 3: Endogenous glucose production with increasing glucagon doses (25, 75, 125, 175 µg) at high, medium and low insulin infusion rates (0.016±0.006 IU/kg/h, 0.032±0.003 IU/kg/h, 0.05±0.0 IU/kg/h) giving plasma insulin concentrations of 17.6±13.0 mIU/L, 29.1±8.9 mIU/L, and 46.0±12.5 mIU/L, respectively. The graph is from El Youssef et al. [28].

at high insulin concentrations match the rates of GN. The results are in agreement with previous studies showing that insulin suppresses GG but does not affect GN. These results suggest that the effect of insulin prevails over glucagon at high insulin concentrations. Some studies indicate that not only does the absolute glucagon concentration affect EGP but also the glucagon rate of change [15, 27].

The glucose kinetics model by Herrero et al. is based on the minimal model and describes the glucose changes with additive effects of insulin and of glucagon [25, 32]. The EGP model by Dalla Man et al. also describes the effects of insulin and of glucagon additively [24]. The glucose kinetics model of Hovorka includes the interaction of insulin and EGP, but does not describe the effect of glucagon on EGP [20]. The only model including the interaction and effect of both glucagon and insulin on EGP is proposed by Emami et al. [26]. This model approximates the EGP response to either glucagon or insulin with linear effects and includes the effect of glucagon rate of change on the EGP. The linear assumption of the EGP response to glucagon is fair as long as the glucagon concentration does not exceed approximately 400 pg/mL. Based on deviance information criterion the novel model outperformed several other models including the one proposed by Herrero et al. The model by Emami et al. assumes that the glycogen stores are never depleted. This is a valid assumption at normal conditions since a recent study showed that small repeated glucagon doses over a short time span did not significantly alter the glycogen stores even after an overnight fast [22].

This technical report presents a novel model of the glucose-insulin-glucagon dynamics by combining the validated insulin-glucose model and the physiological EGP model for the purpose of simulation. The insulin-glucose model by Hovorka et al. forms the basis of the dynamical system [20]. The model by Emami et al. extends the EGP part of the Hovorka model to include glucagon [26]. The EGP model is modified further to ensure saturation of the EGP at high glucagon concentrations in accordance with literature and physiological receptor activation concepts.

Furthermore, this report aims to thoroughly illustrate and explain the mathematical methods applied during the model fitting procedure of the pharmacodynamics model. The final model is fitted to individual
datasets using a multidimensional Bayesian framework. Parameter identifiability is investigated using profile likelihood analysis. Model validity is confirmed by estimating the noise contribution of the system using the grey-box modelling approach with stochastic differential equations (SDEs) [33]. After validation, final parameter estimation is conducted using the white-box modelling approach with ordinary differential equations (ODEs) making the model suitable for simulations. We use a programming environment in R created for continuous time stochastic modelling (CTSM) for the entire model fitting procedure [34].

Previously, PK data of this report were used for model fitting by a different technique and presented as a poster at the 8th International Conference on Advanced Technologies & Treatments for Diabetes (ATTD) in February 2015 [19]. PD results of this report were presented as a poster at the 9th ATTD in February 2016 [35].
Zealand Pharma A/S is developing a new glucagon analogue with increased stability in liquid solution for treatment and better control of hypoglycemia in diabetes patients. The novel compound, ZP-GA-1, was tested against marketed glucagon (GlucaGen®, Novo Nordisk A/S) in pre-clinic. Both compounds are peptides and act as glucagon receptor agonists.

2.1 Data collection
Data originates from a pre-clinical study in dogs designed by Zealand Pharma A/S and conducted at Covance Laboratories Ltd (Covance site, Harrogate UK). The Institutional Animal Care and Use Committee approved the study and all procedures carried out on the dogs were in accordance with the Animals (Scientific Procedures) Act 1986. The study is summarized in Table 1 and described in Section 2.1.1. Data was originally collected for the purpose of showing a PD effect of the glucagon analogue in vivo and to compare it with the PD effect of marketed glucagon.

2.1.1 Study 1
Five healthy Beagle dogs (bodyweight 13.6 ± 1.3 kg; mean ± SD) were included in this randomized cross-over study and named dog 1-5. At four dosing occasions each dog received a subcutaneous (SC) bolus injection of 20 or 120 nmol/kg glucagon or ZP-GA-1. Blood samples were collected at 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 110, 140, and 180 minutes after dose administration. Sample concentrations of glucagon and of ZP-GA-1 were analyzed using an in-house developed LC-MS/MS method. Plasma concentration of insulin was analyzed using a commercially available immunoassay from Meso Scale Discovery (MSD) (catalog no. K152BZC). Although the MSD assay was designed for mouse/rat plasma, an in-house validation showed that it was also valid for analysis of insulin in dog plasma. Plasma concentration of glucose was analyzed using Roche glucose method (UV test) [36]. Figure 4 presents an example of raw data from one dog in study 1.

2.2 Bioavailability
The bioavailability is obtained from the ratio between the dose-normalized area under the curve (AUC) after SC administration compared to IV. The AUC’s were calculated using non-compartmental analysis (reported at Zealand Pharma A/S). The bioavailability varies between the drugs. Thus, for each drug the bioavailability is used in the input to the PK model, see summary in Table 2.

2.3 Unit conversion
In the study, glucagon and analogue concentrations were measured in nmol/L, insulin concentration was measured in pg/mL and glucose concentration was measured in mmol/L. Model parameters concerning glucagon and of insulin are often reported so that concentrations thereof should be in pg/mL and mIU/L,
Figure 4: Raw data from dog 2 in study 1. Plasma concentrations after low or high doses of glucagon and ZP-GA-1 are red crosses or blue dots, respectively.

Table 2: Bioavailability and molar mass of glucagon and glucagon analogue in study 1.

<table>
<thead>
<tr>
<th>Study</th>
<th>Drug</th>
<th>Bioavailability</th>
<th>Molar mass, g/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucagon</td>
<td>37.5%</td>
<td>3482.75</td>
</tr>
<tr>
<td></td>
<td>ZP-GA-1</td>
<td>50%</td>
<td>3339.7</td>
</tr>
</tbody>
</table>

respectively [20,21,25]. The units of the PK and the PD data were converted to these generally used units before modelling.

2.3.1 Glucagon

To convert the plasma concentration of the administered compound from nmol/L to pg/mL, PK data is multiplied by the molar mass of the compound since the following yields units of pg/mL.

\[
\frac{[\text{nmol}]}{[\text{L}]} \times 10^3 \frac{[\text{pmol}]}{[\text{nmol}]} \times \text{Molar Mass} [\text{pg}]/\text{pmol} = \frac{[\text{pg}]}{[\text{mL}]}.
\] (1)

The molar masses of glucagon and the analogue are listed in table 2.

2.3.2 Insulin

To convert the insulin concentration from pg/mL to mIU/L, insulin PD data is multiplied by 0.023 since the following yields units of (mIU/L).

\[
\frac{[\text{pg}]}{[\text{mL}]} \times 10^3 \frac{[\text{mIU}]}{[\mu g]} = \frac{[\text{mIU}]}{[L]}.
\] (2)

The assay used for analysis uses the WHO standard of 0.023 (IU/µg).
2 DATA

Table 3: List of removed outliers in the datasets substituted with linear interpolation of neighbouring observations. *Outlier not substituted by interpolation but removed.

<table>
<thead>
<tr>
<th>Study</th>
<th>Dog</th>
<th>Drug</th>
<th>Dose, nmol/kg</th>
<th>Analyte</th>
<th>Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1M</td>
<td>Glucagon</td>
<td>20</td>
<td>Glucagon</td>
<td>*6</td>
</tr>
<tr>
<td>1</td>
<td>1M</td>
<td>ZP-GA-1</td>
<td>20</td>
<td>Glucose</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>1M</td>
<td>ZP-GA-1</td>
<td>20</td>
<td>Glucose</td>
<td>16</td>
</tr>
<tr>
<td>1</td>
<td>2M</td>
<td>ZP-GA-1</td>
<td>20</td>
<td>Glucose</td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>3M</td>
<td>Glucagon</td>
<td>20</td>
<td>Glucose</td>
<td>15</td>
</tr>
<tr>
<td>1</td>
<td>3M</td>
<td>Glucagon</td>
<td>20</td>
<td>Glucose</td>
<td>40</td>
</tr>
</tbody>
</table>

2.4 Basal concentrations

After an overnight fast in healthy human adults, the glucagon concentration is around 91 pg/mL (range 40-400 pg/mL) and the fasting insulin concentration is approximately 10-15 mIU/L [37]. Based on the two present studies, the basal insulin concentration in dogs are considerably lower with an average around 3 mIU/L and range 1.6-5.7 mIU/L, thus less than one third of the human basal insulin concentration. As there is a negative relationship between basal insulin concentration and insulin sensitivity in humans [38], this lower basal insulin concentration in dogs indicate a higher insulin sensitivity.

Basal glucagon concentration in dogs is around 41-54 pg/mL [27]. The lower level of quantification (LLOQ) for glucagon measured in study 1 was 0.2 nmol/L corresponding to nearly 700 pg/mL. All measurements at time zero were below LLOQ. The basal concentration of glucagon can thus not be determined in this study.

2.5 Data cleaning

No formal tests of the significance of an outlier were used but rather visual inspection. Table 3 lists all data points that the modeller considered to be outliers. The glucagon outlier appeared to have too high concentration after just 6 minutes, whereas the removed glucose observations showed sudden drops in glucose concentration that did not seem physiological. Figure 4 shows an example of such a data point with a drop at 30 minutes after the low dose of glucagon analogue ZP-GA-1. Figures 16-20 in Appendix A present raw data from all datasets including outliers.
3 Models

3.1 PK model

The glucagon pharmacokinetics (PK) are investigated using a simple model as formulated by Haidar et al. [39,40], defined in (3)-(5) and visualized in Figure 5. The PK model is a one-compartment model of the disturbance in plasma concentration from baseline after extravascular drug administration with first order absorption kinetics from the SC tissue to the plasma. The model has two states; the first corresponding to the SC tissue and the second corresponding to the central compartment (plasma and instantaneous equilibrating tissues). The model includes two rate constants; one describing absorption from the SC tissue to the central compartment and another describing elimination from the central compartment. To minimize confusion, the parameter naming is kept as closely to the formulation by Haidar et al. as possible.

\[
\frac{dq_1(t)}{dt} = u(t) - k_1 q_1(t) \quad q_1(0) = 0
\]

\[
\frac{dq_2(t)}{dt} = k_1 q_1(t) - k_2 q_2(t) \quad q_2(0) = 0
\]

\[C(t) = \frac{k_2 q_2(t)}{w Cl} \cdot 10^3 + C_b\]

Model input: \(u(t) = \delta(t) \cdot \text{Dose} \cdot \text{bioavailability}\)

Model observation: \(C(t_n)\)

Model output: \(C(t)\)

Fixed parameter(s): \(w, (C_b)\)

Model parameters: \(k_1, k_2, Cl, C_b\)

\(C(t_n)\) is the measured glucagon concentration in plasma (nmol/L) at discrete timepoints, \(n = 1, ..., N\). \(C(t)\) is the simulated glucagon concentration in plasma at continuous time. \(C_b\) is the basal glucagon concentration in plasma (nmol/L). In case of administration of the glucagon analogue, \(C_b\) is fixed to zero as no basal level exists in the body.

\(w\) is the measured bodyweight (kg). \(Cl\) is the clearance rate normalized by weight (mL/kg/min). \(k_1\) is the absorption rate constant and \(k_2\) is the elimination rate constant (min\(^{-1}\)).

The concentration of glucagon in the central compartment is obtained by multiplying the content with an expression similar to per volume of distribution in (5). In classical PK it is trivial that clearance is equal to the product of the elimination rate constant and volume of distribution [31]. Since clearance is normalized by weight the denominator is multiplied by the bodyweight.

The bioavailability does not influence the fit of the model to data, but is necessary to get physiological parameter estimates of clearance. The Dose (nmol) is multiplied by the Dirac delta function to model the bolus injection at time zero.

A surrogate marker for the onset of action, \(t_{max}\), is obtained analytically from the absorption and elimination rate constants.

\[t_{max} = \frac{\log(k_1/k_2)}{k_1 - k_2}\]

In (6) log is the natural logarithm.
3.2 PD model

3.2.1 Version 1.0 - Integrating Emami in Hovorka

The pharmacodynamics (PD) of glucagon and insulin on glucose are described by combining two published models. The glucose-insulin part of the model was published by Hovorka et al. [20] and listed in equations (7)-(12). A few changes to this model include removal of glucose input, removal of the labelled glucose kinetics, and parameter substitution of insulin sensitivities instead of ratios between activation and deactivation rate constants. To minimize confusion, the parameter naming is kept as closely to the original publication as possible. The model is initially in steady state, but could be initialized in any state.

\[
\frac{dQ_1(t)}{dt} = -F_01 - S_T x_1(t) Q_1(t) + k_{12} Q_2(t) + GG(t) \quad Q_1(0) = Q_{10} \tag{7}
\]
\[
\frac{dQ_2(t)}{dt} = S_T x_1(t) Q_1(t) - [k_{12} + S_D x_2(t)] Q_2(t) \quad Q_2(0) = Q_1(0) \frac{x_1(0)}{x_2(0)} + k_{12} \tag{8}
\]
\[
G(t) = \frac{Q_1(t)}{V} \tag{9}
\]
\[
\frac{dx_1(t)}{dt} = k_{a1}[I(t) - x_1(t)] \quad x_1(0) = I_0 \tag{10}
\]
\[
\frac{dx_2(t)}{dt} = k_{a2}[I(t) - x_2(t)] \quad x_2(0) = I_0 \tag{11}
\]
\[
\frac{dx_3(t)}{dt} = k_{a3}[I(t) - x_3(t)] \quad x_3(0) = I_0 \tag{12}
\]

The model is extended to include a GG model as proposed by Emami et al. [26], defined in equations (14)-(15). The combined PD model is visualized in figure 5.

\[
GG(t) = (1 - S_E x_3(t)) \cdot (S_{gd} E_{gd}(t) + S_g C(t))
\]
where \(1 - S_E x_3(t) \geq 0\) and \(S_{gd} E_{gd}(t) + S_g C(t) \geq 0\) \tag{14}

\[
\frac{dE_{gd}(t)}{dt} = -k_{gd} \left( E_{gd}(t) - \frac{dC(t)}{dt} \right) \tag{15}
\]

Since CTSM-R does not accept if statements to supplement the state equations, the conditional statements are implemented as \(\frac{1}{2} + \frac{1}{2} \tanh(100 \cdot \text{"conditionalstatement"})\) multiplied by the conditional statement. Thus, when the statement is positive the expression equals one, and when the statement is negative the expression is zero.

The PD model not only includes a term for the absolute concentration of glucagon, but also a term describing the glucagon rate of change in (15). The analytical solution to the glucagon rate of change is derived from (4)-(5) yielding units of nmol/L/min.

\[
\frac{dC(t)}{dt} = \frac{k_2}{wC_l} \cdot 10^3 \cdot (k_1 q_1(t) - k_2 q_2(t)) \tag{16}
\]

Model inputs: \(C(t), \frac{dC(t)}{dt}, I(t)\)
Model observation: \(G(t_n)\)
Model output: \(G(t)\)
Fixed parameters: \(V, I_0\)
Model parameters: \(F_{01}, k_{12}, S_T, S_D, S_E, k_{a1}, k_{a2}, k_{a3}, S_g, S_{gd}, k_{gd}\)
\[
C(t) = k_2 q_2 w Cl 10^3 + C_p
\]

\[
dC \over dt = k_2 w Cl 10^3 (k_1 q_1 - k_2 q_2)
\]

\[
Q = k_2 w Cl 10^3 (k_1 q_1 - k_2 q_2)
\]

The full PK-PD model is presented in figure 5.

\[
F_{01} = \text{net total non-insulin-dependent glucose out-flux from the plasma compartment (umol/kg/min).}
\]

\[
S_g = \text{insulin sensitivity on glucose (1/min per mIU/L).}
\]

\[
S_{gd} = \text{insulin sensitivity on glucagon (1/min per pg/mL).}
\]

\[
k_{gd} = \text{delay of glucagon rate of change on EGP (min}^{-1}).
\]

The full PK-PD model is presented in figure 5.
3 MODELS

Figure 6: Comparison of GG model by Emami et al. [26] and modified model capturing the physiological saturation effect of glucagon on GG. The saturation part is proposed roughly to the data presented by Cherrington [15], compare with Figure 1. The graphed models assume basal insulin concentration and constant glucagon concentration. The curves do not start in (0,0) as GN is included.

3.2.2 Version 1.1 - Saturation of EGP

As reviewed in Section 1, GG saturates for some concentration of glucagon at basal insulin concentration, see Figure 1. The model stated in (14) is a linear approximation of the GG response to constant glucagon concentration and only covers the linear range of the dose response curve. No saturated data was available during the model development and therefore this dynamic was not captured in the model. However, the available data from Zealand Pharma A/S takes on very high concentrations of plasma glucagon and thus we assume that the GG response to glucagon is saturated for some parts of the studies if not the entire study duration. The GG model in (14) is modified to saturate GG at $65 \, \frac{\text{mmol}}{\text{kg} \cdot \text{min}}$ at basal insulin level. The saturation is approximated by a simple tangent hyperbolic function and figure 6 confirms that the linear part of the curve resembles the original linear formulation at basal insulin concentration. The GG model with saturation is

$$GG(t) = (1 - S_{E3x3}(t)) \cdot \frac{65}{0.69} \cdot \text{tanh} \left( \frac{2.5}{1400} \cdot \frac{S_{gd}E_{gd}(t) + S_gC(t)}{S_g} \right)$$

where $(1 - S_{E3x3}(t)) \geq 0$ and $(S_{gd}E_{gd}(t) + S_gC(t)) \geq 0$ (17)

3.2.3 Version 1.2 - Basal insulin

Dogs have lower basal insulin concentrations than humans ($\sim 3 \, \text{mIU/L}$ versus $\sim 10 \, \text{mIU/L}$), as described in section 2.4. Therefore, we can not assume that $(1 - S_{E3x3}(t))$ equals 0.69 at basal as in humans (calculated as EGP$_b$/EGP$_0$ from Hovorka et al. [20]). The model is thus changed to

$$GG(t) = \frac{(1 - S_{E3x3}(t))}{(1 - S_{E3x3})} \cdot \frac{65}{(1 - S_{E3x3})} \cdot \text{tanh} \left( \frac{2.5}{1400} \cdot \frac{S_{gd}E_{gd}(t) + S_gC(t)}{S_g} \right)$$

where $(1 - S_{E3x3}(t)) \geq 0$ and $(S_{gd}E_{gd}(t) + S_gC(t)) \geq 0$ (18)
Figure 7: Relationship between plasma glucagon concentration and EGP. GN is constant and the GG is fitted with a sigmoid $E_{\text{max}}$ model. The graph assumes basal insulin concentration. The solid data points originate from [27, 41, 42] and the open dots are approximated from [15] since the original data could not be retrieved. Note that EGP is not zero at zero glucagon due to the glucagon independent GN.

The change ensures that GG saturates at 65 µmol kg$^{-1}$ min$^{-1}$ at basal insulin concentration. Moreover, this addition ensures that at higher insulin levels than basal, the saturation value of GG is lower than 65 µmol kg$^{-1}$ min$^{-1}$. Similarly, at lower insulin levels than basal the saturation value of GG is higher than 65 µmol kg$^{-1}$ min$^{-1}$. Qualitatively speaking, insulin “modulates” the maximum GG response to glucagon.

### 3.2.4 Version 2.0 - Sigmoid $E_{\text{max}}$ model

As discussed by Emami et al., the glucagon rate of change was added to the model to be able to capture the weakened response to a constant plasma glucagon concentration [26], also see Figure 2. Since we do not have data to describe this phenomenon and do not have a physiological explanation for how rate of change affects the GG response, we simplify the GG model to only depend on the absolute concentration of glucagon. We justify this with reference to Emami et al. who found that the GG model using both absolute glucagon concentration and glucagon rate of change was only slightly better than a similar model using only the absolute glucagon concentration [26].

To make the model parameters more physiological interpretable, we substitute the empirical tangent hyperbolic saturation model with the sigmoid $E_{\text{max}}$ model which is used to describe receptor mediated kinetics [43]. The sigmoid $E_{\text{max}}$ model is essentially a first order process at low concentrations, and a zero order process at high concentrations [31]. The model by Emami et al. in (14) describes the first order process and is approximated from data where first order kinetics apply [26]. The effect of plasma glucagon on the GG response is reformulated to comply with literature data [15, 27, 41, 42], and the mean prior parameter values of this model are identified by optimization. The data used to fit the parameters and the optimal solution is presented in Figure 7. The new GG model and the fitted parameters are listed...
Figure 8: Qualitative visualization of the consequences of the multiplicative GG model showing how the total EGP response to glucagon changes at low, basal, high and very high insulin concentrations.

\[
GG(t) = \frac{(1 - S_{E_x3}(t))}{(1 - S_{E_x6})} \left( \frac{(E_{\text{max}} - E_0) C(t)^\gamma}{EC_{50} + C(t)} \right) \quad \text{where} \quad (1 - S_{E_x3}(t)) \geq 0 \quad (19)
\]

\[
E_{\text{max}} = 72.1 \ \mu\text{mol kg}^{-1} \text{min}^{-1}, \quad E_0 = 8 \ \mu\text{mol kg}^{-1} \text{min}^{-1}, \quad EC_{50} = 337.8 \ \text{pg mL}^{-1}, \quad \gamma = 1.25
\]

The model describing the saturation of EGP due to glucagon consists of four parameters - two describing the minimum and maximum effect, \(E_0\) and \(E_{\text{max}}\), and two describing the curvature, \(EC_{50}\) and \(\gamma\). \(E_0\) is describing GN. \(EC_{50}\) is the concentration at the half maximum effect. The parameter \(\gamma\) reflects the number of molecules binding to one receptor and determines the steepness of the curve. We hypothesize that \(E_0\) and \(E_{\text{max}}\) will be identical whether using marketed glucagon or the analogue. However, the parameters describing the curvature, i.e. the potency, might differ. Figure 8 visualizes the GG model with the multiplicative effect of glucagon and insulin qualitatively.
3.2.5 Final PD model

The equations describing the glucose-insulin-glucagon PD model are listed in (20)-(26) and the model visualized in Figure 9.

\[
\frac{dQ_1(t)}{dt} = -F_{01} - S_T x_1(t) Q_1(t) + k_{12} Q_2(t) + GG(t) \quad Q_1(0) = Q_{10} \tag{20}
\]

\[
\frac{dQ_2(t)}{dt} = S_T x_1(t) Q_1(t) - [k_{12} + S_D x_2(t)] Q_2(t) \quad Q_2(0) = Q_1(0) \frac{x_1(0)}{x_2(0) + k_{12}} \tag{21}
\]

\[
GG(t) = \frac{(1 - S_E x_3(t))}{(1 - S_E I_b)} \left( (E_{\text{max}} - E_0) \frac{C(t)^\gamma}{EC_{50}^\gamma + C(t)^\gamma} \right) \quad \text{where } (1 - S_E x_3(t)) \geq 0 \tag{22}
\]

\[
G(t) = \frac{Q_1(t)}{V} \tag{23}
\]

\[
\frac{dx_1(t)}{dt} = k_{a1} [I(t) - x_1(t)] \quad x_1(0) = I_b \tag{24}
\]

\[
\frac{dx_2(t)}{dt} = k_{a2} [I(t) - x_2(t)] \quad x_2(0) = I_b \tag{25}
\]

\[
\frac{dx_3(t)}{dt} = k_{a3} [I(t) - x_3(t)] \quad x_3(0) = I_b \tag{26}
\]

Model inputs: \(C(t), I(t)\)

Model observation: \(G(t_n)\)

Model output: \(G(t)\)

Fixed parameters: \(V, I_b\)

Model parameters: \(F_{01}, k_{12}, S_T, S_D, S_E, k_{a1}, k_{a2}, k_{a3}, E_0, E_{max}, EC_{50}, \gamma\)

The parameters and their units are described in previous sections 3.2.1-3.2.4.

We assume that \(F_{01}\) is constant at all times since we are not measuring any glucose concentrations below 4.5 mmol/L in the datasets to be fitted. However, for simulation purposes it is important to include the extended formulation of \(F_{01}\) taking the current glucose concentration into account [23].
Figure 9: Schematic presentation of the final full model including the PK part at the left and the PD part at the right. The open arrow symbolizes SC input of glucagon. Solid arrows indicate mass transfer to/from a compartment. Dashed arrows indicate effect without mass transfer. Solid lines ending with a dot indicate how an output is derived from the content of a compartment. Plasma compartments of glucagon, insulin, and glucose are colored. A red dashed square surrounds the part of the model published by Hovorka et al. [20].
4 Methods

4.1 Mathematical concepts

4.1.1 Maximum likelihood

The likelihood measures how likely a set of parameters are given data and a model. The likelihood is equal to the probability density considered as a function of the parameter set, \( \theta \), and a time series, \( Y_N \), of \( N \) observations.

\[
L(\theta, Y_N) = p(Y_N|\theta)
\]  

(27)

Different parameters of the model will give different values of the likelihood function. Finding the parameter set that maximizes the likelihood function for given data and a model gives the maximum likelihood (ML).

4.1.2 Profile likelihood

For a parameter, \( \theta_i \), and a time series, \( Y_N \), of \( N \) observations, we can calculate the profile likelihood, which is defined as

\[
L_p(\theta_i, Y_N) = \max_{\theta \neq \theta_i} L(\theta, Y_N)
\]  

(28)

For a fixed value of \( \theta_i \) the likelihood function is maximized across all other parameters of the parameter set \( \theta \) which yields the profile likelihood of parameter \( \theta_i \) [44, 45]. The profile likelihood of a parameter can be used to evaluate whether the parameter in the model is identifiable. Identifiability of parameters are determined by model structure (structural identifiability) and the input dynamics (practical identifiability) [44].

Structural identifiability is related to the transfer function from the input to the output. However, in large complex systems where the transfer function is non-trivial to derive, profile likelihood analysis provides a method for investigating the parameter identifiability.

Practical identifiability is related to the dynamics of the input. Thus, a model can only identify parameters describing dynamics present in data used for model fitting. Also in this case, profile likelihood analysis is a powerful tool.

A parameter is identifiable only if the maximum of the profile likelihood is well defined [44]. Whether the maximum of the profile likelihood of \( \theta_i \) is well defined is evaluated using a 100\((1-\alpha)\)% confidence interval bound by when the natural logarithm of a likelihood ratio test exceeds a chi-squared distribution [45].

\[
\log \left( \frac{L_p(\theta_i, Y_N)}{L(\hat{\theta}, Y_N)} \right) = \log(L_p(\theta_i, Y_N)) - \log(L(\hat{\theta}, Y_N)) > -\frac{1}{2} \chi^2_{1-\alpha}
\]  

(29)

In words, the profile likelihood is log-transformed yielding the profile log-likelihood. The maximum value of the profile log-likelihood is subtracted from the profile log-likelihood so that the maximum function value is zero. The limit of the confidence interval is determined by the 100\((1-\alpha)\) percentile of the chi-squared distribution with one degree of freedom. As an example, a 95% confidence interval of a model parameter is bound by the log-likelihood ratio exceeding approximately \(-1.92\). A profile likelihood confidence interval could be asymmetric, whereas e.g. the Wald statistic applies a quadratic and thus symmetric approximation of the confidence interval [45].
### 4.1.3 Bayesian inference

Bayesian estimates refer to parameters of a model being treated as random variables belonging to some distribution. To fit a parameter in a Bayesian framework a prior distribution of the parameter is needed. The parameters of the prior distribution are called hyper-parameters i.e. if a prior follows a normal distribution, two hyper-parameters define it: mean and standard deviation (SD). The estimated parameter will then be a summary of the posterior probability density function conditioned on the data.

The posterior distribution of a parameter, $\theta$, given the data, $Y_N$, is identified using Bayes’ theorem:

$$
p(\theta, Y_N) = \frac{p(Y_N | \theta) p(\theta)}{p(Y_N)} \quad (30)
$$

where $p(\theta)$ is the prior distribution of $\theta$, $p(Y_N)$ is the marginal distribution and $p(Y_N | \theta)$ is the likelihood of $Y_N$ given $\theta$ as defined in (27).

Finding the set of parameters given data, a model and prior distributions of parameters yielding the maximum of the posterior distribution is called maximum a posteriori (MAP).

### 4.1.4 Maximum a posteriori estimation

MAP estimation is an optimization approach seeking the parameter estimate that maximizes the posterior distribution [46]. Maximizing (30) then reduces to optimizing:

$$
p(\theta, Y_N) \propto p(Y_N | \theta) p(\theta) \quad (31)
$$

MAP estimation reduces to maximizing the likelihood function when the prior is a uniform distribution (i.e. $p(\theta)$ is constant), see (27) and (31). This indicates that ML is a special case of MAP. Also, the weaker a prior is (i.e. having a large standard deviation), the less difference there is between MAP estimation and ML. In general, one distinguishes between informative (highly peaked) and non-informative (not peaked) priors.

Introducing the following notation where $\sigma_\theta$ is a matrix with the prior standard deviations in the diagonal and $R_\theta$ is the prior correlation matrix:

$$
\mu_\theta = E\{\theta\} \quad (32)
$$
$$
\Sigma_\theta = \sigma_\theta R_\theta \sigma_\theta = V\{\theta\} \quad (33)
$$
$$
\epsilon_\theta = \theta - \mu_\theta \quad (34)
$$

Assuming that the priors all follow a Gaussian distribution, the posterior distribution can be rewritten as:

$$
p(\theta | Y_N) \propto \left( \prod_{k=1}^{N} \frac{\exp \left( -\frac{1}{2} \epsilon_k^T R_{k|k-1}^{-1} \epsilon_k \right)}{\sqrt{\det(R_{k|k-1}) (\sqrt{2\pi})}} \right) p(y_0 | \theta) \frac{\exp \left( -\frac{1}{2} \epsilon_\theta^T \Sigma_\theta^{-1} \epsilon_\theta \right)}{\sqrt{\det(\Sigma_\theta) (\sqrt{2\pi})}} \quad (35)
$$

Conditioning the posterior probability on $y_0$ and taking the negative logarithm gives:

$$
- \log(p(\theta | Y_N, y_0)) \propto \frac{1}{2} \sum_{k=1}^{N} \left( \log(\det(R_{k|k-1})) + \epsilon_k^T R_{k|k-1}^{-1} \epsilon_k \right) + \frac{1}{2} \left( \sum_{k=1}^{N} l + p \right) \log(2\pi)
$$
$$
+ \frac{1}{2} \log(\det(\Sigma_\theta)) + \frac{1}{2} \epsilon_\theta^T \Sigma_\theta^{-1} \epsilon_\theta \quad (36)
$$
The MAP solution is found by solving the nonlinear optimization problem:

$$\hat{\theta} = \arg \min_{\theta \in \Theta} \{-\log(p(\theta|Y, y_0))\}$$

(37)

This nonlinear optimization can be solved numerically through gradient-methods. Another method for finding the MAP solution is by using Markov Chain Monte Carlo (MCMC) simulations. MCMC is a brute force method that samples from the posterior distribution to create a rough shape of the posterior distribution and thereby estimates the MAP solution as implemented in WinBUGS [21, 26]. It is computationally time consuming because it can require thousands of samples before reaching convergence. On the contrary, gradient methods converge faster but suffer great difficulties if the objective function is noisy with local gradients not leading to a smaller value of the objective function.

4.1.5 Stochastic differential equations

Modelling a completely known physical system can be done using deterministic ordinary differential equations (ODEs) defined as

$$\frac{dX}{dt} = f(X(t), t)$$

(38)

$$y_k = X(t_k) + e_k$$

(39)

where $X(t)$ is the state of the system, $f()$ is the model, $y_k$ is the discrete observations, and $e_k$ is the measured errors, i.e. observation noise, assumed to be independent and identically distributed (i.i.d.) following a Gaussian distribution [47]. However, in biology one does not always know the true underlying system. In such cases, the discrepancies between the deterministic model and data from the physical system is composed of noise from two sources: measurement errors and systemic model errors. The magnitude of the systemic noise can be identified using stochastic differential equations (SDEs) defined as

$$dx_t = f(x_t, u_t, t, \theta)dt + \sigma(x_t, u_t, t, \theta)dw_t$$

(40)

$$y_k = h(x_k, u_k, t_k, \theta) + e_k$$

(41)

The only difference between the ODE formulation in (38)-(39) and the SDE formulation in (40)-(41) is the diffusion term $\sigma(x_t, u_t, t, \theta)dw_t$ corresponding to the system noise. Thus, solving an SDE with a very small value of $\sigma$ is approximating solving an ODE. The term $f(x_t, u_t, t, \theta)dt$ is called the drift and is the main process driving the system whereas the diffusion term is the system noise. Together, the drift and the diffusion describes the physical state of the system.

4.2 Application in CTSM-R

A team at the Technical University of Denmark (DTU) wrote a package for R allowing to do continuous time stochastic modelling (CTSM) [34,46]. The package was used to obtain the results in this report. This subsection focuses on how the mathematical concepts in the previous subsection are applied in CTSM-R.

4.2.1 Model structure

CTSM-R accepts Itô SDEs in the state space form as presented in (40)-(41). However, CTSM-R does not allow the system noise to depend directly on the state of the system [47], and thus (40) changes to

$$dx_t = f(x_t, u_t, t, \theta)dt + \sigma(u_t, t, \theta)dw_t$$

(42)
Letting the system noise depend on the state can be mitigated in CTSM-R using a transformation of variables called the Lamperti transform [47, 48]. Although the nonlinear equations describing the model in (20)-(26) are presented as ODEs, they are implemented in CTSM-R as SDEs. The ODE presentation is chosen for simplicity.

4.2.2 Initial values

The CTSM-R environment is sensitive to the initial values of the states and thus good initial values are needed to converge to a solution within a reasonable number of iterations. When fitting the PD model, the initial value of the observed state was automatically identified in most datasets as the plasma glucose concentration at time 0. If the initial plasma glucose was not available in one dataset, the initial concentration in the same dog at the other dosing occasions were averaged and used as the initial value of the dataset missing an initial observation. Using the measured or averaged initial glucose concentration the model parameters did not converge to a solution in all datasets. In those cases the initial value was adjusted manually until convergence was reached.

4.2.3 Prior information

In the following all hyper-parameters are fixed, thus all prior probability distributions of parameters are fixed. The values of the hyper-parameters are determined from literature [15,20] and listed in Table 4. All parameters are assumed positive. All parameters of the Hovorka part of the model are assumed to follow a log-normal distribution, except $F_{01}$ which is normally distributed [21]. We also assume that $E_0$, $E_{\text{max}}$, $EC_{50}$, and $\gamma$ follow normal distributions. The insulin sensitivities in [20] were overestimated compared to the results of [21]. As pointed out in Section 2.4, dogs appear to be more sensitive to insulin than humans and thus the estimates listed in [20] are kept as prior information. However, the standard deviation in the logarithmic domain is doubled to allow a different distribution than in humans. The standard deviations of the parameters describing the effect of glucagon on GG are unknown and thus arbitrarily defined as 25% of the mean value estimated from literature [15].

Overall, the prior correlation matrix has the structure presented in Table 5. The correlations of the parameters describing the glucagon part of the model are unknown and thus defined as zero. The values of the prior correlation matrix are calculated from individual parameter fits [20] and presented in Table 6. Fixing one or more of the parameters in the PD model leads to removal of the corresponding parameter rows and columns from the correlation matrix.
Table 4: Prior distributions of PD model parameters listed with source as (mean, SD) in the fitted domain and 95\% confidence interval in non-transformed domain calculated as $\exp(\text{mean} \pm 2 \cdot \text{SD})$ if log-transformed and as $\text{mean} \pm 2 \cdot \text{SD}$ if non-transformed, respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Source</th>
<th>Transformation</th>
<th>Prior distribution</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{12}$</td>
<td>[20]</td>
<td>log</td>
<td>(-2.82, 0.46)</td>
<td>[0.02-0.1]</td>
</tr>
<tr>
<td>$k_{a1}$</td>
<td>[20]</td>
<td>log</td>
<td>(-5.69, 1.12)</td>
<td>[0.0004-0.03]</td>
</tr>
<tr>
<td>$k_{a2}$</td>
<td>[20]</td>
<td>log</td>
<td>(-2.89, 0.70)</td>
<td>[0.01-0.2]</td>
</tr>
<tr>
<td>$k_{a3}$</td>
<td>[20]</td>
<td>log</td>
<td>(-3.74, 0.77)</td>
<td>[0.005-0.1]</td>
</tr>
<tr>
<td>$S_T$</td>
<td>[20]</td>
<td>log</td>
<td>(-5.48, 1.46)</td>
<td>[0.0002-0.08]</td>
</tr>
<tr>
<td>$S_D$</td>
<td>[20]</td>
<td>log</td>
<td>(-7.58, 2.34)</td>
<td>[5-10^{-6}-0.05]</td>
</tr>
<tr>
<td>$S_E$</td>
<td>[20]</td>
<td>log</td>
<td>(-3.19, 1.74)</td>
<td>[0.001-1.3]</td>
</tr>
<tr>
<td>$F_{01}$</td>
<td>[20]</td>
<td>-</td>
<td>(9.68, 2.14)</td>
<td>[5.4-14]</td>
</tr>
<tr>
<td>$E_0$</td>
<td>Section 3.2.4</td>
<td>-</td>
<td>(8.2)</td>
<td>[4-12]</td>
</tr>
<tr>
<td>$E_{max}$</td>
<td>Section 3.2.4</td>
<td>-</td>
<td>(72.1, 18)</td>
<td>[36-108]</td>
</tr>
<tr>
<td>$EC_{50}$</td>
<td>Section 3.2.4</td>
<td>-</td>
<td>(337.8, 85)</td>
<td>[168-508]</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Section 3.2.4</td>
<td>-</td>
<td>(1.25, 0.3)</td>
<td>[0.65-1.85]</td>
</tr>
</tbody>
</table>

Table 5: Overall structure of the full prior correlation matrix.

<table>
<thead>
<tr>
<th></th>
<th>$k_{12}$</th>
<th>$k_{a1}$</th>
<th>$k_{a2}$</th>
<th>$k_{a3}$</th>
<th>$S_T$</th>
<th>$S_D$</th>
<th>$S_E$</th>
<th>$F_{01}$</th>
<th>$E_0$</th>
<th>$E_{max}$</th>
<th>$EC_{50}$</th>
<th>$\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{12}$</td>
<td>1</td>
<td>$\rho_{k_{12},k_{a1}}$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\rho_{k_{12},F_{01}}$</td>
<td>0</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{a1}$</td>
<td>$\rho_{k_{a1},k_{12}}$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\rho_{k_{a1},k_{a2}}$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>0</td>
<td>$\rho_{k_{a1},F_{01}}$</td>
<td>$\cdots$</td>
<td></td>
</tr>
<tr>
<td>$k_{a2}$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\rho_{k_{a2},S_{T}}$</td>
<td>$\cdots$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{a3}$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\rho_{k_{a3},S_{D}}$</td>
<td>$\cdots$</td>
</tr>
<tr>
<td>$S_T$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\rho_{S_{T},F_{01}}$</td>
</tr>
<tr>
<td>$S_D$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
</tr>
<tr>
<td>$S_E$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
</tr>
<tr>
<td>$F_{01}$</td>
<td>$\rho_{F_{01},k_{12}}$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\rho_{F_{01},S_{T}}$</td>
<td>1</td>
<td>0</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
</tr>
<tr>
<td>$E_0$</td>
<td>0</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\rho_{E_0,S_E}$</td>
</tr>
<tr>
<td>$E_{max}$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
</tr>
<tr>
<td>$EC_{50}$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>0</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>0</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>$\cdots$</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 6: Values of the full prior correlation matrix.

<table>
<thead>
<tr>
<th></th>
<th>$k_{12}$</th>
<th>$k_{a1}$</th>
<th>$k_{a2}$</th>
<th>$k_{a3}$</th>
<th>$S_T$</th>
<th>$S_D$</th>
<th>$S_E$</th>
<th>$F_{01}$</th>
<th>$E_0$</th>
<th>$E_{max}$</th>
<th>$EC_{50}$</th>
<th>$\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{12}$</td>
<td>1</td>
<td>0.73</td>
<td>-0.83</td>
<td>-0.14</td>
<td>0.27</td>
<td>0.46</td>
<td>0.22</td>
<td>0.07</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$k_{a1}$</td>
<td>1</td>
<td>1</td>
<td>0.08</td>
<td>0.20</td>
<td>0.05</td>
<td>0.08</td>
<td>0.08</td>
<td>0.22</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$k_{a2}$</td>
<td>1</td>
<td>0.08</td>
<td>0.20</td>
<td>0.05</td>
<td>0.08</td>
<td>0.08</td>
<td>0.22</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$k_{a3}$</td>
<td>1</td>
<td>-0.06</td>
<td>-0.16</td>
<td>-0.28</td>
<td>0.45</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$S_T$</td>
<td>1</td>
<td>0.61</td>
<td>0.77</td>
<td>0.24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$S_D$</td>
<td>1</td>
<td>0.54</td>
<td>-0.51</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$S_E$</td>
<td>1</td>
<td>0.02</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$F_{01}$</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$E_0$</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$E_{max}$</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$EC_{50}$</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
4 METHODS

Log−likelihood
95% LL 95% UL
95% CL 0
Parameter value
95% CL 0
95% UL
95% CL 0

Figure 10: Theoretical examples of three types of profile likelihood plots; from left to right: highly peaked (identifiable), flat (structural non-identifiable), and asymmetric (practical non-identifiable). The 95% confidence limit (CL) is blue. The x-axis shows the 95% lower limit (LL) and upper limit (UL) of the parameter value.

4.2.4 Parameter identifiability

We have performed profile likelihood analysis of each model parameter using graphical presentation to investigate which model parameters are identifiable, see Figure 10 for examples of profile log-likelihood plots. A profile log-likelihood with values only exceeding the 95% confidence limit for the entire physiological range of a parameter indicates that the parameter is not structural identifiable, i.e. if the profile likelihood is flat, the parameter value does not influence the maximum achievable likelihood in fitting the dataset and might as well be fixed. To improve the parameter estimates of the remaining parameters, the unidentifiable parameters were fixed at their prior mean values. On the contrary, if a profile likelihood is highly peaked, the parameter is identifiable and should not be fixed. When a profile likelihood is asymmetric with either no upper or lower limit as seen in Figure 10 at the right, the parameter value is not practically identifiable and could also be fixed if the prior mean value is included in the 95% confidence interval. However, having a prior distribution it is not necessary to fix a parameter with an asymmetric profile log-likelihood but could increase the certainty of the remaining parameters.

As the calculation of a profile likelihood is very time consuming due to optimization of the remaining parameters for each fixed parameter value, the profile log-likelihood plots are initially very coarse with only few points. Profiles are refined as unidentifiable parameters are fixed and calculations are speeding up. Ideally, one should continue the cycle of fixing unidentifiable parameters until all remaining parameters are identifiable. In this study, we have only carried out five cycles of profile likelihood analysis. More parameters could possibly be fixed without changing the likelihood of the model fit significantly. Fitting a model containing unidentifiable parameters using MAP estimation is not wrong, but it comes at the expense of larger confidence intervals of the remaining parameters. The fitted values of parameters having flat or asymmetric profile likelihoods i.e. unidentifiable parameters will mainly be determined by the prior parameter distributions and less determined by data.
4.2.5 Model fitting and validation

Each PK dataset was fitted separately by ML using ODEs by fixing the system noise terms at small values. Since the PK model will be used for simulations we seek the ODE solution instead of the SDE solution. The basal concentration of glucagon was sought estimated. However, the basal level for the analogue was defined as zero and thus not estimated.

Each PD dataset was fitted separately by MAP with the priors listed in Table 4 and the correlation matrix displayed in Table 6. The parameters of each dataset were identified separately based on prior information rather than a population model. All parameters of the Hovorka model except $F_{01}$ were fitted in the logarithmic domain as they are assumed to follow a log-normal distribution [21]. Moreover, all parameters were assumed to be positive. When dealing with small values like the transfer rate constants and sensitivities, log-transformation of the parameters ensure that they are always positive.

During the profile likelihood analysis the PD model was fitted as ODEs. After several model parameters were fixed we fitted the model using SDEs. However, since equations (21) and (24)-(26) were validated by tracer data in a previous publication [20], we fixed the diffusion terms of these states to small values and considered the equations as ODEs. The only modified and thus non-validated state was equation (20) and thus we estimated the diffusion term thereof. As described in Kristensen et al. [33], to validate the model structure, the model was first fitted using SDEs and if the diffusion term was insignificant, i.e. having a large p-value, the model was fitted again using ODEs to obtain the final parameter estimates for simulation purposes. However, if the diffusion term was not insignificant the model structure was incorrect [33]. The necessity of each of the varying model parameters was confirmed by significant p-values less than 0.05.

Furthermore, the model validity was investigated by residual analysis. Residuals should ideally be i.i.d. which was examined by plotting the residuals as a function of time and by plotting the autocorrelation function (ACF). The residuals plot can reveal if there is a drift or change in variance of the residuals over time i.e. if they are identically distributed. The ACF can reveal if there is a pattern in the residuals showing correlation between residuals at different lags i.e. if they are independent [49]. Moreover, very large values of the ACF exceeding the confidence limit imply that the model is not describing the data well.

Simulations of the mean prediction and standard deviations are carried out using extended Kalman filter without updating the states [34]. Predicting future values in a system with very little system noise and thus no updating corresponds to deterministic simulation. It is however possible to perform stochastic simulations in CTSM-R by adding system noise to simulate real life experimental data [34]. Each realization of the stochastic process will be slightly different from another although determined by the size of the noise terms. We can interpret the actual data used for model fitting as one realization of the underlying stochastic process.

5 Results

Despite CTSM-R uses a robust estimation method [46], extreme outliers can largely impact the fit when the number of observations is small. Fitting of the PK data to the simple model was mostly robust to outliers. However, one PK datapoint as listed in Table 3 was so extreme that removing it greatly changed and improved the fit.

The PD model was more sensitive to outliers in both the input data and in the observations due to the large number of model parameters compared to number of observations. Six glucose observations appeared to be outliers as listed in Table 3. Due to the number of model parameters, the glucose observations had to
Table 7: Fitted or fixed PK model parameters (mean, SD). *Non significant. △Fixed.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Glucagon</th>
<th>ZP-GA-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>min$^{-1}$</td>
<td>(0.134, 0.077)</td>
<td>(0.125, 0.072)</td>
</tr>
<tr>
<td>$k_2$</td>
<td>min$^{-1}$</td>
<td>(0.0159, 0.0048)</td>
<td>(0.0116, 0.0031)</td>
</tr>
<tr>
<td>$Cl$</td>
<td>mL/kg/min</td>
<td>(56.9, 13.2)</td>
<td>(88.5, 18.8)</td>
</tr>
<tr>
<td>$C_b$</td>
<td>pg/mL</td>
<td>0*</td>
<td>0\△</td>
</tr>
</tbody>
</table>

Table 8: PK endpoints extracted from PK model fits (mean, SD).

<table>
<thead>
<tr>
<th>PK endpoint</th>
<th>Unit</th>
<th>Dose level</th>
<th>Glucagon</th>
<th>ZP-GA-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{max}$</td>
<td>min</td>
<td>low</td>
<td>(23.9, 11.4)</td>
<td>(25.3, 9.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>high</td>
<td>(19.3, 5.4)</td>
<td>(22.4, 6.6)</td>
</tr>
<tr>
<td>$C_{max}$/Dose</td>
<td>nmol/L per nmol/kg</td>
<td>low</td>
<td>(0.059, 0.018)</td>
<td>(0.043, 0.010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>high</td>
<td>(0.097, 0.017)</td>
<td>(0.059, 0.010)</td>
</tr>
</tbody>
</table>

be replaced by linear interpolation to maintain an identifiable model.

5.1 PK

5.1.1 Parameter estimates

Table 7 lists the average and standard deviation of the PK model parameters for each drug over the populations. As mentioned in section 2.4, it was not possible to measure low glucagon concentrations in any datasets. Due to this lack of data at low concentrations, it was not possible to estimate basal glucagon concentration in plasma. The PK model fit is used as an input to the following PD model fitting and could also be used for simulation purposes.

5.1.2 PK Model fits

After fitting the PK model to data, relevant endpoints were extracted from the fits and presented in Table 8. Paired t-tests of the surrogate marker for onset of action, $T_{max}$, showed no difference between ZP-GA-1 and glucagon (p-value = 0.3).

$C_{max}$ is significantly different for ZP-GA-1 compared to glucagon (p-value = 0.006).

Figure 11 displays examples of PK model fits with 95\% confidence limits of the simulation both with regular and logarithmic base-10 y-axes (log$_{10}$). Figures 21-25 in Appendix B shows all PK fits.

5.2 PD

A few model building cycles have been carried out. The following sections only show results of the last and final model. However, the sections will refer qualitatively to observations made during the model building cycle to justify the decisions made by the model builder.

5.2.1 Reducing variables

Having a large model with twelve parameters and observation noise, some parameters had to be fixed to increase the certainty of other model parameters. To investigate which model parameters were unidentifi-
Figure 11: PK responses to low and high doses of glucagon and ZP-GA-1 in dog 3. Left graphs are with regular y-axes and right graphs are with logarithmic base-10 y-axes (\(\log_{10}\)).

Unidentifiability of \(k_{a2}\) is likely due to the model structure since the parameter describes the insulin transfer that affects the glucose disposal of the non-accessible compartment. Determining the influence of insulin on glucose disposal requires tracer data which is not available.
Figure 12: Profile likelihoods of all twelve model parameters in dog 3 after high dose of glucagon. Red points illustrate fixed parameter values. Red curves illustrate prior parameter distributions. Horizontal blue lines are 95% confidence limits of parameter values.
Table 9: Ratio of datasets with significant p-values less than 0.05 corresponding to 95% confidence level when estimated in SE setting versus OE setting. P-values of fixed parameters do not exist but the parameters are included in the table for completeness.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glucagon</th>
<th>ZP-GA-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SE</td>
<td>OE</td>
</tr>
<tr>
<td>$k_{12}$</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>$k_{a1}$</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>$k_{a2}$</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>$k_{a3}$</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>$S_T$</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>$S_E$</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>$F_{01}$</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>$E_0$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$E_{max}$</td>
<td>7/10</td>
<td>10/10</td>
</tr>
<tr>
<td>$EC_{50}$</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>$\sigma_1$</td>
<td>0/10</td>
<td>-</td>
</tr>
</tbody>
</table>

As an example, the increase in certainty of parameter value when reducing the number of variables is graphically evident for $k_{a1}$ in Figure 12. At the initial cycle the 95% confidence interval of the logarithm of the parameter is approximately [-7 to -3], but reduces to [-5 to -3.5] when four parameters are fixed. Same tendency can be observed for other model parameters.

### 5.2.2 Model validity

After reduction of variables using profile likelihood analysis we investigate using SDEs if the model describes the physiological system adequately i.e. if the model has insignificant system noise. As previously argued, we only examine the noise of equation (20). Table 9 lists the proportion of datasets with significant p-values for each PD model parameter with and without the coefficient of the diffusion term, $\sigma_1$, for both of the compounds. Using SDEs we observe that the model parameters are significant at a 5% confidence level in the majority of datasets and that the diffusion coefficient is insignificant in all datasets. $\sigma_1$ is then fixed at a small value and the PD model parameters re-estimated using ODEs. After fixing $\sigma_1$, all model parameters are significant in all datasets. We can not reject that the model describes the underlying physiological system.

### 5.2.3 Residual Analysis

After the final estimation of PD model parameters using ODEs we analyse the standardized residuals to verify the quality of the model in describing data. Figure 13 shows an example of the standardized residuals plot and ACF showing i.i.d. residuals thus no trends in residuals and no significant correlation between residuals.

However, dealing with data having very few observations makes it challenging to be strict to the rules of i.i.d. residuals. Especially the residuals plot is difficult to interpret in most cases due to few observations. The ACF also has some limitations in that data was not equidistantly sampled. Most ACF are
Figure 13: Example of standardized residuals plot and ACF of residuals of model fit to dataset from dog 3 after high dose of glucagon.

within the confidence limits.
Based on the residual analysis the model describes the dynamics in the data well.

5.2.4 PD Model fits

In Section 5.2.1 we reduced the number of PD model parameters by removing unidentifiable parameters. In Section 5.2.2 we described how the model structure is sufficient to capture the dynamics of the physical system. In Section 5.2.3 we confirmed that the fit did not give rise to trends in the residuals. Finally, we can verify that the model is satisfactory based on visual inspection of the PD model fit together with data. Figure 14 presents examples of PD model fits together with model inputs after administration of glucagon at low and high dose levels in one dog. In the two examples, the model is fitting data well with narrow confidence limits around the simulation. Within 100 minutes we observe a peak in glucose concentration and a return to baseline. At the end of the sampling period the glucose concentrations show a tendency to rise slowly.

Figure 15 presents examples of PD model fits together with model inputs after administration of ZP-GA-1 at low and high dose levels in one dog. The trends in data and the PD model fits are similar to the ones after administration of glucagon described above.

Figures 26-30 in Appendix C present all PD model fits.
Figure 14: Plasma concentrations of PD model inputs glucagon and insulin together with PD model fit of glucose after administration of glucagon.

(a) Dog 3, 20 nmol/kg.
(b) Dog 3, 120 nmol/kg.

Figure 15: Plasma concentrations of PD model inputs ZP-GA-1 and insulin together with PD model fit of glucose after administration of ZP-GA-1.

(a) Dog 3, 20 nmol/kg.
(b) Dog 3, 120 nmol/kg.
Table 10: Posterior distributions of PD model parameters for the population in fitted domain reported as (mean, SD). Parameters not in parenthesis are fixed.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glucagon (mean, SD)</th>
<th>ZP-GA-1 (mean, SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>log($k_{d1}$)</td>
<td>(-2.87, 0.27)</td>
<td>(-3.02, 0.23)</td>
</tr>
<tr>
<td>log($k_{d2}$)</td>
<td>(-5.19, 0.86)</td>
<td>(-5.90, 0.83)</td>
</tr>
<tr>
<td>log($k_{a2}$)</td>
<td>-2.89</td>
<td>-2.89</td>
</tr>
<tr>
<td>log($k_{a3}$)</td>
<td>(-4.88, 0.73)</td>
<td>(-4.70, 0.98)</td>
</tr>
<tr>
<td>log($S_T$)</td>
<td>(-5.65, 0.86)</td>
<td>(-5.35, 0.50)</td>
</tr>
<tr>
<td>log($S_D$)</td>
<td>(-9.11, 0.93)</td>
<td>(-8.72, 1.15)</td>
</tr>
<tr>
<td>log($S_E$)</td>
<td>(-2.71, 0.57)</td>
<td>(-2.95, 0.43)</td>
</tr>
<tr>
<td>$F_{01}$</td>
<td>(9.8, 1.5)</td>
<td>(9.6, 2.0)</td>
</tr>
<tr>
<td>$E_0$</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>$E_{max}$</td>
<td>(50.1, 13.7)</td>
<td>(53.8, 16.5)</td>
</tr>
<tr>
<td>$EC_{50}$</td>
<td>337.8</td>
<td>337.8</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

5.2.5 Parameter estimates

We assume that the variations in parameters within dogs are negligible compared to the variations between dogs. As prior information we used Gaussian distributions and using the individually fitted parameters we can calculate similar posterior distributions of PD model parameters.

Table 10 lists the posterior distributions of each parameter separated by drug and study for the population of dogs. During the PK analysis we noticed that the onset of action and maximum concentration of glucagon were different between the studies. We therefore separate the parameters of the two studies in this analysis, too. During estimation, most parameters are log-transformed.

For ease of comparison to values reported in literature [20], the averages of the log-transformed parameters are transformed back and listed together with ranges in Table 11. Fixed parameters are listed for completeness in both tables.

Table 12 shows the posterior correlation matrix of both PK and PD model parameters.

The following Section describes observations related to these posterior distributions and correlations.
Table 11: Average of PD model parameters in normal domain together with range. Fixed parameters are listed for completeness.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glucagon</th>
<th>ZP-GA-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{12}$</td>
<td>0.057 (0.038-0.085)</td>
<td>0.049 (0.033-0.081)</td>
</tr>
<tr>
<td>$k_{a1}$</td>
<td>0.0056 (0.0019-0.0279)</td>
<td>0.0027 (0.0009-0.0192)</td>
</tr>
<tr>
<td>$k_{a2}$</td>
<td>0.055</td>
<td>0.055</td>
</tr>
<tr>
<td>$k_{a3}$</td>
<td>0.0076 (0.0038-0.0508)</td>
<td>0.0091 (0.0038-0.0657)</td>
</tr>
<tr>
<td>$S_T \cdot 10^{-4}$</td>
<td>35 (13-117)</td>
<td>48 (27-110)</td>
</tr>
<tr>
<td>$S_D \cdot 10^{-4}$</td>
<td>1.1 (0.2-3.8)</td>
<td>1.6 (0.6-35.8)</td>
</tr>
<tr>
<td>$S_E \cdot 10^{-4}$</td>
<td>666 (178-1323)</td>
<td>523 (281-892)</td>
</tr>
<tr>
<td>$F_{01}$</td>
<td>9.8 (8.0-13.1)</td>
<td>9.6 (5.3-12.3)</td>
</tr>
<tr>
<td>$E_0$</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>$E_{max}$</td>
<td>50.1 (26.6-73.2)</td>
<td>53.8 (24.1-71.8)</td>
</tr>
<tr>
<td>$EC_{50}$</td>
<td>337.8</td>
<td>337.8</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 12: Posterior correlation matrix of PK and PD model parameters and body weight.

<table>
<thead>
<tr>
<th></th>
<th>$k_1$</th>
<th>$k_2$</th>
<th>$Cl$</th>
<th>$w$</th>
<th>$\log(k_{12})$</th>
<th>$\log(k_{a1})$</th>
<th>$\log(k_{a3})$</th>
<th>$\log(S_T)$</th>
<th>$\log(S_D)$</th>
<th>$\log(S_E)$</th>
<th>$F_{01}$</th>
<th>$E_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>1</td>
<td>0.26</td>
<td>-0.54</td>
<td>-0.08</td>
<td>-0.25</td>
<td>0.49</td>
<td>0.52</td>
<td>-0.01</td>
<td>-0.13</td>
<td>0.50</td>
<td>-0.55</td>
<td></td>
</tr>
<tr>
<td>$k_2$</td>
<td>1</td>
<td>-0.27</td>
<td>-0.24</td>
<td>0.34</td>
<td>0.17</td>
<td>0.12</td>
<td>0.16</td>
<td>-0.03</td>
<td>-0.11</td>
<td>0.27</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>$Cl$</td>
<td>1</td>
<td>-0.51</td>
<td>-0.37</td>
<td>-0.55</td>
<td>0.44</td>
<td>0.60</td>
<td>0.11</td>
<td>-0.22</td>
<td>0.35</td>
<td>-0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$w$</td>
<td>1</td>
<td>0.10</td>
<td>0.32</td>
<td>-0.46</td>
<td>-0.63</td>
<td>0.02</td>
<td>0.19</td>
<td>-0.55</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log(k_{12})$</td>
<td>1</td>
<td>0.78</td>
<td>-0.13</td>
<td>-0.21</td>
<td>0.02</td>
<td>0.01</td>
<td>0.11</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log(k_{a1})$</td>
<td>1</td>
<td>-0.45</td>
<td>-0.68</td>
<td>-0.44</td>
<td>0.31</td>
<td>-0.07</td>
<td>0.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log(k_{a3})$</td>
<td>1</td>
<td>0.60</td>
<td>0.00</td>
<td>-0.80</td>
<td>0.78</td>
<td>-0.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log(S_T)$</td>
<td>1</td>
<td>0.36</td>
<td>-0.32</td>
<td>0.53</td>
<td>-0.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log(S_D)$</td>
<td>1</td>
<td>0.00</td>
<td>-0.45</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log(S_E)$</td>
<td>1</td>
<td>-0.57</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{01}$</td>
<td>1</td>
<td>-0.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{max}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
Table 13: P-values of two-tailed paired t-tests comparing PD model parameter estimates of glucagon versus analogue within study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glucagon vs. ZP-GA-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>0.29</td>
</tr>
<tr>
<td>$k_{a1}$</td>
<td>0.09</td>
</tr>
<tr>
<td>$k_{a2}$</td>
<td>-</td>
</tr>
<tr>
<td>$k_{a3}$</td>
<td>0.42</td>
</tr>
<tr>
<td>$S_T$</td>
<td>0.19</td>
</tr>
<tr>
<td>$S_D$</td>
<td>0.42</td>
</tr>
<tr>
<td>$S_E$</td>
<td>0.14</td>
</tr>
<tr>
<td>$F_{01}$</td>
<td>0.81</td>
</tr>
<tr>
<td>$E_0$</td>
<td>-</td>
</tr>
<tr>
<td>$E_{max}$</td>
<td>0.51</td>
</tr>
<tr>
<td>$EC_{50}$</td>
<td>-</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>-</td>
</tr>
</tbody>
</table>

5.2.6 Native glucagon versus glucagon analogue

The reason for doing cross-over studies of glucagon and the glucagon analogue is to be able to compare the dynamics of the compounds without too many confounding factors like biological variations. Table 13 presents p-values of paired t-tests between glucagon and the glucagon analogue. No model parameters are significantly different on a 5% confidence level.

6 Discussion

In this report, we present a novel model describing how insulin and glucagon contribute to the EGP in dogs. The model is based on physiological knowledge and parameter estimates are based on data from pre-clinical studies in five dogs. The PD model description is contentious compared to most existing models in several ways: claiming that GG is completely suppressed when insulin concentration exceeds a threshold as in Hovorka et al. [20], claiming that GG saturates when glucagon concentration is high, and claiming a multiplicative effect of insulin and glucagon as in Emami et al. [26]. Moreover, we have used the model to compare PD characteristics of marketed glucagon and a novel glucagon analogue invented by Zealand Pharma A/S.

According to our PD model there exists a certain threshold of insulin concentration at which the GG is completely suppressed. From equation (22) the threshold can easily be identified using the insulin sensitivity on GG as $S_E^{-1}$. Using the average parameter estimate listed in Table 11 we find a threshold of less than 20 mIU/L. This threshold is considerably lower than the threshold identified by Cherrington as 45 mIU/L [15]. Also, El Youssef et al. found that insulin concentrations exceeding 40 mIU/L only results in EGP of roughly 20 mg/kg during 60 minutes [28]. This EGP production of ~0.33 mg/kg/min is similar magnitude as GN of ~0.5 mg/kg/min observed by Cherrington [15]. Thus, the results by El Youssef et al. at insulin concentrations exceeding 40 mIU/L could be explained by insulin’s suppression of GG. The human prior of $S_E$ suggested an insulin threshold of 19 mIU/L [20], whereas a later publication suggested an average insulin threshold of 85 mIU/L based on the value of $S_E$ [21]. However, both of these
estimates of $S_E$ were based on human data. As discussed in Section 2.4 dogs have lower basal insulin levels than humans and are therefore more sensitive to insulin than humans. It is thus reasonable that the threshold at which the GG is completely suppressed is lower in dogs than in humans.

The graphs of raw data in Appendix A reveal that the dog’s insulin concentrations exceed even the human threshold of GG suppression during at large parts of the study time. In some datasets we notice a slight increase in blood glucose concentrations during the last part of the study which could be explained by GG no longer being suppressed by insulin and a plasma glucagon concentration that is still much higher than basal levels. However, we only observe this slight increase in plasma glucose in some datasets. This could be explained by the delay in insulin action on plasma glucose described by the small rate constant with a half-life of approximately 90 minutes which corresponds to the remaining of the sampling period after plasma insulin concentrations have returned to baseline.

The diabetes community is speculating whether glucagon works when insulin inhibits GG. El Youssef et al. found in diabetes patients that increasing glucagon doses of 25-175 $\mu$g increases EGP at insulin concentrations less than 30 mIU/L [28], but increasing doses up to 175 $\mu$g have no effect on EGP at insulin concentrations exceeding 40 mIU/L as explained previously. Blauw et al. investigated the glucose response in patients with diabetes to various doses of glucagon from 0.1-1 mg at different blood glucose levels and concluded that blood glucose level was irrelevant to the glucose response to glucagon [50]. Unfortunately, the study does not report insulin concentrations. Ranjan et al. investigated the glucose response in diabetes patients to glucagon doses of 100-300 $\mu$g at insulin concentrations of 8-20 mIU/L and found no significant increase in glucose response after a glucagon dose of 300 $\mu$g compared to 200 $\mu$g [51]. A study by Graf et al. in healthy showed no further effect size of glucagon doses larger than 250 $\mu$g [52]. The studies by Ranjan et al. and Graf et al. suggest a saturation effect of glucagon in both healthy and patients with diabetes.

The glucagon doses used in the pre-clinical dog study ranged 20-120 nmol/kg (0.07-0.4 mg/kg) corresponding to human equivalent doses of 0.04-0.2 mg/kg using allometric scaling. Thus, the previous studies suggesting a saturation effect of glucagon doses exceeding 0.2 mg supports our observations that the glucose responses of the dogs were saturated at all dose levels.

According to Cherrington the GG response to glucagon is almost saturated for glucagon concentrations exceeding approximately 1000 pg/mL [15]. From the graphs of raw data in Figures 16-20 we observe that plasma glucagon concentrations are higher than 1000 pg/mL most of the study duration. We only have very limited data when glucagon concentrations are low. During the reduction of variables we recognized this fact since we were not able to identify the parameters describing GG response to glucagon at low concentrations, but only at saturated concentrations. We still believe that the sigmoid $E_{\text{max}}$ model presented here is valid in describing the effect of glucagon on GG because it builds on knowledge from literature in particular Cherrington [15]. Moreover, we believe that this novel model is more physiologically correct than previous models based on the minimal model or a linear approximation since these models do not describe the saturation effect of glucagon on GG [24–26]. Future studies should be designed so that the plasma glucagon concentration does not yield saturated EGP response for the entire study duration.

The multiplicative effect of insulin and glucagon on GG was proposed by Emami et al. [26]. The idea was derived from Hovorka et al. who states that with increasing insulin concentration the total EGP decreases linearly [20]. The model by Emami et al. multiplies the effect of insulin as described by Hovorka et al. with an expression stating that GG increases linearly when glucagon increases.

In this report, we have extended the model by Emami et al. to include saturation of glucagon. As the glucose response to glucagon was saturated during the entire study time for most datasets, the sophisticated sigmoid $E_{\text{max}}$ model practically reduces to a constant value and thus the expression for GG originally
Despite the saturated GG response due to glucagon, the model assumes that the glucogen stores in the liver are never depleted. As the study was conducted over short time, this is a fair assumption. Moreover, the glucose response from breakdown of glycogen is suppressed by insulin most of the study time. Recent data shows that small frequent glucagon boluses do not deplete the glucogen stores [22]. The study by Castle et al. has a few limitations in that it was carried out over short time and all participants were well-fed and had good control of their diabetes. It is however especially the poorly controlled patients that would need the glucagon bolus regularly. The effects of repeated daily and long term use of glucagon remain unknown. Studies investigating the long term effects are needed to verify that the glucose response to glucagon does not change over time.

The used datasets posed other challenges than not covering low glucagon concentrations. The study was not optimal for the purpose of fitting models describing the glucose-insulin-glucagon dynamics nor designed for identifying how insulin and glucagon affect EGP. The datasets were sparsely sampled which made it necessary to fix some parameters in order to increase the certainty of the estimates of the remaining parameters. We used profile likelihood analysis to justify fixation of four PD model parameters. Residual analysis of time series with only 14 observations is challenging and should not be considered as strict as an analysis using ten times the number of observations. Not all residuals plot and ACFs showed i.i.d. but visual inspection of model fits confirmed that the model described data well for the purpose of simulation.

We chose not to do cross-validation of the model, as this would be a waste of our limited amount of data. Also, with inter and intra biological variation, we would not expect to get good PD model fits testing parameters estimated in one dataset in another. Only in cases with constant conditions can such cross validation methods lead to meaningful and fair results.

In this report we focused on fitting data from individual trials using prior information in order to obtain a model suitable for simulation of the glucose-insulin-glucagon dynamics. The posterior parameter distributions and correlation matrix form a population from which a parameter set can be sampled for simulation of a subject. The estimated model parameters depend on the prior parameter distributions to some extend. However, comparing the prior parameter distributions in Table 4 with the posterior parameter distributions in Table 10, we observe that most posterior distributions are much narrower than the initial prior distributions of parameters, i.e. the parameter distributions are more informative. The parameter estimation could be re-done by performing population modelling thus determining, not only the individual model parameters, but the hyper-parameters, i.e. population parameters, too. We also expect this simulation model to be valid in describing human glucose-insulin-glucagon dynamics although possibly with different population parameter distributions and parameter correlations.

We used a simple PK model together with the novel PD model to compare glucagon with a novel glucagon analogue referred to as ZP-GA-1 invented by Zealand Pharma A/S. Comparing PK between compounds, we did not find any significant differences for ZP-GA-1 compared to glucagon. However, we did find a significantly higher peak concentration of the analogue compared to glucagon. This is in agreement with the higher bioavailability of the analogue compared to glucagon, see Table 2 in Section 2.2. Comparing PD model parameters between glucagon and the analogue we did not find any significant differences at a 95% confidence level. Therefore, we can not reject that the analogue has similar PD effect on the glucose response and has similar PK characteristics to marketed glucagon.
In conclusion, we developed a novel model of the complex glucose-insulin-glucagon dynamics based on physiology and data. We demonstrated that the model describes the glucoregulatory system well and enables simulations of glucose dynamics knowing insulin and glucagon plasma concentrations. Comparisons of marketed glucagon with the novel glucagon analogue did not show any differences in PK or PD characteristics. This report presents parameter estimates for simulations of the glucose-insulin-glucagon dynamics in dogs but could be extended to simulations of the human dynamics after obtaining parameter estimates based on similar studies in humans.
References


REFERENCES


REFERENCES


Appendix

A Raw data

Figure 16: Raw PK and PD data with outliers measured in dog 1. Data from low or high doses of glucagon and ZP-GA-1 are red crosses or blue dots, respectively.

Figure 17: Raw PK and PD data with outliers measured in dog 2. Data from low or high doses of glucagon and ZP-GA-1 are red crosses or blue dots, respectively.
Figure 18: Raw PK and PD data with outliers measured in dog 3. Data from low or high doses of glucagon and ZP-GA-1 are red crosses or blue dots, respectively.

Figure 19: Raw PK and PD data with outliers measured in dog 4. Data from low or high doses of glucagon and ZP-GA-1 are red crosses or blue dots, respectively.
Figure 20: Raw PK and PD data with outliers measured in dog 5. Data from low or high doses of glucagon and ZP-GA-1 are red crosses or blue dots, respectively.
B PK Model fits

Figure 21: PK model fit after low and high doses of glucagon (green) and ZP-GA-1 (blue) in dog 1. Left graphs are with regular axes and right graphs are with log_{10} axes.
Figure 22: PK model fit after low and high doses of glucagon (green) and ZP-GA-1 (blue) in dog 2. Left graphs are with regular axes and right graphs are with log\(_{10}\) axes.

Figure 23: PK model fit after low and high doses of glucagon (green) and ZP-GA-1 (blue) in dog 3. Left graphs are with regular axes and right graphs are with log\(_{10}\) axes.
Figure 24: PK model fit after low and high doses of glucagon (green) and ZP-GA-1 (blue) in dog 4. Left graphs are with regular axes and right graphs are with log$_{10}$ axes.

Figure 25: PK model fit after low and high doses of glucagon (green) and ZP-GA-1 (blue) in dog 5. Left graphs are with regular axes and right graphs are with log$_{10}$ axes.
C  PD model fits
Figure 26: Plasma concentrations of PD model inputs glucagon and insulin together with PD model fit of glucose in dog 1. Administered doses and drugs are written in each subfigure.
Figure 27: Plasma concentrations of PD model inputs glucagon and insulin together with PD model fit of glucose in dog 2. Administered doses and drugs are written in each subfigure.

(a) 20 nmol/kg of glucagon.
(b) 120 nmol/kg of glucagon.
(c) 20 nmol/kg of ZP-GA-1.
(d) 120 nmol/kg of ZP-GA-1.
Figure 28: Plasma concentrations of PD model inputs glucagon and insulin together with PD model fit of glucose in dog 3. Administered doses and drugs are written in each subfigure.

(a) 20 nmol/kg of glucagon.
(b) 120 nmol/kg of glucagon.
(c) 20 nmol/kg of ZP-GA-1.
(d) 120 nmol/kg of ZP-GA-1.
Figure 29: Plasma concentrations of PD model inputs glucagon and insulin together with PD model fit of glucose in dog 4. Administered doses and drugs are written in each subfigure.
Figure 30: Plasma concentrations of PD model inputs glucagon and insulin together with PD model fit of glucose in dog 5. Administered doses and drugs are written in each subfigure.
This appendix presents the 1-page paper with the title "Modelling of Glucose-Insulin-Glucagon Pharmacodynamics in Man" accepted at the 38th annual international conference of the IEEE Engineering in Medicine and Biology Society (EMBC’16) in Orlando, Florida during August 2016 [19].
Modelling of Glucose-Insulin-Glucagon Pharmacodynamics in Man
S. L. Wendt1,2, J. K. Møller2, A. Haidar3, C. B. Knudsen1, H. Madsen2, J. B. Jørgensen2

Abstract—The purpose is to build a simulation model of the glucoregulatory system in man. We estimate individual human parameters of a physiological glucose-insulin-glucagon model. We report posterior probability distributions and correlations of model parameters.

I. INTRODUCTION
In healthy individuals, insulin and glucagon work in a complex fashion to maintain blood glucose levels within a narrow range. Recent studies suggest a multiplicative effect of insulin and of glucagon on endogenous glucose production (EGP) [1].

II. MATERIALS AND METHODS
A. PD Model
The pharmacodynamics (PD) model is mainly inspired by Hovorka et al. [2].

\begin{align*}
Q_1(t) &= -F_{01} - S_T x_1(t) Q_1(t) + k_{12} Q_2(t) + F_{IC}(t) \\
Q_2(t) &= S_T x_1(t) Q_1(t) - (k_{12} + S_D x_3(t)) Q_2(t) \\
\dot{x}_i(t) &= \frac{k_i (I(t) - x_i(t))}{V} \quad i = 1, 2, 3
\end{align*}

\(Q_1(t)\) and \(Q_2(t)\) are the masses of glucose per bodyweight (μmol/kg) in the accessible and non-accessible compartments. Glucose concentration (mmol/L) in the accessible compartment is \(Q_1(t)/V\) with \(V\) fixed at 160 mL/kg. \(I(t)\) is the insulin concentration (mIU/L) in the accessible compartment. \(x_1(t)\) are the remote effects of insulin (mIU/L). \(F_{01}\) is the non-insulin-dependent glucose flux. \(k_{12}\) and \(k_1\) are transfer rate constants. \(S_D, S_E, \) and \(S_T\) are insulin sensitivities.

The model in (1) is modified so \(F_{IC}(t)\) is the insulin and glucagon dependent EGP [3].

\begin{align*}
F_{IC}(t) &= \frac{1 - S_E x_3(t)}{1 - S_E b_{01g}} \left( E_{max} - E_0 \right) C(t) \\
C(t) &= \frac{C_{E50}}{C_{E50} + C(t)}
\end{align*}

\(C(t)\) is the glucagon concentration (pg/mL) in the accessible compartment. \(b_{01g}\) is the fixed basal insulin concentration (mIU/L) for subject \(i\), and \(E_0\) is the minimum EGP fixed at 8 μmol/(kg-min). \(E_{max}\) is the maximum EGP at \(I_{b,g}. \ C_{E50}\) is the glucagon concentration at half maximum EGP.

B. Parameter Estimation
We used maximum a posteriori to estimate PD model parameters and profile likelihood analysis to reduce unidentifiable parameters in data with measurements of glucose, insulin and glucagon from ten healthy male subjects who received a 1 mg subcutaneous bolus of marketed glucagon.

III. RESULTS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_{E50})</td>
<td>pg/mL</td>
<td>407</td>
<td>39</td>
</tr>
<tr>
<td>(E_{max})</td>
<td>μmol/kg/min</td>
<td>38.8</td>
<td>5.0</td>
</tr>
<tr>
<td>(F_{01})</td>
<td>μmol/kg/min</td>
<td>10.5</td>
<td>0.95</td>
</tr>
<tr>
<td>(k_{12})</td>
<td>min⁻¹</td>
<td>-3.48</td>
<td>0.26</td>
</tr>
<tr>
<td>(k_1)</td>
<td>min⁻¹</td>
<td>-2.11</td>
<td>0.03</td>
</tr>
<tr>
<td>(k_2)</td>
<td>min⁻¹</td>
<td>-4.20</td>
<td>0.74</td>
</tr>
<tr>
<td>(k_3)</td>
<td>min⁻¹</td>
<td>-3.19</td>
<td>0.67</td>
</tr>
<tr>
<td>(S_D)</td>
<td>pg/mL per mIU/L</td>
<td>-3.73</td>
<td>0.54</td>
</tr>
<tr>
<td>(S_E)</td>
<td>pg/mL per mIU/L</td>
<td>-3.69</td>
<td>*</td>
</tr>
<tr>
<td>(S_T)</td>
<td>pg/mL per mIU/L</td>
<td>-7.58</td>
<td>*</td>
</tr>
</tbody>
</table>

* Fixed unidentifiable parameter.

IV. CONCLUSIONS
The model enables simulations of the glucose-insulin-glucagon dynamics in man at the following concentrations: glucagon (180-8000 pg/mL), insulin (1.2-81.9 mIU/L) and glucose (3.3-11.5 mmol/L).

REFERENCES
This appendix presents the journal paper by Wendt et al. with the title "Cross-Validation of a Glucose-Insulin-Glucagon Pharmacodynamics Model for Simulation using Data from patients with Type 1 Diabetes" published in Journal of Diabetes Science and Technology in February 2017 [20].
The treatment goal for patients with type 1 diabetes is near-normalization of plasma glucose levels. Few patients achieve this even with intensive insulin treatment. New approaches with automatic glucose controlled insulin and glucagon delivery, known as a dual-hormone artificial pancreas (AP), may offer a solution to improve glycemic control. To design and tune control algorithms for AP devices prior to in vivo tests, a validated simulation model capturing the dynamics between glucose, insulin and glucagon is needed to perform helpful in silico experiments.

Glucagon primarily affects hepatic glucose production by increasing glycogenolysis, while the rate of gluconeogenesis seems less affected by changes in both insulin and glucagon concentrations. Currently marketed glucagon is approved as a 1 mg rescue-treatment for severe hypoglycemia, although the interest in mini-dose glucagon is increasing. Recent research has focused on developing models to simulate glucagon effects for glycemic control.

The treatment goal for patients with type 1 diabetes is near-normalization of plasma glucose levels. Few patients achieve this even with intensive insulin treatment. New approaches with automatic glucose controlled insulin and glucagon delivery, known as a dual-hormone artificial pancreas (AP), may offer a solution to improve glycemic control. To design and tune control algorithms for AP devices prior to in vivo tests, a validated simulation model capturing the dynamics between glucose, insulin and glucagon is needed to perform helpful in silico experiments.

Glucagon primarily affects hepatic glucose production by increasing glycogenolysis, while the rate of gluconeogenesis seems less affected by changes in both insulin and glucagon concentrations. Currently marketed glucagon is approved as a 1 mg rescue-treatment for severe hypoglycemia, although the interest in mini-dose glucagon is increasing. Recent research has focused on developing models to simulate glucagon effects for glycemic control.

The treatment goal for patients with type 1 diabetes is near-normalization of plasma glucose levels. Few patients achieve this even with intensive insulin treatment. New approaches with automatic glucose controlled insulin and glucagon delivery, known as a dual-hormone artificial pancreas (AP), may offer a solution to improve glycemic control. To design and tune control algorithms for AP devices prior to in vivo tests, a validated simulation model capturing the dynamics between glucose, insulin and glucagon is needed to perform helpful in silico experiments.

Glucagon primarily affects hepatic glucose production by increasing glycogenolysis, while the rate of gluconeogenesis seems less affected by changes in both insulin and glucagon concentrations. Currently marketed glucagon is approved as a 1 mg rescue-treatment for severe hypoglycemia, although the interest in mini-dose glucagon is increasing. Recent research has focused on developing models to simulate glucagon effects for glycemic control.

The treatment goal for patients with type 1 diabetes is near-normalization of plasma glucose levels. Few patients achieve this even with intensive insulin treatment. New approaches with automatic glucose controlled insulin and glucagon delivery, known as a dual-hormone artificial pancreas (AP), may offer a solution to improve glycemic control. To design and tune control algorithms for AP devices prior to in vivo tests, a validated simulation model capturing the dynamics between glucose, insulin and glucagon is needed to perform helpful in silico experiments.

Glucagon primarily affects hepatic glucose production by increasing glycogenolysis, while the rate of gluconeogenesis seems less affected by changes in both insulin and glucagon concentrations. Currently marketed glucagon is approved as a 1 mg rescue-treatment for severe hypoglycemia, although the interest in mini-dose glucagon is increasing. Recent research has focused on developing models to simulate glucagon effects for glycemic control.
studies proved that the glycemic response to low-dose glucagon is dependent on ambient insulin levels,\textsuperscript{13} but neither on plasma glucose level\textsuperscript{14,15} nor on prior glucagon dosing.\textsuperscript{16} At high circulating insulin concentrations (50-60 mU/l), the endogenous glucose production (EGP) is completely inhibited,\textsuperscript{11} and at insulin levels exceeding ~40 mU/l the EGP cannot be stimulated by glucagon.\textsuperscript{13}

The ability of insulin to suppress the glycogenolytic response to glucagon at high insulin concentration is not reflected in previously published models of glucose-glucagon dynamics.\textsuperscript{18-20} A comparative study found that a multiplicative relationship was needed to describe insulin’s inhibitory effect and glucagon’s stimulating effect on glycogenolysis with insulin overriding the effect of glucagon at high concentrations of both hormones.\textsuperscript{21} Recently, we extended the multiplicative model by incorporating the interaction between insulin and glucagon on glycogenolysis.\textsuperscript{13,22} The model extension was developed using preclinical data from dogs and was fitted to clinical human data in previous studies.\textsuperscript{23,24} In this article, we aim to validate the multiplicative glucose-insulin-glucagon model for simulation studies in humans using data from eight patients with type 1 diabetes.

**Methods**

**Data Collection**

Clinical data originated from a glucagon dose-finding study in eight well-controlled patients with type 1 diabetes (5 females, age range: 19-64 years, BMI range: 20.0-25.4 kg/m\textsuperscript{2}, HbA1c range: 6.1-7.4%), who were insulin pump-treated and had no endogenous production of insulin.\textsuperscript{25} Table S1 summarizes the patient characteristics. In brief, the patients completed four similar study days in random order. On each study day, patients arrived at the research facility in the morning in a fasting state. A subcutaneous (SC) insulin bolus (NovoRapid\textsuperscript{®}, Novo Nordisk A/S, Bagsværd, Denmark) was administered via the patient’s insulin pump, aiming to lower plasma glucose to 54 mg/dl or 240 minutes after the saline/glucagon bolus the experiment was stopped. Basal insulin infusion continued throughout the experiment. From t = –x to t = 0, plasma glucose was measured every 15-30 minutes, while plasma glucagon and serum insulin were measured every 60 minutes. Plasma glucose was measured every 5 minutes from t = 0 to t = 60, every 10 minutes from t = 60 to t = 120 and then every 15 minutes. Plasma glucagon and serum insulin were measured every 5 minutes from t = 0 to t = 15, every 15 minutes from t = 15 to t = 60, every 30 minutes from t = 60 to t = 120, and then every 60 minutes.

**Models**

When applying a pharmacokinetic (PK) model, we assume that all increases in insulin and glucagon concentrations are due to exogenously dosed drugs so that endogenous production is constant or negligible.

**Insulin Pharmacokinetic Model.** Previous studies showed that a simple two-state model with identical time constants for absorption and elimination could be used to describe the PK of insulin aspart after SC dosing.\textsuperscript{25}

\[
\frac{dX_i(t)}{dt} = u_i(t) \frac{X_i(t)}{t_{\text{max}}} - \frac{X_i(t)}{t_{\text{max}}}
\]

\[
\frac{dX_j(t)}{dt} = \frac{X_j(t)}{t_{\text{max}}} - \frac{X_j(t)}{t_{\text{max}}}
\]

\[
l(t) = \frac{1}{t_{\text{max}}} \frac{X_i(t)}{W \cdot C_{\text{f,j}}} 10^6 + I_i
\]

Table 1 lists the interpretations of the insulin PK model parameters and their units. The insulin concentration in serum is the sum of external rapid acting insulin dosage and basal infusion. The model assumes steady state insulin
concentration, $I_b$, maintained by the basal infusion when no exogenous rapid acting insulin is dosed.

**Glucagon Pharmacokinetic Model.** A two-state model with different absorption and elimination rate constants can describe glucagon PK after SC dosing.$^{23}$

$$\frac{dZ_1(t)}{dt} = u_C(t) - k_1 Z_1(t)$$

$$\frac{dZ_2(t)}{dt} = k_1 Z_1(t) - k_2 Z_2(t)$$

$$C(t) = \frac{k_2 Z_2(t)}{W C_{I,F,C}} + C_b$$

Table 1 lists the interpretations of the glucagon PK model parameters and their units. The glucagon concentration in plasma is the sum of constant endogenous glucagon, $C_b$, and external glucagon dosage. The model does not include an endogenous response to hypoglycemia.

**Glucose Pharmacodynamic Model.** The glucose PD model was originally derived by Hovorka et al.$^{29,30}$ and further extended by Wendt et al.$^{23}$

$$\frac{dQ(t)}{dt} = -F_{01} - F_R - S_3 x_3(t) Q_1(t) + k_{12} Q_2(t) + G_{GG}(t) + G_{GNG}$$

$$\frac{dQ(t)}{dt} = S_3 x_3(t) Q_1(t) - [k_{12} + S_3 x_2(t)] Q_2(t)$$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1(t)$</td>
<td>U</td>
<td>Insulin mass due to exogenous dosing, in SC tissue</td>
</tr>
<tr>
<td>$X_2(t)$</td>
<td>U</td>
<td>Insulin mass due to exogenous dosing, in serum</td>
</tr>
<tr>
<td>$u_I(t)$</td>
<td>U/minute</td>
<td>Insulin dose</td>
</tr>
<tr>
<td>$t_{	ext{max}}$</td>
<td>minutes</td>
<td>Time from dose to maximum serum concentration</td>
</tr>
<tr>
<td>$W$</td>
<td>kg</td>
<td>Body weight</td>
</tr>
<tr>
<td>$C_{I,F}$</td>
<td>ml/kg/minute</td>
<td>Apparent insulin clearance</td>
</tr>
<tr>
<td>$I_b$</td>
<td>mU/l</td>
<td>Steady state insulin concentration</td>
</tr>
<tr>
<td>$I(t)$</td>
<td>mU/l</td>
<td>Insulin concentration in serum</td>
</tr>
<tr>
<td>$Z_1(t)$</td>
<td>pg</td>
<td>Glucagon mass due to exogenous dosing, in SC tissue</td>
</tr>
<tr>
<td>$Z_2(t)$</td>
<td>pg</td>
<td>Glucagon mass due to exogenous dosing, in plasma</td>
</tr>
<tr>
<td>$u_C(t)$</td>
<td>pg/minute</td>
<td>Glucagon dose</td>
</tr>
<tr>
<td>$k_1$</td>
<td>minute$^{-1}$</td>
<td>Absorption rate constant</td>
</tr>
<tr>
<td>$k_2$</td>
<td>minute$^{-1}$</td>
<td>Elimination rate constant</td>
</tr>
<tr>
<td>$C_{I,C}$</td>
<td>ml/kg/minute</td>
<td>Apparent glucagon clearance</td>
</tr>
<tr>
<td>$C_b$</td>
<td>pg/ml</td>
<td>Steady state glucagon concentration</td>
</tr>
<tr>
<td>$C(t)$</td>
<td>pg/ml</td>
<td>Glucagon concentration in plasma</td>
</tr>
<tr>
<td>$Q_1(t)$</td>
<td>µmol/kg</td>
<td>Glucose mass per W in the accessible compartment</td>
</tr>
<tr>
<td>$Q_2(t)$</td>
<td>µmol/kg</td>
<td>Glucose mass per W in the nonaccessible compartment</td>
</tr>
<tr>
<td>$x_1(t)$</td>
<td>mU/l</td>
<td>Remote effects of insulin on glucose transport</td>
</tr>
<tr>
<td>$x_2(t)$</td>
<td>mU/l</td>
<td>Remote effects of insulin on glucose disposal</td>
</tr>
<tr>
<td>$x_3(t)$</td>
<td>mU/l</td>
<td>Remote effects of insulin on glycogenolysis</td>
</tr>
<tr>
<td>$G(t)$</td>
<td>mmol/l</td>
<td>Glucose concentration in plasma</td>
</tr>
<tr>
<td>$G_{GG}(t)$</td>
<td>µmol/kg/minute</td>
<td>Glucose production due to glycogenolysis</td>
</tr>
<tr>
<td>$G_{GNG}(t)$</td>
<td>µmol/kg/minute</td>
<td>Glucose production due to gluconeogenesis</td>
</tr>
<tr>
<td>$F_{01}$</td>
<td>µmol/kg/minute</td>
<td>Insulin independent glucose flux</td>
</tr>
<tr>
<td>$F_R$</td>
<td>µmol/kg/minute</td>
<td>Renal glucose clearance</td>
</tr>
<tr>
<td>$S_3$</td>
<td>minute$^{-1}$/(mU/l)</td>
<td>Insulin sensitivity of glucose transport</td>
</tr>
<tr>
<td>$S_1$</td>
<td>minute$^{-1}$/(mU/l)</td>
<td>Insulin sensitivity of glucose disposal</td>
</tr>
<tr>
<td>$S_2$</td>
<td>l/mU</td>
<td>Insulin sensitivity on glycogenolysis</td>
</tr>
<tr>
<td>$k_{12}$</td>
<td>minute$^{-1}$</td>
<td>Transfer rate constant from the nonaccessible to the accessible compartment</td>
</tr>
<tr>
<td>$k_{13}$</td>
<td>minute$^{-1}$</td>
<td>Insulin deactivation rate constant</td>
</tr>
<tr>
<td>$k_{23}$</td>
<td>minute$^{-1}$</td>
<td>Insulin deactivation rate constant</td>
</tr>
<tr>
<td>$k_{33}$</td>
<td>minute$^{-1}$</td>
<td>Insulin deactivation rate constant</td>
</tr>
<tr>
<td>$E_{max}$</td>
<td>µmol/kg/minute</td>
<td>Maximum EGP at basal insulin concentration</td>
</tr>
<tr>
<td>$C_{E50}$</td>
<td>pg/ml</td>
<td>Glucagon concentration yielding half of maximum EGP</td>
</tr>
<tr>
<td>$V$</td>
<td>ml/kg</td>
<td>Glucose volume of distribution</td>
</tr>
</tbody>
</table>
\[ G(t) = \frac{Q(t)}{C(t)} \]

\[ \frac{dx_1(t)}{dt} = k_{a1} \left[ I(t) - x_1(t) \right] \]

\[ \frac{dx_2(t)}{dt} = k_{a2} \left[ I(t) - x_2(t) \right] \]

\[ \frac{dx_3(t)}{dt} = k_{a3} \left[ I(t) - x_3(t) \right] \]

Table 1 lists the interpretations of the glucose PD model parameters and their units. The endogenous glucose production is the sum of glycogenolysis, \( G_{GOG} \), and gluconeogenesis, \( G_{NG} \). The gluconeogenesis is fixed at \( 6 \) µmol/kg/minute. \(^{10}\) The renal glucose clearance is zero when plasma glucose concentrations do not exceed \( 162 \) mg/dl. \(^{30}\) The glucose volume of distribution is fixed at \( 160 \) ml/kg. \(^{29}\)

Model Fitting

All model fitting was executed in R version 3.1.0 Spring Release using the additional packages CTSMS-R and numDeriv. \(^{31}\) Additional data handling was carried out using Microsoft Excel 2013. Unless stated otherwise, the results are reported as means with \( 95\% \) Wald confidence intervals (CIs) derived from the inverse Hessian, which provides the curvature of the log-likelihood function. \(^{32}\)

We fitted the insulin PK model using ordinary differential equations (ODEs) and estimated the log-normally distributed observation noise variance using maximum likelihood (ML). \(^{33}\) Due to missing insulin data around the expected time of maximum insulin concentration both \( t_{\text{max}} \) and \( C_{\text{max}} \) were estimated using maximum a posteriori (MAP) while \( I_b \) was estimated using ML. Prior distributions of \( t_{\text{max}} \) and \( C_{\text{max}} \) were reported in Haidar et al. \(^{34}\) and further information regarding \( t_{\text{max}} \) was extracted from the product monograph on insulin aspart. \(^{34}\) Table S2 lists the prior parameter distributions. No prior correlation between \( t_{\text{max}} \) and \( C_{\text{max}} \) was assumed.

Insulin PK parameters were optimized on a subject basis to datasets from all four visits (8 parameter sets reported). Despite SC infusion rates of short acting insulin (ie, the basal rates) were similar per subject for all study visits, the baseline insulin concentration varied as evident from the raw data plotted in Figures S1-S7. Therefore, the parameter describing the steady state insulin level was estimated separately for each visit. Using the subject specific optimized parameters, the insulin PK was simulated every minute and used as input to the PD model.

We fitted the glucagon PK model for visits B, C, and D using ODEs and estimated the log-normally distributed observation noise variance using ML. Plasma glucagon was sampled adequately to perform ML estimation of all parameters in the glucagon PK model. There was some uncertainty regarding the exact dosing time of the glucagon bolus, which was given after the blood sampling at time zero but before the next blood sampling five minutes after. Due to this uncertainty, we estimated the dosing time by choosing the discrete dosing time within the five-minute interval yielding the fit with the highest likelihood value and kept this updated dosing time throughout the data fitting and handling.

As the absolute elimination rate of glucagon is limited by the absorption rate, glucagon exerts flip-flop kinetics. \(^{35}\) To avoid the flip-flop phenomenon and to reduce the population variation in the two time constants, \( k_{a} \) was parameterized such that it was greater than \( k_{b} \) in all datasets.

The glucagon PK parameters were estimated to the datasets from visits with glucagon dosing (24 parameter sets, data not shown) and the PK simulated every minute to be used as input when fitting the PD model. On a subject basis, the glucagon PK parameters were optimized to datasets from all three glucagon visits (8 parameter sets reported). Due to the limited amount of data, we assumed the parameters did not differ between the visits.

The data following administration of saline (visits A) were not fitted to the glucagon PK model but described using linear interpolation between measurements. These interpolated data were used as inputs to the PD model.

The PD model was fitted using ODEs and the log-normally distributed observation noise variance estimated using ML. The remaining parameters \( \left( E_{\text{max}}, C_{E50}, k_{a1}, k_{a2}, k_{a3}, k_{b1}, k_{b2}, S_{b1}, S_{b2} \right) \) were estimated using MAP with priors inspired by literature. \(^{12,29}\) We used priors for the time constants rather than fixing the four parameters. \(^{36}\) The time constants and the insulin sensitivities were log-transformed during the parameter estimation. Table S2 lists the prior PD model parameter distributions. The PD model parameters have units yielding a glucose output measured in mmol/l, but the output is converted and graphically displayed with units of mg/dl. We assumed no prior correlation between parameters. As previously mentioned, glucose volume of distribution and gluconeogenesis were both fixed based on literature. \(^{16,20}\) \( I_b \) was fixed for each subject based on their average steady state insulin concentration. The final PD model parameters were obtained by optimizing the fit to all nonoutlier visits by each subject (8 parameter sets reported).

Pharmacodynamic Model Validation

To quantify the simulation accuracy of the model on datasets not used for parameter optimization, the bias was calculated...
by the mean prediction error (MPE) and the precision calculated by the mean absolute prediction error (MAPE). MPE and MAPE were calculated as percentages.36

\[
MPE = \frac{1}{N} \sum_{j=1}^{N} \left( \frac{\text{pred}_j - \text{obs}_j}{\text{obs}_j} \right) \times 100
\]

\[
MAPE = \frac{1}{N} \sum_{j=1}^{N} \left( \left| \frac{\text{pred}_j - \text{obs}_j}{\text{obs}_j} \right| \right) \times 100
\]

The variables \text{pred}_j and \text{obs}_j are the \(j\)th predicted and observed value, respectively, of a total of \(N\) observations. If the MPE is less than ±15% and the MAPE is less than 20%, we regard the model fit as accurate, precise and suitable for simulations. Cut-off limits were based on categorizing some fits as “good,” “medium,” and “bad” prior to knowledge of those fits’ MPE and MAPE values by two independent raters. The limits were chosen so that all fits categorized as “good” by both raters would be accepted and all fits categorized as “bad” by both raters would not meet the acceptance criteria. The PD model validation was carried out as a fourfold leave-one-out cross-validation leaving all data from one visit out per fold. As each subject participated in four visits, each subject had four training datasets comprised of data from three visits and four corresponding test datasets with data from one visit:

- **Training:** B-C-D, **Test:** A
- **Training:** A-C-D, **Test:** B
- **Training:** A-B-D, **Test:** C
- **Training:** A-B-C, **Test:** D

Thus, all four visits were used for testing once without being used for optimization during that fold. If the MAPE of a test fit exceeded 50%, the test visit was considered an outlier and removed from further analysis. After removal of the outlier dataset another round of leave-one-out was performed on the remaining three datasets. To validate the PD model in a subject, we required that at least one PD model test fit of a dataset from a glucagon visit (B, C or D) was accepted.

**Results**

Table 2 lists the estimated insulin PK model parameters. The fasting steady state insulin concentration had day-to-day variation within patients of up to 6 mU/l and ranged from 3.0 mU/l to 22.6 mU/l between subjects. The mean of all steady state insulin concentrations was 9.7 mU/l. The time to maximum concentration ranged from 40.8 to 68.5 minutes and the apparent clearance ranged from 4.7 to 9.1 minutes -1, yielding a calculated time to maximum concentration of 7.5-19.1 minutes. The apparent clearance ranged from 91 to 200 ml/kg/minute.

Table 4 provides an overview of the leave-one-out cross-validation procedure of the PD model. The MPE and MAPE for the test fits are listed together with a dichotomous decision of acceptance or not using the criteria outlined in the “Pharmacodynamic Model Validation” section. Based on the PD model during leave-one-out, we excluded four outlier datasets from further analysis and these four patients had a second round of leave-one-out including the remaining three datasets. Overall, the test fit was accepted two to three times out of three in three patients, and one to two times out of four in four patients. In patient 8 we did not accept any of the test fits even after removal of an outlier dataset. Figure 2 presents examples of PD model test fits and corresponding MPE and MAPE values of the test fits both passing and violating the acceptance criteria. In summary, the PD model successfully predicted unseen glucose data at least once in seven patients and therefore we regard the PD model as validated and suitable for simulation studies of these seven type 1 diabetes patients.

Table 5 lists the PD model parameters optimized to all nonoutlier visits in each patient with mean parameter values and 95% CI. The parameter describing the maximum EGP at steady state insulin concentration, \(E_{\text{max}}\), ranged from 56 to 84 \(\mu\text{mol/kg/minute}\). The glucagon concentration at which the effect is half maximum, \(C_{\text{g,50}}\), ranged from 141 to 436 pg/ml. Extrapolated to zero insulin and at basal glucagon concentration, the EGP ranged from 7 to 13.3 \(\mu\text{mol/kg/minute}\). According to the inverse of the parameter describing the insulin sensitivity to EGP, \(S_g\), the calculated insulin concentration at which the effect of glucagon shuts off ranged from 22 to 71 mU/l. Figures 3 and S1-S7 provide simulations of patient optimized PD model fits and data.

**Discussion**

We fitted simple PK models of serum insulin and plasma glucagon after SC bolus administrations of the hormones. The
simulated concentrations of insulin and glucagon were used as inputs to the PD model. We sought to validate the PD model for simulations in eight type 1 diabetes patients and succeeded in seven. Finally, we estimated the patient’s individual PD model parameters.

The fitted insulin PK model assumes that all changes in serum insulin concentration are due to SC insulin dosing. This is a valid assumption as no patients had measurable endogenous insulin secretion after glucagon stimulation. Patients’ insulin levels are at steady state when no insulin bolus is administered.

The clinical study focused on generating data describing the effect of glucagon on glucose, and therefore only few data points describing the insulin PK were obtained. The insulin PK data were sampled very sparsely around the expected time of maximum concentration. The missing data did not allow for ML estimation of the insulin PK model. However, using literature informed prior distributions of both $t_{max}$ and $Cl_{F,C}$ and optimizing for all four visits simultaneously we obtained reasonable fits by MAP estimation.

As the insulin PK model was fitted to in-hospital sedentary patients, its application in patients with type 1 diabetes outside the hospital setting may be limited due to numerous factors affecting insulin absorption rate, sensitivity and bioavailability. Such factors could be accounted for by introducing time-variant model parameters, which was beyond the scope of this work. Especially, differences in insulin absorption could explain the observed intrapatient variation in steady state insulin concentration despite equal basal rates at all four visits.

Patients with type 1 diabetes have a blunted glucagon response to hypoglycemia compared to healthy subjects. The fitted glucagon PK model assumes that all changes in plasma glucagon concentration are due to SC dosing and that the endogenous production is constant or negligible. To verify this assumption, we determined the size of the endogenous response during these days was negligible compared to the exogenous dosed glucagon. However, the endogenous response was not negligible during the saline day and therefore the glucagon PK model was not applicable to those datasets.

The glucagon PK fit was challenged by the short time to maximum concentration combined with the uncertainty of the exact dosing time of glucagon. This could potentially result in an error in time to maximum concentration of up to ±4 minutes. However, this possible deviation has minor impact on the PD model fit when the glucagon PK fit is used as an input. Despite the dosing time uncertainty, the calculated times to maximum concentration are within reasonable range of population averages reported in the literature. In the model by Haidar et al, the glucagon absorption rate and elimination rate were identical which we only observed in patient 4. In the remaining seven patients, the elimination rate was significantly higher than the absorption rate. Moreover, having different absorption and elimination rate constants we observed a higher clearance rate. Compared to Haidar et al, we found lower basal concentration of glucagon, which could be attributed to differences in the assays for analysis of plasma glucagon concentration.

Despite using informed priors for all PD model parameters, some optimized parameters are very different from the population mean and vary considerably more than originally listed in Hovorka et al. However, the original reference is based on a population of only six subjects, which makes it unlikely that all true population variations were captured, and we believe, therefore, that our parameter estimates are still valid. Similarly, with a population of eight subjects, we did not fit a population model but focused on estimating parameters for each subject individually.

The limited human data on EGP response to glucagon are consistent with data from dogs. As the human response to high glucagon concentrations has not been thoroughly investigated, the dog data provide best guesses of the human values. The maximum EGP due to glucagon and glucagon concentration at half-maximum effect at basal insulin average around 60 µmol/kg/minute and 300 pg/ml in dogs. Our results match the reference values and therefore seem plausible.

We found that EGP at zero insulin and basal glucagon is somewhat lower than previous publications, which state

<table>
<thead>
<tr>
<th>Patient</th>
<th>$C_0$ (pg/ml)</th>
<th>$k_1$ (min$^{-1}$)</th>
<th>$k_2$ (min$^{-1}$)</th>
<th>$Cl_{F,C}$ (ml/kg/min)</th>
<th>$t_{max}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.7 (9.4-12.0)</td>
<td>0.042 (0.03-0.06)</td>
<td>0.14 (0.10-0.22)</td>
<td>94 (83-105)</td>
<td>12.2</td>
</tr>
<tr>
<td>2</td>
<td>7.6 (6.9-8.3)</td>
<td>0.056 (0.05-0.06)</td>
<td>0.26 (0.18-0.38)</td>
<td>106 (96-116)</td>
<td>7.5</td>
</tr>
<tr>
<td>3</td>
<td>7.6 (5.9-9.3)</td>
<td>0.022 (0.01-0.03)</td>
<td>0.10 (0.06-0.17)</td>
<td>114 (96-132)</td>
<td>19.1</td>
</tr>
<tr>
<td>4</td>
<td>10.9 (9.2-12.6)</td>
<td>0.058 (0.01-0.31)</td>
<td>0.058 (NA)</td>
<td>159 (133-184)</td>
<td>17.3</td>
</tr>
<tr>
<td>5</td>
<td>8.7 (7.7-9.8)</td>
<td>0.038 (0.03-0.04)</td>
<td>0.19 (0.13-0.29)</td>
<td>200 (176-223)</td>
<td>10.7</td>
</tr>
<tr>
<td>6</td>
<td>8.9 (7.8-10.0)</td>
<td>0.035 (0.031-0.040)</td>
<td>0.28 (0.19-0.41)</td>
<td>125 (111-138)</td>
<td>8.6</td>
</tr>
<tr>
<td>7</td>
<td>11.6 (10.1-13.0)</td>
<td>0.035 (0.030-0.041)</td>
<td>0.25 (0.16-0.39)</td>
<td>136 (120-152)</td>
<td>9.2</td>
</tr>
<tr>
<td>8</td>
<td>19.0 (16.1-22.0)</td>
<td>0.052 (0.037-0.072)</td>
<td>0.090 (0.04-0.26)</td>
<td>91 (78-105)</td>
<td>14.5</td>
</tr>
</tbody>
</table>
10-20 \textmu mol/kg/minute\textsuperscript{29} and \textsim\textsim 30 \textmu mol/kg/minute\textsuperscript{22}. This might be due to the fixation of gluconeogenesis at 6 \textmu mol/kg/minute\textsuperscript{10}, which is increased in subjects with poorly controlled type 1 diabetes compared to the present well-controlled patients or healthy subjects\textsuperscript{25,41}. Assuming the proposed model of EGP is correct, the insulin concentration at which the glycogenolysis, hence the effect of glucagon, shuts off is reasonable compared to the limited publications showing glycogenolysis at various insulin concentrations\textsuperscript{22,42}. Rizza et al found that the glucose production was suppressed by insulin beyond approximately 60 mU/l\textsuperscript{17}. El Youssef et al found that at serum insulin concentrations beyond 40 mU/l glucagon concentrations below 450 pg/ml did not stimulate EGP\textsuperscript{13}. Further clinical studies are needed to investigate whether high insulin concentrations completely suppress the effect of glucagon or whether the maximum EGP is still attainable though at higher glucagon concentrations.

A major limitation to some of the previously published models describing the effect of glucagon on glucose production is lack of validation\textsuperscript{18,21}. We were able to mimic never-before-seen glucose data at least once and at most three times in seven of the eight subjects using the presented glucose PD model. We did not expect to accept the test fit of all nonoutlier datasets in each subject as the visits often described complimentary dynamics of the glucose-insulin-glucagon relationship; for instance the placebo day had very limited information on how different glucagon concentrations affects EGP as glucagon levels were

<table>
<thead>
<tr>
<th>Patient</th>
<th>Training visits</th>
<th>Test visits</th>
<th>MPE (%)</th>
<th>MAPE (%)</th>
<th>Accept? (Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BCD A</td>
<td>–25.0</td>
<td>25.0</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACD B</td>
<td>–11.3</td>
<td>13.7</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABD C</td>
<td>78.8</td>
<td>78.8</td>
<td>N*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABC D</td>
<td>3.3</td>
<td>25.5</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BD A</td>
<td>–10.3</td>
<td>11.1</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AD B</td>
<td>10.4</td>
<td>13.1</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AB D</td>
<td>4.0</td>
<td>21.3</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>BCD A</td>
<td>29.1</td>
<td>29.8</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACD B</td>
<td>–18.2</td>
<td>18.7</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABD C</td>
<td>–6.3</td>
<td>7.5</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABC D</td>
<td>6.3</td>
<td>10.0</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>BCD A</td>
<td>10.3</td>
<td>17.4</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACD B</td>
<td>–2.3</td>
<td>8.6</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABD C</td>
<td>23.4</td>
<td>24.6</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABC D</td>
<td>–20.1</td>
<td>20.1</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>BCD A</td>
<td>–17.3</td>
<td>18.9</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACD B</td>
<td>–9.4</td>
<td>11.1</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABD C</td>
<td>–23.6</td>
<td>23.7</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABC D</td>
<td>38.2</td>
<td>38.4</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>BCD A</td>
<td>–13.4</td>
<td>13.4</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACD B</td>
<td>–30.0</td>
<td>30.4</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABD C</td>
<td>–16.3</td>
<td>21.3</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABC D</td>
<td>74.6</td>
<td>74.6</td>
<td>N*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BC A</td>
<td>–1.7</td>
<td>4.5</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC B</td>
<td>–9.8</td>
<td>14.1</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AB C</td>
<td>–7.5</td>
<td>17.4</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>BCD A</td>
<td>–23.5</td>
<td>24.2</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACD B</td>
<td>–4.5</td>
<td>12.0</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABD C</td>
<td>59.0</td>
<td>59.0</td>
<td>N*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABC D</td>
<td>–8.6</td>
<td>16.3</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BD A</td>
<td>–13.7</td>
<td>16.9</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AD B</td>
<td>16.7</td>
<td>17.5</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AB D</td>
<td>4.7</td>
<td>15.8</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>BCD A</td>
<td>43.0</td>
<td>43.3</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACD B</td>
<td>–19.0</td>
<td>19.0</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABD C</td>
<td>–2.9</td>
<td>19.0</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABC D</td>
<td>6.0</td>
<td>8.0</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>BCD A</td>
<td>–8.0</td>
<td>12.4</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACD B</td>
<td>–32.9</td>
<td>33.0</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABD C</td>
<td>–14.5</td>
<td>24.2</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABC D</td>
<td>174.1</td>
<td>174.1</td>
<td>N*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BC A</td>
<td>–26.2</td>
<td>26.2</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC B</td>
<td>–24.6</td>
<td>24.6</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AB C</td>
<td>42.5</td>
<td>42.5</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Summary of PD Model Parameters for Simulation With Mean and 95% CI.

<table>
<thead>
<tr>
<th>ID</th>
<th>Data</th>
<th>$C_{50}$ (µmol/kg)</th>
<th>$E_{\text{max}}$ (µmol/kg/min)</th>
<th>$E_0$ (µmol/kg/min)</th>
<th>$k_{12} \times 10^{-4}$ (min$^{-1}$)</th>
<th>$k_{21} \times 10^{-4}$ (min$^{-1}$)</th>
<th>$k_{32} \times 10^{-4}$ (min$^{-1}$)</th>
<th>$S_{1} \times 10^{-4}$ (min$^{-1}$)</th>
<th>$S_{2} \times 10^{-4}$</th>
<th>$S_{3} \times 10^{-4}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ABD</td>
<td>436 (355-517)</td>
<td>56.4 (51.1-61.8)</td>
<td>14.2 (12.9-15.5)</td>
<td>244 (181-330)</td>
<td>16 (7-35)</td>
<td>5.5 (2.2-12.3)</td>
<td>215 (59-778)</td>
<td>1.5 (0.6-3.3)</td>
<td>155 (83-289)</td>
</tr>
<tr>
<td>2</td>
<td>ABCD</td>
<td>405 (339-471)</td>
<td>67.4 (59.3-75.5)</td>
<td>13.8 (12.8-14.7)</td>
<td>285 (223-363)</td>
<td>15 (7-35)</td>
<td>4.9 (2.3-10.3)</td>
<td>234 (137-389)</td>
<td>1.2 (0.6-2.3)</td>
<td>334 (232-481)</td>
</tr>
<tr>
<td>3</td>
<td>ABCD</td>
<td>401 (327-475)</td>
<td>57.4 (49.8-65.0)</td>
<td>15.5 (14.2-16.8)</td>
<td>397 (277-568)</td>
<td>18 (8-42)</td>
<td>5.8 (2.9-12.1)</td>
<td>227 (168-368)</td>
<td>1.4 (0.7-2.5)</td>
<td>211 (183-308)</td>
</tr>
<tr>
<td>4</td>
<td>ABCD</td>
<td>285 (226-344)</td>
<td>84.4 (73.9-94.8)</td>
<td>12.8 (11.3-14.4)</td>
<td>213 (157-289)</td>
<td>18 (9-36)</td>
<td>4.3 (1.8-10.4)</td>
<td>68 (42-113)</td>
<td>2.0 (1.0-3.8)</td>
<td>415 (347-516)</td>
</tr>
<tr>
<td>5</td>
<td>ABC</td>
<td>339 (251-427)</td>
<td>65.4 (53.8-77.1)</td>
<td>12.0 (10.6-13.5)</td>
<td>281 (194-406)</td>
<td>15 (7-32)</td>
<td>5.1 (2.3-12.0)</td>
<td>235 (95-596)</td>
<td>1.1 (0.4-2.6)</td>
<td>229 (127-451)</td>
</tr>
<tr>
<td>6</td>
<td>ABD</td>
<td>424 (333-515)</td>
<td>60.1 (46.3-74.0)</td>
<td>13.1 (11.7-14.5)</td>
<td>238 (172-330)</td>
<td>10 (4-22)</td>
<td>3.5 (1.4-12.2)</td>
<td>74 (23-232)</td>
<td>2.6 (1.4-6.2)</td>
<td>404 (185-882)</td>
</tr>
<tr>
<td>7</td>
<td>ABCD</td>
<td>141 (96-187)</td>
<td>78.0 (68.9-87.1)</td>
<td>14.2 (12.2-16.1)</td>
<td>358 (252-509)</td>
<td>49 (23-105)</td>
<td>6.2 (3.1-12.2)</td>
<td>178 (69-459)</td>
<td>4.4 (3.2-6.0)</td>
<td>140 (99-199)</td>
</tr>
<tr>
<td>8</td>
<td>ABC</td>
<td>307 (228-386)</td>
<td>75.3 (61.5-89.1)</td>
<td>13.4 (11.4-15.4)</td>
<td>289 (197-424)</td>
<td>37 (18-75)</td>
<td>5.1 (2.0-13.2)</td>
<td>154 (68-348)</td>
<td>4.2 (2.8-6.5)</td>
<td>463 (377-569)</td>
</tr>
</tbody>
</table>

Table 5. Summary of PD Model Parameters for Simulation With Mean and 95% CI.
changing very little. On the contrary, the placebo datasets were rich in information about the effects of insulin on plasma glucose. Some glucagon datasets had few observations of the effects of insulin on EGP as the plasma glucose some days reached the bolus threshold of 70 mg/dl quickly, for example, in subjects 2 and 7 shown in Figure S2 and Figure 3, respectively. As an example, this difference in data sampling can explain why it was not possible to validate the model using subject 2’s visit B as the test dataset. For this particular patient, the placebo visit was stopped early and therefore does not contain much information about the insulin dynamics. Moreover, the insulin only phase of visit B lasted nearly five hours and only two hours during visits C and D. Leaving visit B out of the training dataset does not provide the model with enough information to predict the insulin dynamics present in visit B. We noted that in most cases when the test fit was not accepted there was a monotone bias in the residuals yielding almost equal values of absolute MPE and MAPE (see Table 4). This bias indicates that the test fit would either over- or undershoot compared to data and thus both insulin and glucagon dynamics of the test dataset were not well described by the training datasets. Analyzing the PD model parameters during leave-one-out in Tables S3-S10, we observed that when a test fit could not be accepted, usually one or more parameters were outside the CI obtained when fitting to all nonoutlier data. Therefore, failing to accept the test fit during a fold is not necessarily a sign of an incorrect model structure. Rather it could emphasize that the test dataset contains unique information about the dynamics, which are not present in any of the training datasets.43 However, in four patients one dataset was so different from the other three datasets that it had to be excluded from the final PD model estimation as it would otherwise affect the parameters and yield bad fits for all four study days.

Simulation models are rarely validated on unseen data. The only glucose model including glucagon that is currently validated and FDA approved has undisclosed parameter values and can only be accessed by payment.19,44 We believe that this article is a step toward more openly sharing simulation models that will allow more research groups to test dual-hormone dosing strategies and control algorithms for managing diabetes before carrying out expensive simulations or clinical trials.

**Conclusion**

We have successfully validated a model describing the glucose-insulin-glucagon dynamics in seven type 1 diabetes subjects using leave-one-out cross-validation. We have reported model parameter sets with uncertainties for each subject, which could be used for in silico experiments. Simulations could also aid in optimizing treatment for type 1 diabetes patients such as glucagon dosing strategies for preventing hypoglycemia and tuning control strategies for an AP.
Abbreviations
AP, artificial pancreas; BMI, body mass index; BW, body weight; CI, confidence interval; EGP, endogenous glucose production; FDA, Food and Drug Administration; HbA1c, glycated hemoglobin A1c; MAP, maximum a posteriori; MAPE, mean absolute prediction error; ML, maximum likelihood; MPE, mean prediction error; ODE, ordinary differential equation; PD, pharmacokinetics; PK, pharmacokinetics; SC, subcutaneous; SD, standard deviation.

Declaration of Conflicting Interests
The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: SLW is a full-time employee of Zealand Pharma. SS serves on the continuous glucose monitoring advisory board of Roche Diabetes Care and as a consultant to Unomedical. CBK is a full-time employee of Zealand Pharma and owns shares in Zealand Pharma. JIH has consulted for Merck Sharp & Dome, Novo Nordisk, and Roche. SM has served as a consultant or adviser to Amgen, AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Eli Lilly, Intarcia Therapeutics, Johnson & Johnson, Merck Sharp & Dohme, Novo Nordisk, Novartis Pharma, and Sanofi, has received a research grant from Novo Nordisk, and has received fees for speaking from Astra-Zeneca, Bristol-Myers Squibb, Eli Lilly, Merck, Sharp & Dohme, Novo Nordisk, Novartis Pharma, and Sanofi. KN serves as an adviser to Medtronic, Abbott, and Novo Nordisk, owns shares in Novo Nordisk, has received research grants from Novo Nordisk, and has received fees for speaking from Medtronic, Roche, Rubin Medical, Sanofi, Novo Nordisk, Bayer, and Zealand Pharma. JBJ has served as a consultant for Novo Nordisk.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by Zealand Pharma A/S and the Innovation Fund Denmark. Further, the study was supported by the Danish Diabetes Academy funded by the Novo Nordisk Foundation.

Supplemental Material
The supplemental materials are available at http://journals.sagepub.com/doi/suppl/10.1177/1932296817693254

References


This appendix presents the technical report with the title "Simulating Clinical Studies of the Glucoregulatory System: in Vivo Meets in Silico" published by the Technical University of Denmark in February 2017 [21].
Simulating clinical studies of the glucoregulatory system: *in vivo* meets *in silico*

Sabrina Lyngbye Wendt*1,2*, Ajenthen Ranjan3,4, Jan Kloppenborg Møller2, Carsten Boye Knudsen1, Jens Juul Holst5, Sten Madsbad3,5, Henrik Madsen2, Kirsten Nørgaard1, and John Bagterp Jørgensen2

1Department of Bioanalysis and Pharmacokinetics, Zealand Pharma, Glostrup, Denmark
2Department of Applied Mathematics and Computer Science, Technical University of Denmark, Kongens Lyngby, Denmark
3Department of Endocrinology, Hvidovre University Hospital, Hvidovre, Denmark
4Danish Diabetes Academy, Odense, Denmark
5Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Version 1
January, 2017


* Email: slw@zealandpharma.com or slwe@dtu.dk

**Abstract:** In this report we use a validated model of the glucoregulatory system including effects of insulin and glucagon for simulation studies in seven type 1 diabetes patients. Using simulations, we replicate the results from a clinical study investigating the effect of micro-doses of glucagon on glucose metabolism at varying ambient insulin levels. The report compares *in vivo* and *in silico* results head-to-head, and discusses similarities and differences. We design and simulate simple studies to emphasize the implications of some glucoregulatory dynamics which are ignored in most previous clinical studies: the effect of discontinuing insulin and glucose infusions prior to glucagon administration, the delayed effect of insulin, timing of data sampling, and carry-over effects from multiple subcutaneous doses of glucagon. We also use simulations to discuss two hypotheses of how insulin and glucagon might interact in influencing the glucose response. Following the simulations we propose a study design that potentially could explore if the hypotheses are true or false.

**Keywords:** Glucagon, Glucoregulatory system, Glucose, Insulin, Simulation
Preface

This technical report aims to discuss how to conduct clinical studies seeking to elucidate the dynamics in the glucoregulatory system with focus on glucagon. The discussion is based on published clinical data and simulation experiments using a newly validated glucose-insulin-glucagon model [1]. Simulation models describing insulin and glucagon pharmacokinetics and glucose pharmacodynamics are presented in the first section along with subject specific model parameters and their interpretations.

Second section presents an in silico replication of the highly cited study by El Youssef et al. from 2014 with the title "Quantification of the Glycemic Response to Microdoses of Subcutaneous Glucagon at Varying Insulin Levels" [2]. All results and graphs of the original paper are replicated using simulations. We present a comparison between the in silico and the in vivo results.

Third section describes a simulation study exploiting the ability of computer simulations to conduct infinite number of trials thereby creating smooth dose-response curves for glucagon at varying insulin levels with glucagon doses ranging from 1 µg to 10 mg. This section also discusses two possible hypotheses describing the interaction between insulin and glucagon, and suggests a study design that could evaluate the hypotheses. Based on simulation studies and published clinical studies, fourth section discusses pearls and pitfalls for conducting clinical studies of the glucoregulatory system with focus on trials including glucagon. This last section contains a thorough discussion of the importance of clamp study designs and limitations to identify dynamics in data.
Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>AUC$_{60}$</td>
<td>AUC over 60 minutes</td>
</tr>
<tr>
<td>EGP</td>
<td>endogenous glucose production</td>
</tr>
<tr>
<td>HbA$_{1c}$</td>
<td>glycated hemoglobin A1c</td>
</tr>
<tr>
<td>IIR</td>
<td>insulin infusion rate</td>
</tr>
<tr>
<td>IQR</td>
<td>inter quartile range</td>
</tr>
<tr>
<td>MPC</td>
<td>model predictive control</td>
</tr>
<tr>
<td>PD</td>
<td>pharmacodynamic</td>
</tr>
<tr>
<td>PID</td>
<td>proportional integral derivative</td>
</tr>
<tr>
<td>PK</td>
<td>pharmacokinetic</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SS</td>
<td>steady state</td>
</tr>
<tr>
<td>SC</td>
<td>subcutaneous</td>
</tr>
<tr>
<td>Tmax</td>
<td>time to maximum concentration</td>
</tr>
<tr>
<td>T1D</td>
<td>type 1 diabetes</td>
</tr>
</tbody>
</table>
Contents

Preface 2

Abbreviations 3

1 Simulation Model 5
   1.1 Insulin Pharmacokinetics Model ................. 5
   1.2 Glucagon Pharmacokinetics Model ............... 6
   1.3 Glucose Pharmacodynamics Model ............... 6
   1.4 Model Parameters .......................... 7

2 The Study by El Youssef et al. 10
   2.1 Study Design ................................ 10
   2.2 Simulation Study Details ...................... 10
      2.2.1 Determining Infusion Rates ............... 10
      2.2.2 Proportional Integral Derivative Controller 11
      2.2.3 Calculation of Endogenous Glucose Production 11
   2.3 Results and Discussion ....................... 12
      2.3.1 Insulin and Glucose Infusion Rates .......... 12
      2.3.2 Insulin and Glucose Concentrations .......... 14
      2.3.3 Glucagon ................................ 15
      2.3.4 Endogenous Glucose Production .......... 17
      2.3.5 Examples of Simulated Raw Data .......... 21

3 Dose-Response Studies 23
   3.1 Study Design ................................ 23
   3.2 Results and Discussion ....................... 24
   3.3 Dose Selection for an in Vivo Study ........ 25

4 Pearls & Pitfalls 27
   4.1 Glucose Clamps ................................ 27
      4.1.1 Glucose Level and Glucose Infusion .......... 27
      4.1.2 Insulin Level and Insulin Infusion .......... 27
   4.2 Dynamics in Data ............................ 29
      4.2.1 Identifying Steady State .................... 29
      4.2.2 Repeated Glucagon Boluses ................. 31
      4.2.3 The Glucagon Evanescent Effect ........... 31
   4.3 Summary .................................... 33

References 34
1 Simulation Model

The simulation models in this section including equations and parameter values were published by Wendt et al. [1]. For details on the model validation and parameter estimation the reader is kindly referred to the original publication. This section serves as a summary of the model providing the information necessary to use the model for simulations.

1.1 Insulin Pharmacokinetics Model

The insulin pharmacokinetics (PK) model is adopted from Haidar et al. [3] and described by equations (1)-(3).

\[
\frac{dX_1(t)}{dt} = u_I(t) - \frac{X_1(t)}{t_{\text{max}}} 
\]

(1)

\[
\frac{dX_2(t)}{dt} = \frac{X_1(t)}{t_{\text{max}}} - \frac{X_2(t)}{t_{\text{max}}} 
\]

(2)

\[
I(t) = \frac{1}{t_{\text{max}}} X_2(t) W \cdot Cl_{F,I} 10^6 + I_b 
\]

(3)

The steady state conditions of the system are both states, \(X_1\) and \(X_2\), equal to zero. The interpretations of the insulin PK model variables are listed in Table 1. Individual model parameter values are presented in Section 1.4.

<table>
<thead>
<tr>
<th>Class</th>
<th>Variable</th>
<th>Unit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>States</td>
<td>(X_1(t))</td>
<td>U</td>
<td>insulin mass due to SC dosing, in SC tissue</td>
</tr>
<tr>
<td></td>
<td>(X_2(t))</td>
<td>U</td>
<td>insulin mass due to SC dosing, in serum</td>
</tr>
<tr>
<td>Input</td>
<td>(u_I(t))</td>
<td>U/min</td>
<td>insulin dose</td>
</tr>
<tr>
<td>Output</td>
<td>(I(t))</td>
<td>mU/L</td>
<td>insulin concentration in serum</td>
</tr>
<tr>
<td>Parameters</td>
<td>(t_{\text{max}})</td>
<td>min</td>
<td>time to maximum serum concentration</td>
</tr>
<tr>
<td></td>
<td>(W)</td>
<td>kg</td>
<td>body weight</td>
</tr>
<tr>
<td></td>
<td>(Cl_{F,I})</td>
<td>mL/kg/min</td>
<td>apparent insulin clearance</td>
</tr>
</tbody>
</table>

Table 1: Interpretation of insulin PK model variables
1.2 Glucagon Pharmacokinetics Model

The glucagon PK model is adopted from Wendt et al. [4] and described by equations (4)-(6).

\[
\frac{dZ_1(t)}{dt} = u_C(t) - k_1 Z_1(t) \quad (4)
\]

\[
\frac{dZ_2(t)}{dt} = k_1 Z_1(t) - k_2 Z_2(t) \quad (5)
\]

\[
C(t) = \frac{k_2 Z_2(t)}{W \cdot Cl_{F,C}} + C_b \quad (6)
\]

The steady state conditions of the system are both states, \(Z_1\) and \(Z_2\), equal to zero. Table 2 lists the interpretations of glucagon PK model variables. Individual model parameter values are presented in Section 1.4.

Table 2: Interpretation of glucagon PK model states, input, output and parameters.

<table>
<thead>
<tr>
<th>Class</th>
<th>Variable</th>
<th>Unit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>States</td>
<td>(Z_1(t))</td>
<td>pg</td>
<td>glucagon mass due to SC dosing, in SC tissue</td>
</tr>
<tr>
<td></td>
<td>(Z_2(t))</td>
<td>pg</td>
<td>glucagon mass due to SC dosing, in plasma</td>
</tr>
<tr>
<td>Input</td>
<td>(u_C(t))</td>
<td>pg/min</td>
<td>glucagon dose</td>
</tr>
<tr>
<td>Output</td>
<td>(C(t))</td>
<td>pg/mL</td>
<td>glucagon concentration in plasma</td>
</tr>
<tr>
<td>Parameters</td>
<td>(C_b)</td>
<td>pg/mL</td>
<td>steady state glucagon concentration</td>
</tr>
<tr>
<td></td>
<td>(k_1)</td>
<td>min(^{-1})</td>
<td>absorption rate constant</td>
</tr>
<tr>
<td></td>
<td>(k_2)</td>
<td>min(^{-1})</td>
<td>elimination rate constant</td>
</tr>
<tr>
<td></td>
<td>(W)</td>
<td>kg</td>
<td>body weight</td>
</tr>
<tr>
<td></td>
<td>(Cl_{F,C})</td>
<td>mL/kg/min</td>
<td>apparent glucagon clearance</td>
</tr>
</tbody>
</table>

1.3 Glucose Pharmacodynamics Model

The glucose pharmacodynamics (PD) model was first developed using preclinical data from healthy dogs [4] and then tested with data from healthy humans [5]. Finally, the PD model was validated for simulations in seven type 1 diabetes patients [1]. The model structure is described by equations (7)-(13).
1.4 Model Parameters

The majority of PK and PD model parameters are subject specific and listed in Table 4. A few parameters are fixed for all subjects including the rate of gluconeogenesis, $G_{GNG}$, at 6 $\mu$mol/kg/min [6], and the glucose volume of distribution, $V$, at 160 mL/kg [7]. The renal clearance of glucose is zero unless the plasma glucose concentration exceeds 9 mmol/L in which case it is calculated as $0.003 \cdot (G - 9) \cdot V$ [8]. Similarly, the insulin independent glucose flux is calculated as $F_{01} \cdot G/4.5$ when the plasma glucose concentration falls below 4.5 mmol/L [8].

The glucose PD model was validated using leave-one-out cross-validation in seven out of eight type 1 diabetes patients. The model parameters of patient 8 are reported although the model could not be validated in this subject. Therefore, simulations in the following chapters are carried out using only subjects 1-7.

In equation (9), $1 - S_{E_3}(t)$ is always greater than or equal to zero. Interpretations of PD model states, inputs, outputs and parameters are listed in Table 3. Subject specific model parameters are presented in Section 1.4. The steady state conditions of the model are listed in equations (14)-(18).

\[
\begin{align*}
\frac{dQ_1(t)}{dt} &= -F_{01} - F_R - S_{t,x_1}(t)Q_1(t) + k_{12}Q_2(t) + G_{GG}(t) + G_{GNG} \quad (7) \\
\frac{dQ_2(t)}{dt} &= S_{t,x_1}(t)Q_1(t) - [k_{12} + S_{d,x_2}(t)]Q_2(t) \quad (8) \\
G_{GG}(t) &= \frac{1 - S_{E_3}(t)}{1 - S_{E_1}} \cdot \left( \frac{E_{max} - G_{GNG}}{C(t)} + C(t) \right) \quad (9) \\
G(t) &= \frac{Q_1(t)}{V} \quad (10) \\
\frac{dx_1(t)}{dt} &= k_{a1}[I(t) - x_1(t)] \quad (11) \\
\frac{dx_2(t)}{dt} &= k_{a2}[I(t) - x_2(t)] \quad (12) \\
\frac{dx_3(t)}{dt} &= k_{a3}[I(t) - x_3(t)] \quad (13)
\end{align*}
\]

\[
\begin{align*}
Q_{1,SS} &= G_{SS} \cdot V \quad (14) \\
Q_{2,SS} &= Q_{1,SS} \frac{x_{1,SS}}{x_{2,SS} + k_{12}} \quad (15) \\
x_{1,SS} &= I_b \quad (16) \\
x_{2,SS} &= I_b \quad (17) \\
x_{3,SS} &= I_b \quad (18)
\end{align*}
\]
Table 3: Interpretation of glucose PD model states, input, output and parameters.

<table>
<thead>
<tr>
<th>Class</th>
<th>Variable</th>
<th>Unit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>States</td>
<td>$Q_1(t)$</td>
<td>$\mu\text{mol/kg}$</td>
<td>glucose mass per W in the accessible compartment</td>
</tr>
<tr>
<td></td>
<td>$Q_2(t)$</td>
<td>$\mu\text{mol/kg}$</td>
<td>glucose mass per W in the non-accessible compartment</td>
</tr>
<tr>
<td></td>
<td>$x_1(t)$</td>
<td>mU/L</td>
<td>remote effects of insulin on glucose transport</td>
</tr>
<tr>
<td></td>
<td>$x_2(t)$</td>
<td>mU/L</td>
<td>remote effects of insulin on glucose disposal</td>
</tr>
<tr>
<td></td>
<td>$x_3(t)$</td>
<td>mU/L</td>
<td>remote effects of insulin on glycogenolysis</td>
</tr>
<tr>
<td>Inputs</td>
<td>$I(t)$</td>
<td>mU/L</td>
<td>insulin concentration in serum</td>
</tr>
<tr>
<td></td>
<td>$C(t)$</td>
<td>pg/mL</td>
<td>glucagon concentration in plasma</td>
</tr>
<tr>
<td>Outputs</td>
<td>$G(t)$</td>
<td>nmol/L</td>
<td>glucose concentration in plasma</td>
</tr>
<tr>
<td></td>
<td>$G_{GG}(t)$</td>
<td>$\mu\text{mol/kg/min}$</td>
<td>glucose production due to glycogenolysis</td>
</tr>
<tr>
<td>Parameters</td>
<td>$G_{GNG}$</td>
<td>$\mu\text{mol/kg/min}$</td>
<td>glucose production due to gluconeogenesis</td>
</tr>
<tr>
<td></td>
<td>$C_{E}$</td>
<td>pg/mL</td>
<td>glucagon concentration yielding half of maximum EGP</td>
</tr>
<tr>
<td></td>
<td>$E_{\text{max}}$</td>
<td>$\mu\text{mol/kg/min}$</td>
<td>maximum EGP at basal insulin concentration</td>
</tr>
<tr>
<td></td>
<td>$F_{01}$</td>
<td>$\mu\text{mol/kg/min}$</td>
<td>insulin independent glucose flux</td>
</tr>
<tr>
<td></td>
<td>$F_R$</td>
<td>$\mu\text{mol/kg/min}$</td>
<td>renal glucose clearance</td>
</tr>
<tr>
<td></td>
<td>$k_{12}$</td>
<td>$10^{-4}$</td>
<td>transfer rate constant from $Q_2$ to $Q_1$</td>
</tr>
<tr>
<td></td>
<td>$k_{a1}$</td>
<td>$10^{-4}$</td>
<td>insulin deactivation rate constant</td>
</tr>
<tr>
<td></td>
<td>$k_{a2}$</td>
<td>$10^{-4}$</td>
<td>insulin deactivation rate constant</td>
</tr>
<tr>
<td></td>
<td>$k_{a3}$</td>
<td>$10^{-4}$</td>
<td>insulin deactivation rate constant</td>
</tr>
<tr>
<td></td>
<td>$S_D$</td>
<td>$10^{-4}$</td>
<td>insulin sensitivity of glucose disposal</td>
</tr>
<tr>
<td></td>
<td>$S_E$</td>
<td>$10^{-4}$</td>
<td>insulin sensitivity of glycogenolysis</td>
</tr>
<tr>
<td></td>
<td>$S_T$</td>
<td>$10^{-4}$</td>
<td>insulin sensitivity of glucose transport</td>
</tr>
<tr>
<td></td>
<td>$V$</td>
<td>mL/kg</td>
<td>glucose volume of distribution</td>
</tr>
</tbody>
</table>
Table 4: Subject specific PK/PD model parameters used for simulations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>$W$ [kg]</td>
<td>54</td>
<td>50</td>
<td>81</td>
<td>69</td>
<td>87</td>
<td>72</td>
<td>73</td>
<td>59</td>
</tr>
<tr>
<td>$I_b$ [mU/L]</td>
<td>7.3</td>
<td>10.6</td>
<td>11.7</td>
<td>8.7</td>
<td>6.6</td>
<td>5.5</td>
<td>19.7</td>
<td>7.4</td>
</tr>
<tr>
<td>$t_{max}$ [min]</td>
<td>57.6</td>
<td>57.3</td>
<td>40.8</td>
<td>67.9</td>
<td>48.5</td>
<td>46.5</td>
<td>68.5</td>
<td>55.4</td>
</tr>
<tr>
<td>$CI_{F,I}$ [mL/kg/min]</td>
<td>18.9</td>
<td>18.5</td>
<td>14.8</td>
<td>17.4</td>
<td>17.3</td>
<td>24.6</td>
<td>23.7</td>
<td>26.8</td>
</tr>
<tr>
<td>$C_b$ [pg/mL]</td>
<td>10.7</td>
<td>7.6</td>
<td>7.6</td>
<td>10.9</td>
<td>8.7</td>
<td>8.9</td>
<td>11.6</td>
<td>19.0</td>
</tr>
<tr>
<td>$k_1$ [min$^{-1}$]</td>
<td>0.042</td>
<td>0.056</td>
<td>0.022</td>
<td>0.058</td>
<td>0.038</td>
<td>0.035</td>
<td>0.035</td>
<td>0.052</td>
</tr>
<tr>
<td>$k_2$ [min$^{-1}$]</td>
<td>0.14</td>
<td>0.26</td>
<td>0.10</td>
<td>0.058</td>
<td>0.19</td>
<td>0.28</td>
<td>0.25</td>
<td>0.090</td>
</tr>
<tr>
<td>$CI_{F,C}$ [mL/kg/min]</td>
<td>94</td>
<td>106</td>
<td>114</td>
<td>159</td>
<td>200</td>
<td>125</td>
<td>136</td>
<td>91</td>
</tr>
<tr>
<td>$C_{E0}$ [pg/mL]</td>
<td>436</td>
<td>405</td>
<td>401</td>
<td>285</td>
<td>339</td>
<td>424</td>
<td>141</td>
<td>307</td>
</tr>
<tr>
<td>$E_{max}$ [µmol/kg/min]</td>
<td>56.4</td>
<td>67.4</td>
<td>57.4</td>
<td>84.4</td>
<td>65.4</td>
<td>60.1</td>
<td>78.0</td>
<td>75.3</td>
</tr>
<tr>
<td>$F_{01}$ [µmol/kg/min]</td>
<td>14.2</td>
<td>13.8</td>
<td>15.5</td>
<td>12.8</td>
<td>12.0</td>
<td>13.1</td>
<td>14.2</td>
<td>13.4</td>
</tr>
<tr>
<td>$k_{12}$ $\cdot 10^{-4}$ [min$^{-1}$]</td>
<td>244</td>
<td>285</td>
<td>397</td>
<td>213</td>
<td>281</td>
<td>238</td>
<td>358</td>
<td>289</td>
</tr>
<tr>
<td>$k_{a1}$ $\cdot 10^{-4}$ [min$^{-1}$]</td>
<td>16</td>
<td>15</td>
<td>18</td>
<td>18</td>
<td>15</td>
<td>10</td>
<td>49</td>
<td>37</td>
</tr>
<tr>
<td>$k_{a2}$ $\cdot 10^{-4}$ [min$^{-1}$]</td>
<td>522</td>
<td>495</td>
<td>548</td>
<td>437</td>
<td>517</td>
<td>353</td>
<td>624</td>
<td>518</td>
</tr>
<tr>
<td>$k_{a3}$ $\cdot 10^{-4}$ [min$^{-1}$]</td>
<td>215</td>
<td>231</td>
<td>327</td>
<td>68</td>
<td>235</td>
<td>74</td>
<td>178</td>
<td>182</td>
</tr>
<tr>
<td>$S_D$ $\cdot 10^{-4}$ [min$^{-1}$/mU/L]</td>
<td>1.5</td>
<td>1.2</td>
<td>1.4</td>
<td>2.0</td>
<td>1.1</td>
<td>2.6</td>
<td>4.4</td>
<td>4.2</td>
</tr>
<tr>
<td>$S_E$ $\cdot 10^{-4}$ [mU/L$^{-1}$]</td>
<td>155</td>
<td>334</td>
<td>237</td>
<td>415</td>
<td>229</td>
<td>404</td>
<td>140</td>
<td>463</td>
</tr>
<tr>
<td>$S_T$ $\cdot 10^{-4}$ [min$^{-1}$/mU/L]</td>
<td>23</td>
<td>19</td>
<td>25</td>
<td>18</td>
<td>31</td>
<td>21</td>
<td>21</td>
<td>29</td>
</tr>
</tbody>
</table>
2 The Study by El Youssef et al.

In this section, using simulations we aim to replicate the clinical study by El Youssef et al. published in Diabetes Care November 2014 with the title "Quantification of the Glycemic Response to Microdoses of Subcutaneous Glucagon at Varying Insulin Levels" [2].

2.1 Study Design

The study by El Youssef et al. [2] included 11 type 1 diabetes (T1D) patients (5 females, age IQR: 36.5-46.0 years, BMI IQR: 23.0-31.1 kg/m², HbA₁c IQR: 7.0-8.2%). The patients participated in three study days of each 10 hours duration with constant intravenous insulin infusion rate (IIR) of either low, medium or high. Average results during low, medium and high IIR are based on 10, 9, and 10 subjects, respectively. Glucose infusion rates were controlled using a proportional integral derivative (PID) controller aiming at a blood glucose concentration of 85 ± 20 mg/dL. When blood glucose read below 60 mg/dL the controller regulated the glucose infusion rate every five minutes, otherwise every ten minutes. After an initial two hours run-in period the subjects received the first glucagon bolus. They received the second glucagon bolus after another two hours until a total of four glucagon boluses were delivered and observed for the following two hours. The glucagon boluses were delivered in a pseudo-random order by varying the initial dose, but keeping the order: 25 µg, 75 µg, 125 µg, and 175 µg (25-75-125-175, 75-125-175-25, 125-175-25-75, 175-25-75-125). Each subject received the same pseudo-random order of glucagon boluses during each study day. The study used regular human insulin (Humulin R, Eli Lilly and Company) and glucagon (GlucaGen, Novo Nordisk).

2.2 Simulation Study Details

In the in silico study we used the validated patient specific PK/PD models describing seven T1D patients (4 females, age range: 19-64 years, BMI range: 20.0-25.4 kg/m², HbA₁c range: 6.1-7.4 %) presented in Section 1 [1]. All virtual subjects participated in experiments with low, medium and high IIRs. We followed the study design of the clinical study described in Section 2.1. We allowed initialization of patients at steady state (SS) at the beginning of the two hours run-in period by solving the patient specific equations for SS.

The insulin and glucagon PK/PD model parameters were based on a study using insulin aspart (NovoRapid, Novo Nordisk) and glucagon (GlucaGen, Novo Nordisk). As the IIRs are constant during the experiment the possible differences in insulin PK between Humulin R and NovoRapid are not confounding the study. Differences in insulin PD effects are relevant, however, we assume that the insulins have identical PD effects.

2.2.1 Determining Infusion Rates

The low and medium IIR were chosen based on individual basal infusion rates and the high IIR was fixed at 0.05 U/kg/h for all subjects. In the clinical study they used either
0.01 U/kg/h or the patient’s average daytime basal rate as the lowest IIR, but the latter information was not available in the simulation study. Therefore, the individual low IIR was maximized to either 0.01 U/kg/h or the infusion rate yielding an insulin level equal to one and a half times the fasting serum insulin concentration. The medium IIR was chosen halfway between the low and high IIR.

The SS glucose infusion rate was calculated by solving individual glucoregulatory models at SS given the pre-specified insulin infusion rate.

2.2.2 Proportional Integral Derivative Controller

We implemented a simple proportional integral derivative (PID) controller with clipping to control the blood glucose concentration by adjusting the glucose infusion rate, as listed in equations (19)-(22).

\[
\begin{align*}
    e_k &= G_{SS} - G_k \\
    I_k &= I_{k-1} + k_i \cdot \Delta t \cdot e_k \\
    d_e_k &= \frac{e_k - e_{k-1}}{\Delta t} \\
    U_k &= \max(0, U_{SS} + k_p \cdot e_k + I_k + k_d \cdot d_e_k)
\end{align*}
\]

\(G_{SS}\) is the glucose concentration at SS (set point of 85 mg/dL), \(G_k\) is the \(k^{th}\) glucose observation, and \(e_k\) is the deviation from set point of the \(k^{th}\) observation. \(I_k\) is the discretization of the integral of errors until \(k\), calculated as the sum of the previous integral of errors, \(I_{k-1}\), and the current integral of error weighted by \(k_i\). \(d_e_k\) is the discretization of the error derivative at \(k\) calculated by the backward difference. The updated glucose infusion rate, \(U_k\), is the sum of the SS glucose infusion rate, \(U_{SS}\), the error weighted by \(k_p\), the integral of errors, and the error derivative weighted by \(k_d\), unless the sum is negative, in which case the glucose infusion rate is set to zero. We used \(k_p = 4\), \(k_i = 1\), and \(k_d = -2\).

2.2.3 Calculation of Endogenous Glucose Production

The endogenous glucose production (EGP) due to glucagon was directly calculated using the PD model. EGP was baseline-corrected by subtracting the EGP level at the time of the most recent glucagon dose to avoid carry-over effects from previous glucagon doses or from baseline production maintained by the constant insulin infusion and SS glucagon concentration.

\[
    EGP_{Corrected}(t) = EGP(t) - EGP(t_{Dose,n}) \quad n = 1, \ldots, 4
\]

The baseline-corrected EGP can thus become negative when current EGP is less than at the time of the most recent glucagon dose. However, it can not be more negative than the difference between EGP at SS and EGP at the time of the most recent glucagon dose.
2.3 Results and Discussion

2.3.1 Insulin and Glucose Infusion Rates

![Box plot of glucose infusion rate (mg/kg/min) across all studies, by insulin infusion group.](image)

Figure 1: Box plot of glucose infusion rate (mg/kg/min) across all studies, by insulin infusion group.

Table 5 compares the reported in vivo and simulated in silico average insulin and glucose infusion rates at low, medium and high IIRs. At a first glance, insulin infusion rates are very similar in the two studies based on averages and medians. However, the virtual study contained one patient (no. 7) having a very high basal IIR yielding a low IIR of 0.042 U/kg/h and a medium IIR of 0.046 U/kg/h. Ultimately, there was little differences between the three IIRs in this subject and therefore only minor differences in responses during the various insulin infusion rates. The high basal IIR indicates that the subject is not very sensitive to insulin and therefore the response to glucagon during the high IIR was little attenuated by the insulin level. No formal test was performed to exclude this subject. However, the low IIR and medium IIR are more than two standard deviations from the mean infusion rates reported in vivo which justifies the exclusion of the subject from the analysis of EGP response to glucagon at various insulin levels.

Table 5: Summary of insulin and glucose infusion rates in vivo and in silico. Infusion rates are reported as mean ± SD and median [IQR]. L = low, M = medium, H = high.

<table>
<thead>
<tr>
<th>IIR</th>
<th>In Vivo</th>
<th>In Silico</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>0.016 ± 0.006</td>
<td>0.018 ± 0.011</td>
</tr>
<tr>
<td>M</td>
<td>0.032 ± 0.003</td>
<td>0.034 ± 0.006</td>
</tr>
<tr>
<td>H</td>
<td>0.05 ± 0.00</td>
<td>0.05 ± 0.00</td>
</tr>
<tr>
<td>U/kg/h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>0.7 ± 0.5</td>
<td>0.5 ± 0.8</td>
</tr>
<tr>
<td>M</td>
<td>2.9 ± 1.3</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>H</td>
<td>4.5 ± 2</td>
<td>3.8 ± 1.2</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>0.6 [0.2-1]</td>
<td>0.5 [0.0-1.0]</td>
</tr>
<tr>
<td>M</td>
<td>3.2 [1.9-4]</td>
<td>2.3 [1.9-2.9]</td>
</tr>
<tr>
<td>H</td>
<td>5.1 [2.9-6.2]</td>
<td>4.0 [3.2-4.8]</td>
</tr>
<tr>
<td>mg/kg/min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summary statistics of glucose infusion rates are similar although the interquartile range
2.3 Results and Discussion

The study by El Yousef et al. shows that the metabolic response is narrower in silico than in vivo. This difference is also evident in Figure 1 showing smaller boxes but several outliers in silico compared to in vivo. Looking at the glucose infusion rates over time in Figure 2b, we observe a difference in the response to glucagon, thus the amount of decrease in glucose infusion rate compared to Figure 2a. Moreover, the glucose infusion rate at SS is slightly lower during medium and high IIRs. To the authors it is not clear how the large decrease in glucose infusion rate during high IIR displayed in Figure 2a relates to the small EGP area under the curve (AUC) displayed in Figure 8a.

(a) In vivo from [2].

(b) In silico (n=7).

Figure 2: Mean glucose infusion (mg/kg/min) over time by insulin infusion rate group and glucagon dose: top left, all insulin infusion rates together; top right, bottom left, and bottom right, low, medium, and high insulin infusion rates, respectively.
2.3.2 Insulin and Glucose Concentrations

Table 6 compares reported and simulated serum insulin concentrations and plasma glucose concentrations after the two hours run-in period. The distribution of serum insulin levels are very similar in the clinical and virtual studies as seen in Figure 3. This confirms that the insulin PK model is applicable despite not being estimated from optimally sampled data as described in [1]. On the contrary, the plasma glucose concentrations differ. The glucose concentration is lower with less variation during the in silico experiment especially during low and medium IIR. This is probably due to differences in the PID controller settings achieving better control in the virtual population than in real subjects. Moreover, using the SS equations of the individual subjects we calculated the exact needed glucose infusion rate to counter the IIR. This is unfortunately not possible in real life. The plasma glucose concentration is equally well controlled during the high IIR which is probably due to the attenuated EGP response to glucagon.

Table 6: Serum insulin and plasma glucose concentrations in vivo and in silico. Concentrations are reported as mean ± SD and median [IQR]. L = low, M = medium, H = high.

<table>
<thead>
<tr>
<th>IIR</th>
<th>In Vivo</th>
<th>In Silico</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Plasma</td>
</tr>
<tr>
<td></td>
<td>Insulin (mU/L)</td>
<td>Glucose (mg/dL)</td>
</tr>
<tr>
<td>L</td>
<td>17.6 ± 13.0</td>
<td>14.0 ± 7.2</td>
</tr>
<tr>
<td>M</td>
<td>29.1 ± 8.9</td>
<td>29.7 ± 4.8</td>
</tr>
<tr>
<td>H</td>
<td>46.0 ± 12.5</td>
<td>44.4 ± 7.8</td>
</tr>
<tr>
<td></td>
<td>28.1 [25.5-31.5]</td>
<td>30.5 [28.0-31.9]</td>
</tr>
<tr>
<td></td>
<td>41.7 [37.5-46.8]</td>
<td>45.1 [37.4-48.1]</td>
</tr>
<tr>
<td>L</td>
<td>150.8 ± 68.3</td>
<td>100.5 ± 28.0</td>
</tr>
<tr>
<td>M</td>
<td>92.9 ± 21.3</td>
<td>83.6 ± 14.6</td>
</tr>
<tr>
<td>H</td>
<td>88.0 ± 16.0</td>
<td>83.4 ± 13.4</td>
</tr>
</tbody>
</table>

(a) In vivo from [2].  
(b) In silico (n=7).

Figure 3: Box plot of serum insulin levels (mU/L) at low, medium, and high insulin infusion rates.
2.3.3 Glucagon

Table 7 compares time to maximum concentration (Tmax) of glucagon between the clinical and virtual studies stratified by glucagon dose. In both studies, Tmax did not dependent on glucagon dose. We found smaller Tmax with smaller variation \textit{in silico} than \textit{in vivo}. One should keep in mind that blood was only sampled every 10 minutes in the clinical study whereas data used for estimating glucagon PK model parameters for simulations were sampled every 5 minutes. Blood sampling every 10 minutes does not allow for accurate determination of glucagon’s Tmax.

Comparisons of glucagon AUCs in Figure 4 and concentration time profiles in Figure 5 to the \textit{in vivo} findings should be made with caution as absolute glucagon concentration highly depends on the assay [9]. Moreover, the \textit{in vivo} study measured glucagon in serum whereas the \textit{in silico} study simulated glucagon in plasma. Overall, \textit{in silico} glucagon levels seem more variable, although with clearly separated average PK profiles for each dose.

Figure 5a and 5b show that the plasma glucagon concentration is still above SS two hours after most glucagon doses. Therefore, repeating glucagon dosing after only two hours will likely introduce some carry-over effects from the previous dose, even when baseline-corrected at the time of dose.

<table>
<thead>
<tr>
<th>Dose</th>
<th>In Vivo</th>
<th>In Silico</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 µg</td>
<td>23.2 ± 13.5</td>
<td>11.1 ± 3.5</td>
</tr>
<tr>
<td>75 µg</td>
<td>17.1 ± 8.1</td>
<td>12.1 ± 4.3</td>
</tr>
<tr>
<td>125 µg</td>
<td>19.6 ± 6.1</td>
<td>12.1 ± 4.3</td>
</tr>
<tr>
<td>175 µg</td>
<td>20 ± 9.6</td>
<td>12.1 ± 4.3</td>
</tr>
</tbody>
</table>

Figure 4: Box plot of glucagon plasma level AUC over 60 min, stratified by dose.
2.3 Results and Discussion

2. THE STUDY BY EL YOUSSEF ET AL.

(a) In vivo from [2].

(b) In silico (n=21).

Figure 5: Mean incremental change in glucagon plasma levels (baseline corrected at time = 0).

---

16

Technical Report 2
Simulation Studies

Figure 5: Mean incremental change in glucagon plasma levels (baseline corrected at time = 0).
2.3 Results and Discussion

2.3.4 Endogenous Glucose Production

The results in the following section highly depend on the method used for calculating EGP. In the in vivo study they derived the EGP from tracer data by fitting a two-compartment model. In the in silico study we calculated the EGP directly from the model description.

As mentioned in Section 2.3.1, one patient was considered an outlier because of nearly no difference in IIRs and was excluded from the following analysis of EGP due to glucagon at various insulin levels.

Figure 6b replicates Figure 6a with many similarities but also some differences. Most importantly, the magnitudes of average peak EGP to the four glucagon doses are similar. The EGP increase appears to be more rapid in silico than in vivo yielding a faster Tmax, which can be partly explained by the faster glucagon Tmax. However, with the sampling of every ten minutes the observed Tmax could be anywhere between 10-30 minutes and the simulated Tmax could be between 0-20 minutes (the average is in fact 12 minutes). If the true Tmax of EGP in response to glucagon is between 10-20 minutes, this fits with both the observed and simulated results.

The in vivo estimated EGP returned fast to baseline and after 60 minutes it was below the production before injection of the preceding glucagon bolus. The simulated EGP has slower return to baseline and we only observe slightly negative values after the lowest glucagon dose.

The average EGP over the first 60 minutes is somewhat higher in silico than in vivo as visualized in Figure 7b compared to Figure 7a. This difference is expected based on the simulated slower return to baseline just described. The simulated averages are however within the standard error of measurement of the observed data.

Perhaps the most interesting graph is Figure 8a which is replicated by simulation in Figure 8b. The averages of the simulated data are different from the observed averages. However, considering the standard error of measurement of both datasets the simulated data is not different from the observed data. The EGP responses to doses of glucagon during medium IIR were very similar to the EGP responses during low IIR in the measured data, whereas we observe a difference between the responses during the two IIRs when simulating the experiments. We also find a small increase in response to increasing glucagon boluses even at high IIR which is not pronounced in the original observed data. In general, the standard error of measurements are smaller in silico than in vivo.

Figure 9b is a replication of Figure 9a but without extrapolation. The original graph shows the actual data in the dose-range of 25-175 μg glucagon and extrapolates the presumed trends down to 1 μg and up to 10 mg. Note, this is a wild extrapolation with no data to support it. Within the data-range the simulated results match the observed results although the simulated EGP at low IIR tends to be higher than the observed. Having only four points very closely spaced on a log-scale, a single point can largely influence the overall interpretation of the curves.
2.3 Results and Discussion

(a) In vivo from [2].

(b) In silico (n=6).

Figure 6: Time profiles of calculated EGP by glucagon dose, baseline corrected for EGP at the time of dose.
2.3 Results and Discussion

2 THE STUDY BY EL YOUSSEF ET AL.

(a) In vivo from [2].

(b) In silico (n=18).

Figure 7: Mean EGP AUC over 60 min after the dose.

(a) In vivo from [2].

(b) In silico (n=6).

Figure 8: Mean EGP AUC separated by glucagon dose and insulin infusion rate.
2.3 Results and Discussion

(a) In vivo from [2].

(b) In silico (n=6 or 18).

Figure 9: Dose-response curve across all doses, and for low and high insulin infusion rate experiments, estimated from simulated data.
2.3 Results and Discussion

2.3.5 Examples of Simulated Raw Data

Figures 10 and 11 show examples of raw data from the simulation study. The points mark blood sampling times during the *in vivo* study.

The first figure presents data during low IIR and reveals that the blood glucose level cannot be kept within ±20 mg/dL of the set point at all times. Especially after the higher doses of glucagon the blood glucose exceeds the upper limit. The graph also reveals that the glucose infusion rate is zero during most of the experiment. The explanation to this observation is that during low IIR the glucose infusion rate needed to maintain SS is equally low and cannot be lowered sufficiently after the glucagon boluses to maintain the blood glucose within the boundaries. Moreover, the plasma glucose concentration and glucose infusion rate do not return to SS before the next glucagon bolus administration.

The second figure shows data during medium IIR where the glucose infusion rate is never zero although decreased in response to glucagon boluses. The blood glucose is mostly kept within the boundaries.

The raw data from the study by El Youssef et al. [2] are not available, making it im-
Figure 11: Example of simulated raw data from patient 6 during the medium IIR with first bolus being 75 µg. Notice how the glucose infusion is regulated to control the blood glucose close to the set point.

possible to compare our examples to actual data.
3 Dose-Response Studies

In this section, we demonstrate the advantage of using simulations to conduct large cross-over studies that would not be feasible in real life. Moreover, we use simulations to design guidelines for realistically sized studies that, if the simulation model is correct, will provide the same information as the large in silico study, while exposing the patients to a limited number of experiments.

3.1 Study Design

The in silico study included seven T1D virtual patients [1], that each underwent 115 cross-over study days. Model equations and subject specific model parameters are listed in Section 1. At each study day, the IIR was constant at either 1, 2, 3, 4, or 5 times the basal IIR and the glucose infusion rate controlled every five minutes as described previously in Section 2.2.2 to maintain a glucose clamp of 5 mmol/L. After 60 minutes SS run-in period, a glucagon bolus was administered and simulation continued till 5 hours after the bolus. We simulated the effect of the following glucagon boluses: 1 µg, 2.5 µg, 5 µg, 10 µg, 25 µg, 50 µg, 75 µg, 100 µg, 125 µg, 175 µg, 200 µg, 300 µg, 400 µg, 500 µg, 750 µg, 1 mg, 1.5 mg, 2 mg, 3 mg, 4 mg, 5 mg, 7.5 mg, and 10 mg.

Figure 12: Simulated average of seven T1D patients’ dose-response curves of glucagon boluses ranging from 1 µg to 10 mg at various insulin levels expressed as multiples of the basal IIR.
3.2 Results and Discussion

The EGP AUCs over 60 minutes (AUC₆₀) were calculated as described in Section 2.2.3. The average EGP AUC₆₀ for each dose stratified by IIR were calculated and plotted in Figure 12. The response to glucagon doses below approximately 25 µg are very similar independent of IIR. However, with increasing glucagon doses the curves for each IIR separate. The higher the IIR, the less response to a glucagon bolus. Small increases in glucagon dose during low IIR increase the response significantly although it seems to saturate for some glucagon dose.

The results in Figure 12 represent classical dose-response curves and can be described mathematically by the Michaelis-Menten equation:

\[
\text{EGP AUC}_{60\text{min}} = R_{\text{max}} \cdot \frac{\text{Dose}}{E_{\text{D}_{50}} + \text{Dose}}
\]

(24)

\(R_{\text{max}}\) is the maximum response and \(E_{\text{D}_{50}}\) is the dose yielding the half-maximum response. The fitted \(R_{\text{max}}\) and \(E_{\text{D}_{50}}\) for each IIR are summarized in Table 8. The \(E_{\text{D}_{50}}\) does not seem to depend on the insulin level. On the contrary, \(R_{\text{max}}\) is highly dependent on the insulin level according to Table 8. This observation is expected, as the model used for simulations describes how insulin modulates the maximum achievable EGP response to glucagon, but does not influence the concentration yielding half-maximum response.

Table 8: Fitted parameters for dose-response relationship between glucagon and EGP at multiples of the average basal IIR.

<table>
<thead>
<tr>
<th>IIR x basal</th>
<th>(R_{\text{max}}, \text{mg/kg})</th>
<th>(E_{\text{D}_{50}}, \text{mg})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>609</td>
<td>0.220</td>
</tr>
<tr>
<td>2</td>
<td>385</td>
<td>0.226</td>
</tr>
<tr>
<td>3</td>
<td>183</td>
<td>0.244</td>
</tr>
<tr>
<td>4</td>
<td>96</td>
<td>0.256</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>0.237</td>
</tr>
</tbody>
</table>
3.3 Dose Selection for an in Vivo Study

There is speculation on how the ambient insulin level affects the EGP response to glucagon. Two hypotheses are proposed:

- insulin level influences the maximum response to glucagon, $R_{\text{max}}$,
- insulin level influences the glucagon dose at which half-maximum response is achieved, $ED_{50}$

The hypotheses could be examined by carrying out a smaller in vivo study. However, the glucagon doses must be carefully chosen to make sure to capture the essential parts of the dose response curve. If all tested doses are below the true $ED_{50}$, both hypotheses would describe the data equally well. This pitfall is illustrated in the following example.

The in silico study just described in Section 3.1 was inspired by the results presented in Figure 9 in Section 2.3.4. Assuming the in vivo study was carried out again with the same glucagon doses of 25, 75, 125, and 175 $\mu$g at one to three times the basal IIR, would one be able to decide which parameter in equation (24) insulin affects? To answer this question, we simulated the small study and fitted the parameters of (24) twice; first assuming $ED_{50}$ was constant across insulin levels and then assuming $R_{\text{max}}$ was constant across insulin levels. The results are presented in Figure 13. Because the four doses are within a narrow dose range and all doses are below the simulated "true" $ED_{50}$, both hypotheses fit the simulated data equally well. Although the case with equal $ED_{50}$ represents the simulated "truth", the commonly identified $ED_{50}$ is much lower than the parameter value presented in Table 8.
3.3 Dose Selection for an in Vivo Study

3 DOSE-RESPONSE STUDIES

(a) \(ED_{50}\) independence of IIR.

(b) \(R_{\text{max}}\) independence of IIR.

Figure 14: Fitted dose response curves when using SC glucagon doses of 25, 100, and 1000 \(\mu\)g assuming independence of insulin for either \(ED_{50}\) or \(R_{\text{max}}\).

If the glucagon doses had been distributed across a larger dose range encompassing the "true" \(ED_{50}\), would it then be possible to determine how insulin affects EGP? Realistically, one can not administer more than 1 mg glucagon as a SC bolus injection which causes some limitations to the maximum possible dose range in an in vivo study. We simulated a small realistic study with three SC glucagon boluses of 25 \(\mu\)g, 100 \(\mu\)g, and 1 mg at one to three times the basal IIR. We then fitted (24) assuming either \(ED_{50}\) or \(R_{\text{max}}\) constant and independent of the ambient insulin level. Figure 14 presents the results using the model analyzed in Section 3.2 where \(ED_{50}\) is constant and independent of the ambient insulin level. The graphs visualize a clear difference in the fitness of the two hypotheses making one more plausible than the other; that \(ED_{50}\) does not depend on ambient insulin levels, but that \(R_{\text{max}}\) does. Moreover, the identified common \(ED_{50}\) is similar although a bit lower than the values listed in Table 8.

If this study was to be carried out in real life, biological variation between subjects might be dominating making it difficult to determine which hypothesis to accept and which to reject. Instead of relying on the inter-subject variation being low, one could fit (24) to data from individual subjects and hopefully reach the same conclusion in all subjects. The individually confirmed hypothesis could then be transferred to the population mean.
4 Pearls & Pitfalls

This section focuses on the **DOs** and **DON'Ts** when conducting clinical studies of the glucoregulatory system with focus on experiments involving glucagon. The points will be exemplified through simulations and references to literature. We hope that this section serves as an inspiration to researchers who are planning *in vivo* studies of the glucoregulatory system.

4.1 Glucose Clamps

The blood glucose clamp is a procedure used to maintain the same glucose level throughout an experiment either hypoglycaemic (below normal), euglycaemic (normal), or hyperglycaemic (above normal). The purpose of clamping the blood glucose is to eliminate the influence of varying glucose levels during an experiment where glucose is believed to affect the investigated mechanism. As an example, the glucose clamp procedure could be used during a gastric emptying study to eliminate the negative feedback mechanism between blood glucose concentration and gastric emptying rate [10].

The glucose clamp can be controlled using intravenous (IV) infusions of insulin and glucose. Somatostatin may be infused to inhibit endogenous production of hormones like insulin and glucagon in healthy subjects. The glucose regulating hormones are then clamped at continuous rates and as a minimum clamped at the basal rates to substitute for baseline concentrations. Somatostatin may not be necessary in clamp studies when investigating effects of exogenous supraphysiological glucagon doses in patients with type 1 diabetes having no endogenous insulin production.

4.1.1 Glucose Level and Glucose Infusion

An *in vivo* study by Hinshaw et al. points to that the glucose level does not influence the effect of glucagon [11]. However, Cherrington advocates there is an inhibitory effect of hyperglycemia on EGP [12]. Therefore, we recommend that the blood glucose concentration is kept close to a set point throughout a clamp experiment involving glucagon to minimize potential influence of the blood glucose concentration on EGP.

The simplest and fastest way to control the glucose level during a clamp is through IV glucose infusion. The glucose infusion can be controlled automatically using various controllers based on PID or Model Predictive Control (MPC). In Section 2.3.5 we demonstrated that a simple PID controller was sufficient to maintain the blood glucose close to a predefined set point while administrating glucagon. Moreover, a simple PID controller is easy to implement and may assist investigators in keeping the blood glucose close to the predefined set point level. This is however only possible in cases when the insulin and glucose infusions are sufficiently high to allow for the glucose infusion to be reduced corresponding to the EGP contribution from the glucagon bolus.

4.1.2 Insulin Level and Insulin Infusion

Glucose clamps are not recommended to be controlled by IV insulin infusion although it occurs. Studies have showed that high insulin levels during euglycaemia suppress the
Figure 15: *In silico* demonstration of the dynamics when stopping or continuing IV insulin and glucose infusion during a clamp study in subject 4. Blood glucose was clamped at 5 mmol/L by twice the basal IIR and constant glucose infusion. After 30 minutes SS, glucose and insulin infusions were stopped (left column) and 20 minutes after either no bolus (dashed line) or a 0.5 mg SC glucagon bolus (solid line) was administered. In a different scenario, the insulin and glucose infusions continued throughout the study and a 0.5 mg SC glucagon bolus was administered (right column).

The first *in silico* study design is inspired by Blauw et al. [14] to demonstrate that it is difficult to interpret the glucose response to glucagon when too many dynamics influence the response. This situation is illustrated in Figure 15 by simulations and explained in the caption. When insulin and glucose infusions are stopped during a clamp procedure, the immediate response is a drop in glucose levels both due to the lack of glucose infusion and because the effect of insulin persists after the infusion is stopped.

Although the glucose responses to glucagon in Figure 15 look fairly similar, more EGP is produced when the infusions are stopped as measured by the AUC. The increased EGP is hiding the drop in glucose that would have been seen if no glucagon bolus was administered.
administered. Even when no glucagon bolus is administered the EGP increases slightly after infusion stop of insulin and glucose because of the fading insulin level.

It should be noted, that the insulin clearance parameters were estimated from data following SC insulin administration rather than IV administration, which could underestimate the actual clearance since it is limited by the slow and variable SC absorption.

The second in silico study design explores what happens if one uses insulin to control a glucose clamp rather than glucose. The blood glucose of a patient undergoing a glucose clamp responds immediately to the changes in the glucose infusion whereas the effect of insulin is delayed. An example to illustrate the delayed effect of insulin is demonstrated in Figure 16 by simulations and explained in the caption. Although the same glucagon bolus of 0.5 mg was given at the same insulin concentration, the responses were different - the larger the prior SC insulin bolus, the smaller response to glucagon. However, not all virtual patients seem to have as pronounced delayed response to insulin as in this example. The size of the delay highly depends on the parameter $k_{a3}$ in the PD model which represents the rate constant of remote insulin action on EGP, see Table 4 in Section 1.4.

The simulated examples show that the insulin level highly influences the EGP response to glucagon and the effect of insulin can be delayed.

4.2 Dynamics in Data

A model can only be expected to describe dynamics present in the data used for model development and parameter estimation, if data is sampled sufficiently. To correctly estimate $T_{\text{max}}$ after a bolus administration in a PK model, data must be sampled densely around $T_{\text{max}}$. If $T_{\text{max}}$ of a compound is expected to be 50 minutes, and no samples are collected the first two hours after dose administration, it is practically impossible to determine $T_{\text{max}}$ without inferring prior knowledge. More importantly, if $T_{\text{max}}$ is very short the exact notation of the dosing time is absolutely necessary in order to fit a meaningful PK model to data.

Furthermore, factors influencing the model parameters can not be included in the description of the parameters if the factors do not vary in the training dataset. As an example, exercise and stress are long known to alter the insulin sensitivity, and recently Ranjan et al. found that diet might influence the response to glucagon [15]. None of these factors are accounted for in the glucose PD model used for simulations throughout this report [1]. Moreover, the model does not include a feedback mechanism of the glucose levels to the endogenous production of insulin and glucagon.

4.2.1 Identifying Steady State

As described, the final fitted model is limited by the data used for estimating the model parameters. Thus identifying the correct steady state can be difficult if the data do not contain much information thereof. Figure 17 illustrates that the PD model described in [1] does not estimate the correct steady state. Initializing patient 7 at euglycaemia with
Figure 16: *In silico* demonstration of the delayed insulin effect on EGP. Blood glucose was clamped at 5 mmol/L by basal IIR and controlled via IV glucose infusion every 5 minutes using the PID controller explained in Section 2.2.2. After 30 minutes steady state, subject 4 received a SC insulin bolus of either 5 U (left), 3 U (middle) or 1 U (right). When the insulin concentration dropped below 13 mU/L a SC glucagon bolus of 0.5 mg was administered. The EGP responses are displayed in the bottom row; the 120 minutes AUCs are 323, 429, and 612 mg/kg, respectively.

baseline levels of insulin and glucagon as in Figure 17a, one would expect the glucose level to stay constant or at least approach a level similar to either of the observed initial values. However, the glucose concentration drops to a level below 50 mg/dL. The reason for this behaviour is explained in the data used for estimating the model parameters of patient 7 [1,16], see Figure 17b. The data contains very little information about the insulin, glucagon and glucose levels before the system is disturbed with an insulin bolus. On the contrary, it appears that a SS is achieved towards the end of the experiment when the glucose concentration is around 50 mg/dL and both glucagon and insulin have returned to their baseline levels. This phenomenon explains why the model assumes glucose SS lower than one would expect at baseline levels of insulin and glucagon.
4.2 Dynamics in Data

4.2.2 Repeated Glucagon Boluses

When conducting experiments in vivo one naturally wishes to maximize the information from those experiments. Clinical studies are often set up to investigate multiple glucagon doses during each trial day [2, 14]. However, depending on the size of the glucagon bolus, it can take several hours before all administered drug is cleared from the system and plasma concentration has returned to baseline. As evident in Figure 17b, plasma glucagon has only just returned to baseline four hours after a SC bolus of 300 µg glucagon. Be reminded of Figure 10 in Section 2 where the continuous increase in glucose concentration is due to residual glucagon from the previous dose. One must allow sufficient time in-between experiments and perhaps lower ones expectations to what is practically possible to avoid carry-over effects from previous doses, rather than rushing too many experiments in short time.

4.2.3 The Glucagon Evanescence Effect

The glucagon evanescence effect is well known and documented since the early 1980s [11, 17, 18]. As implied in the name, the effect of glucagon tends to fade away over time. This trend is observed during clamp studies with constant insulin and glucagon levels where the EGP tends to return to baseline after approximately two hours although the glucagon level is still significantly elevated above the baseline level [11, 18]. Mechanisms to explain this phenomenon could be degradation/aggregation of infused glucagon, hyperglycemia, intra-hepatic negative feedback mechanism or simply glycogen depletion. As the evanescence effect is observed even during clamped euglycaemia, hyperglycemia can not solemnly explain the vanishing effect of glucagon [11]. Glycogen depletion seems like an easy explanation. However, the amount of infused glucagon during the study by Hinshaw et al. [11] was 0.54 µg/kg over three hours during the highest glucagon infusion rate. A study by Castle et al. [19] found that repeated boluses of 2 µg/kg did not deplete the liver even after an overnight fast. Therefore, it does not seem likely that the evanescence effect observed during clamp studies of glucagon is due to depletion of the glycogen stores in the liver.

In vitro data suggest that the glucagon evanescence effect is due to desensitization of the receptor regulated by cyclic AMP [20]. The reduced responsiveness to glucagon was fully expressed after 2 hours which fits well with in vivo data [11, 18]. Hinshaw et al. [11] proposed a mathematical expression to capture the waning effect of glucagon. However, it is unclear for how long this evanescence effect persists and if a sudden increase in the glucagon infusion can overrule the evanescence phenomenon. More studies of the glucagon evanescence effect are needed to fully understand the underlying mechanisms and how the effect should be accounted for in a glucoregulatory model including glucagon.
4.2 Dynamics in Data

(a) Simulation of subject 7’s modelled SS. Insulin and glucagon are at baseline levels, and glucose concentration initiated at euglycaemia.

(b) Raw data and PK/PD model fits in subject 7: insulin PK (top), glucagon PK (middle), glucose PD (bottom). Increasing glucagon boluses left to right: 0, 100, 200, 300 µg. Triangles indicate time of insulin bolus (blue) and glucagon bolus (red). Please see [1] for further study details.

Figure 17: Comparison of modelled SS and data used for model building.
4.3 Summary

The learnings of the previous sections can be summed up in the following statements that should aid in the design of clinical studies:

- The glucose level during hypo- or euglycaemia does not influence the glucose response to glucagon.
- Theoretically, a simple PID controller can control the needed glucose infusion to maintain constant glucose levels when the insulin infusion is sufficiently high.
- The insulin infusion should be constant throughout the study duration.
- Nominal sampling times must be chosen carefully and actual sampling times noted meticulously.
- A model can only be expected to account for dynamics present in the dataset used for model building and parameter estimation.
- Less is more. Avoid multiple dynamics simultaneously by allowing enough time between disturbances of the glucoregulatory system.
- The effect of glucagon wanes over time despite constant infusion.
- Glucagon doses should be distributed across a wide range encompassing the true half maximum response in order to correctly identify the dose-response relationship.
References


This appendix presents the draft journal paper by Ranjan et al. with the title "Relationship between Optimum Mini-doses of Glucagon and Insulin Levels when Treating Mild Hypoglycaemia in Patients with Type 1 Diabetes - A Simulation Study" to be submitted to a high impact journal in Summer 2017 [22].
Relationship between Optimum Mini-doses of Glucagon and Insulin Levels when Treating Mild Hypoglycaemia in Patients with Type 1 Diabetes - A Simulation Study

Ranjan A1,2, Wendt SL3,4, Schmidt S1,2, Madsbad S1, Holst JJ5, Madsen H4, Knudsen CB3, Jørgensen JB3, Nørgaard K1

1Department of Endocrinology, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark.
2Danish Diabetes Academy, Odense, Denmark.
3Department of Bioanalysis and Pharmacokinetics, Zealand Pharma A/S, Glostrup, Denmark.
4Department of Applied Mathematics and Computer Science, Technical University of Denmark, Kgs. Lyngby, Denmark.
5NFF Center for Basic Metabolic Research and Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark.

Running title: Optimum glucagon treatment of mild hypoglycaemia

Keywords: In silico, dose selection, insulin on board, mini-bolus, PK/PD models, glucoregulatory model

Corresponding author:
Ajenthen Ranjan
Copenhagen University Hospital Hvidovre
Department of Endocrinology
Kettegaard Alle 30
2650 Hvidovre
Denmark
Email: Ajenthen.Ranjan.02@Regionh.dk
Phone: +45 38 62 63 78

Running title: 42
Abstract: 256
Word Count: 2668
Number of tables: 1
Number of figures: 5
Number of references: 23
Abstract

Objective: To develop an insulin-dependent optimum glucagon dosing regimen for treatment of mild hypoglycaemia in patients with type 1 diabetes based on simulations.

Methods: A validated glucose-insulin-glucagon model was used to describe seven virtual patients with insulin pump-treated type 1 diabetes. In each simulation, one of ten different and individualised subcutaneous insulin boluses was administered to decrease plasma glucose (PG) from 7.0 to ≤3.9 mmol/l. Insulin levels were estimated as ratio of actual to baseline serum insulin concentration (se/ba-insulin), insulin on board (IOB) or percentage of IOB to total daily insulin dose (IOB/TDD). Insulin bolus sizes were chosen to provide predefined insulin levels when PG reached 3.9 mmol/l, where one of 17 subcutaneous glucagon boluses was administered. Optimum glucagon bolus to treat mild hypoglycaemia at varying insulin levels was the lowest dose that in most patients caused PG peak between 5.0 and 10.0 mmol/l and sustained PG≥3.9 mmol/l for 2 hours after the bolus.

Results: PG response to glucagon declined with increasing insulin levels. The glucagon dose to optimally treat mild hypoglycaemia depended exponentially on insulin levels, regardless of how insulin was estimated. A 125 µg glucagon dose was needed to optimally treat mild hypoglycaemia when insulin levels were equal to baseline levels. In contrast, glucagon doses >500 µg were needed when se/ba-insulin >2.5, IOB >2.0U or IOB/TDD >6%.

Conclusions: Although the proposed model-based glucagon regimen needs confirmation in clinical trials, this is the first attempt to develop an insulin-dependent glucagon dosing regimen for treatment of insulin-induced mild hypoglycaemia in patients with type 1 diabetes.
**Introduction**

Intensive insulin therapy increases the risk of hypoglycaemia in patients with type 1 diabetes (1). The fear of hypoglycaemia impedes many in seeking optimum glycaemic control and affects their quality of life negatively (2). Different adjunct therapies and advances in insulin therapy have been tested, but none markedly improved glycaemic control, risk of hypoglycaemia and/or quality of life (3).

Some studies have suggested that low-dose glucagon as an add-on to the intensified insulin therapy may optimise glycaemic control and reduce the risk of hypoglycaemia (4). This dual-hormone approach has mainly been tested in settings with automatic delivery of the drugs (closed-loop therapy) (5,6). However, manual delivery of insulin and glucagon (open-loop therapy) may equally improve diabetes management as demonstrated by Haymond *et al.* in children with type 1 diabetes and gastroenteritis (7). To our knowledge, proposals for optimum dosing of glucagon to treat mild hypoglycaemia in an open-loop setting have never been developed for adults with type 1 diabetes.

Although the anti-hypoglycaemic effect of glucagon highly depends on ambient insulin levels, no commercially available devices are able to measure insulin concentrations in real-time (8). Bolus calculators in insulin pumps have addressed this issue by providing “insulin on board” (IOB) feedback to reduce the risk of insulin stacking and hypoglycaemia (9,10). Basal insulin is not included in the calculation of IOB, which is an approximation of the remaining effect of an insulin bolus, measured in units of subcutaneously (SC) administered bolus insulin (11).

Based on simulations using pharmacokinetic (PK) and pharmacodynamic (PD) models for glucose, insulin and glucagon, our aim was to develop an optimum glucagon dosing regimen for treatment of mild hypoglycaemia depending on ambient insulin levels. We used a validated glucoregulatory model to simulate how different insulin levels would affect the glucose response to different glucagon doses (12). The success of each glucagon dose in treating mild hypoglycaemia was
evaluated. Our criteria for the optimum glucagon dose to treat mild hypoglycaemia at varying insulin levels was the lowest dose that in most patients caused a plasma glucose concentration (PG) peak between 5.0 and 10.0 mmol/l and sustained PG above or equal to 3.9 mmol/l for 2 hours after the bolus.

**Methods**

**Data:** We used insulin and glucagon PK models in combination with a validated glucose-insulin-glucagon PD model to simulate data from seven virtual type 1 diabetes patients (12). MATLAB 2016b (The MathWorks, Inc., Natick, MA) was used for model implementation and simulations.

The population of virtual patients described seven “real” adults (4 females, age range: 19-64 years, BMI range: 20.0-25.4 kg/m²) with insulin pump-treated type 1 diabetes, who previously had participated in a study investigating the glucose response to different mini-doses of glucagon during insulin-induced mild hypoglycaemia (13). Patients were in good glycaemic control (HbA1c range: 6.1-7.4%) and had no endogenous insulin production (13).

The PD model is an extension of Hovorka’s glucoregulatory model with the effects of glucagon on the endogenous glucose production (14) and was validated in a previous study (12). The PK models assumed that changes in insulin and glucagon concentrations were only due to the administered drugs: insulin aspart (NovoRapid®, Novo Nordisk) and glucagon (GlucaGen®, Novo Nordisk).

**In silico experiments:** We executed three simulations to investigate the glucose response to different glucagon doses depending on the ambient insulin levels during insulin-induced mild hypoglycaemia (Figure 1). In each simulation, a SC insulin bolus was administered at PG of 7.0 mmol/l, followed by a SC glucagon bolus that was administered when PG was 3.9 mmol/l. The simulation of one experiment lasted for ten hours following the insulin bolus. The individual insulin bolus size was chosen to achieve a predefined insulin level at the time of glucagon administration.
Thus, patients received different insulin boluses to achieve the same predefined insulin levels, due to differences in insulin PK/PD profiles. For each simulation, one of the following predefined insulin levels was achieved when PG was 3.9 mmol/l:

1) Ratio of actual to baseline serum insulin concentration (se/ba-insulin): 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 3.0, 3.5 or 4.0

2a) Insulin on board (IOB): 0.0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, or 3.5 U

2b) Percentage of insulin on board to total daily insulin dose (IOB/TDD): 0, 1, 2, 3, 4, 5, 6, 7, 8, or 10%

The insulin PK model was used to estimate the actual serum insulin level which was divided by the individual baseline level before the insulin bolus was given (se/ba-insulin). A linear function of patient’s insulin action time was used to estimate IOB. TDD was an average of seven days.

In all experiments, when PG reached 3.9 mmol/l, one of following 17 glucagon boluses was administered SC: 25, 50, 75, 100, 125, 150, 175, 200, 250, 300, 400, 500, 1000, 1500, 2000, or 2500 µg.

**Treatment assessment:** For each experiment, the success of a glucagon dose in treating mild hypoglycaemia but avoiding rebound hyperglycaemia was evaluated based on three criteria: peak PG ≥ 5.0 mmol/l (PG≥5), peak PG ≤ 10.0 mmol/l (PG≤10) and PG ≥ 3.9 mmol/l for 120 min after the glucagon bolus (PG_{120}≥3.9) (Figure 1). The success rate of a glucagon bolus in achieving each of these criteria was calculated at various insulin levels. For each combination of glucagon dose and insulin level, the overall treatment success was calculated as a weighted harmonic mean (H) of the three criteria:
where \( S \) is the success rate, equal to the number of subjects fulfilling a criterion divided by the total number of subjects. Arbitrarily, the weighted harmonic mean prioritises the criteria for peak PG (PG\( \geq 5 \) and PG\( \leq 10 \)) higher than the PG level two hours after dose (PG\(_{120} \geq 3.9 \)), since we consider the acute rescue of hypoglycaemia and the avoidance of rebound hyperglycaemia to be more important than the duration of the anti-hypoglycaemic effect. For each insulin level, the lowest glucagon dose with the highest \( H \)-value was the optimum bolus.

**Results**

Figures 2-4 show the proportion of patients achieving the predefined treatment criteria as functions of the glucagon dose, stratified by se/ba-insulin (Figure 2), IOB (Figure 3), and IOB/TDD (Figure 4). The proportion of patients achieving the criterion of PG\( \geq 5 \) (green line) and of PG\(_{120} \geq 3.9 \) (red line) increased with increasing glucagon doses. The curves for the PG\( \geq 5 \) criterion were left-shifted compared to the curves for the PG\(_{120} \geq 3.9 \) criterion, meaning that less glucagon was needed to fulfil the criterion of PG\( \geq 5 \) compared to PG\(_{120} \geq 3.9 \). On the other hand, the proportion of patients avoiding rebound hyperglycaemia, PG\( \leq 10 \), declined with increasing glucagon doses (blue line). For instance, when patients had a PG of 3.9 mmol/l and IOB of 1.5 U, a glucagon dose of 100 µg would increase PG \( \geq 5.0 \) mmol/l in less than 60% of patients, keep PG \( \geq 3.9 \) mmol/l for two hours in more than 40% of patients, and keep PG \( \leq 10 \) mmol/l in all patients.

Figure 5 shows the optimum glucagon dosing regimens for treatment of mild hypoglycaemia in the virtual population as a function of insulin levels extracted from Figures 2-4 (vertical black lines). The relationship between insulin level and the corresponding optimum glucagon dose could be approximated by an exponential function, regardless of the method used for estimating insulin levels. A 125 µg glucagon dose was needed to optimally treat mild hypoglycaemia when insulin...
levels were equal to baseline levels. In contrast, glucagon doses >500 µg were needed when serum insulin exceeded 2.5 times baseline insulin concentrations, IOB were above 2.0 U or IOB/TDD were above 6%.

**Discussion**

In this *in silico* study, we used a validated PK/PD model to develop optimum glucagon dosing regimens to treat mild hypoglycaemia at varying levels of serum insulin ratio (i.e. the actual serum insulin level divided by patients’ baseline insulin level before the insulin bolus), “insulin on board” and percentage of “insulin on board” to total daily insulin use in patients with type 1 diabetes. As expected, the anti-hypoglycaemic effect of glucagon was highly dependent on ambient insulin levels. El Youssef *et al.* previously showed this relation *in vivo* by quantifying the glycaemic effects of glucagon at various insulin levels (8). Notably, the PK/PD model used in the present study was able to replicate the findings by El Youssef *et al.* with simulations (15). Furthermore, the PD model was validated using data from another cross-over *in vivo* study with three different SC injections of glucagon for treatment of insulin-induced mild hypoglycaemia (13). Therefore, we consider the model to be valid for estimating the optimum glucagon dose for treatment of mild hypoglycaemia at varying levels of insulin.

The strength of *in silico* studies is the ability to simulate large scale cross-over trials that are not feasible in real-life settings. In this study, we estimated the optimum glucagon dose at varying insulin levels based on virtual patients, each undergoing 170 cross-over visits per study, resulting in 510 simulations per patient. We defined an optimum glucagon dose to increase PG from 3.9 mmol/l to a peak between 5.0 and 10.0 mmol/l, and sustain PG above 3.9 mmol/l for at least 120 minutes following the glucagon bolus. These success criteria were arbitrarily set as no consensus exists regarding post-rescue glucose excursions or postprandial glucose excursions (16). We based our criteria on the recommendations of American Diabetes Association (17) that were considered
clinically reasonable in most patients. However, not all criteria could be achieved in all patients with the same glucagon dose. We considered the acute rescue of mild hypoglycaemia and the following avoidance of rebound hyperglycaemia to be more important than the duration of the anti-hypoglycaemic effect. This priority of peak PG over the 2-hour PG level was applied because patients will benefit from the acute rescue and still have time to avoid subsequent hypoglycaemia by suspending their insulin infusion and/or consuming carbohydrates. Alternatively, not reported here, a second bolus of glucagon could be given which has shown to give similar glucose response as the first glucagon bolus (18).

The glucagon dosing regimens were stratified in relation to different methods of estimating ambient insulin levels. We included IOB because no real-time monitors of serum insulin concentrations are currently available (19). For decades, insulin pumps with bolus calculators have used IOB feedback as standard to prevent insulin stacking. Depending on the manufacturer, the bolus calculators estimate IOB differently, i.e. using a linear or a curvilinear time profile (9). Most bolus calculators use a curvilinear time profile because it resembles the insulin time-action profile (9). However, we chose the linear approach due to the unambiguous implementation compared with the curvilinear functions. Further, we consider the differences in IOB time profiles to be negligible for the success of glucagon treatment.

In this study, we found an exponential relationship between the optimum glucagon doses to treat mild hypoglycaemia and the ambient insulin levels. However, the relationship was approximately linear in ranges of serum insulin from 1 to 2 times basal insulin levels, IOB from 0 to 2 U, and IOB/TDD from 0 to 5%. The lowest glucagon dose to optimally treat mild hypoglycaemia was 125 µg when actual insulin levels were equal to baseline levels, which is in accordance with findings from previous studies (20, 21). In contrast, at very high insulin levels (se/ba-insulin >3, IOB ≥3 U, IOB/TDD >8%), the optimum glucagon doses exceeded the amount (1000 µg) normally used for
treating severe hypoglycaemia. Further, at some point the estimated optimum glucagon dose was, in our opinion, too high (>500 µg) as treatment option for mild hypoglycaemia, especially due to the increased risk of side effects. In a previous study, a single bolus of 500 µg glucagon tended to cause more nausea than 100 µg glucagon during mild hypoglycaemia (22). In the present study, we found that 500 µg glucagon was needed if serum insulin was 2.5 times baseline insulin levels, IOB was 2 U, or IOB was 6% of TDD. Therefore, if patients have mild hypoglycaemia, but insulin levels above these critical limits, ingestion of carbohydrates rather than mini-dose glucagon may be a better treatment for restoring PG.

We applied the same success criteria for optimum glucagon dosing to the results of a previous in vivo dose finding study (supplemental table 1). Here in a comparison of glucagon doses, 200 and 300 µg had almost similar success rate to restore mild hypoglycaemia with serum insulin levels 1.9 times higher than baseline levels or with an average IOB of 0.9 U, equivalent to 2.5% of TDD. The lowest optimum dose was similar to the dose suggested in the current in silico (Figure 5).

This study has limitations. First, the glucagon dose regimen was based on a simulation model, which may not completely capture real life events. Second, the model parameters were based on an in vivo study with glucagon doses from 100 to 300 µg given to treat mild hypoglycaemia when ambient insulin levels were 0.19-2.3 U of IOB, 0.7-6.1% of IOB/TDD, and 1.2-2.9 times the baseline concentrations. Therefore, the simulations with glucagon doses greatly exceeding 300 µg should be interpreted with caution, as should simulations at insulin levels greater than those used for the model building. Third, the used patient sample may not be transferable to a large-scale population, since our virtual population only consisted of seven lean and well-controlled patients and the results may differ in other patients with type 1 diabetes. Therefore, each patient may benefit from adjusting the glucagon dosing regimen in order to account for, i.e. glucagon sensitivity (23). Further, the glucagon doses used were predefined and categorically analysed, meaning the “real”
optimum glucagon dose may be between the doses tested in this study. The validated model is based on human in-patient studies and do not account for, i.e. low carbohydrate diet (22), alcohol, exercise and “stress”. Finally, the model has only been validated in lean patients with type 1 diabetes. Since the optimum glucagon dose was not weight adjusted, the responses may be different in an obese population. To our knowledge, no studies have found a significant effect of weight on the glucose response to glucagon in patients with type 1 diabetes.

Glucagon is currently only available in 1 mg vials and has to be reconstituted immediately before use. At the moment, the proposed glucagon approach to treat mild hypoglycaemia may not be feasible. Though, stable soluble glucagon formulations may soon be on the market, and multidosing may be of interest in the future. Hypothetically, an advanced bolus calculator advising for insulin injections, carbohydrate intake and glucagon injections could account for side effects, treatment success, and IOB; providing the best option for prevention and treatment of hypoglycaemia and leading to improved glucose control. However, as the proposed glucagon dosing regimen is based on reconstituted GlucaGen® from Novo Nordisk, the model parameters of the simulation model may need adjustments to describe other glucagon products.

To our knowledge, this is the first study to propose a dosing regimen of glucagon in an open-loop setting using simulations. The focus has primarily been on the closed-loop dual-hormonal settings in which micro-doses of glucagon were frequently given to prevent hypoglycaemia (5). This frequent administration cannot be applied in open-loop settings due to inconvenience of multiple glucagon injections. Rather, we consider low dose glucagon as an alternative to oral carbohydrate intake in treatment of mild hypoglycaemia. We can only speculate that low dose glucagon treatment, in combination with an advanced bolus calculator, may provide more predictable glucose responses than oral carbohydrate ingestion in treatment of mild hypoglycaemia, and may also reduce the risk of overeating and post-rescue hyperglycaemia.
Conclusion

In this study, we used a mathematical PK/PD model to develop insulin-dependent optimum glucagon dosing regimens for treatment of insulin-induced mild hypoglycaemia. The glucagon doses depend on insulin levels evaluated as serum insulin concentration normalised to basal, insulin on board and ratio of insulin on board to TDD. The regimens were based on simulations of glucagon doses ranging from 25 to 2500 µg and insulin doses yielding predefined insulin levels when blood glucose reached the hypoglycaemia threshold. The next step is to evaluate the proposed glucagon dosing regimen in a clinical trial including different phenotypes of patients with type 1 diabetes.

Funding

The study was supported by the Danish Diabetes Academy funded by the Novo Nordisk Foundation. Further, the study was supported by Zealand Pharma A/S and the Innovation Fund Denmark.

Author disclosure statement

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: SLW is a full-time employee of Zealand Pharma. SS serves on the continuous glucose monitoring advisory board of Roche Diabetes Care and as a consultant to Unomedical. CBK is a full-time employee of Zealand Pharma and owns shares in Zealand Pharma. JJH has consulted for Merck Sharp & Dome, Novo Nordisk, and Roche. SM has served as a consultant or adviser to Amgen, Astra-Zeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Eli Lilly, Intarcia Therapeutics, Johnson & Johnson, Merck Sharp & Dohme, Novo Nordisk, Novartis Pharma, and Sanofi, has received a research grant from Novo Nordisk, and has received fees for speaking from Astra-Zeneca, Bristol-Myers Squibb, Eli Lilly, Merck, Sharp & Dohme, Novo Nordisk, Novartis Pharma, and Sanofi. JBJ has served as a consultant for Novo Nordisk.
Nordisk. KN serves as adviser to Medtronic, Abbott, and Novo Nordisk, owns shares in Novo Nordisk, has received research grants from Novo Nordisk, and has received fees for speaking from Medtronic, Roche, Rubin Medical, Sanofi, Novo Nordisk, Bayer, and Zealand Pharma.
References

17. ADA. Lifestyle Management - Standard of Medical Care. Diabetes Care 2017;40:S33–43
Figure 1: Schematic description of study design and treatment assessment. In seven virtual patients, 1 of 10 boluses of subcutaneous insulin was administered (t=-x) to decrease PG from 7.0 mmol/l to below 3.9 mmol/l. The insulin bolus size had to achieve predefined insulin levels when PG was 3.9 mmol/l. When PG reached 3.9 mmol/l (t=0), 1 of 17 subcutaneous glucagon boluses was administered. Treatment success of each glucagon dose was assessed on whether following peak PG was within 5 mmol/l (Green line: treatment limit) and 10 mmol/l (Blue line: Hyperglycaemia limit), and whether PG 120 min after the glucagon bolus was above 3.9 mmol/l (Red lines: Hypoglycaemia limit).
Figure 2: Proportion of patients achieving treatment criteria as a function of glucagon dose, stratified by actual to baseline serum insulin concentrations. Treatment criteria were achieved if glucagon could increase PG to a peak above 5 mmol/l (Green line) and below 10 mmol/l (Blue line), and keep PG above 3.9 mmol/l for 120 min after the glucagon bolus (Red lines). The optimum glucagon dose for each serum insulin level (Black vertical line) was chosen as the lowest dose yielding the maximal weighted success rate of the three treatments criteria.
Figure 3: Proportions of patients achieving treatment criteria as a function of glucagon dose stratified by insulin on board. Treatment criteria were achieved if glucagon could increase PG to a peak above 5 mmol/l (Green line) and below 10 mmol/l (Blue line), and keep PG above 3.9 mmol/l for 120 min after the glucagon bolus (Red lines). The optimum glucagon dose for each insulin on board (Black vertical line) was chosen as the lowest dose yielding the maximal weighted success rate of the three treatment criteria.
Figure 4: Proportion of patients achieving treatment criteria as a function of glucagon dose and stratified by the percentage of insulin on board to the total daily insulin dose. Treatment criteria were achieved if glucagon could increase PG to a peak above 5 mmol/l (Green line) and below 10 mmol/l (Blue line), and keep PG above 3.9 mmol/l for 120 min after the glucagon bolus (Red lines). The optimum glucagon dose for each percentages of insulin on board (Black vertical line) was chosen as the lowest dose yielding the
maximal weighted success rate of the three treatment criteria.
Figure 5: Optimum glucagon dose as a function of ambient insulin levels stratified by actual to baseline serum insulin concentration (upper panel), insulin on board (middle panel), and percentage of insulin on board to total daily insulin dose (lower panel).

Virtual patients performed 170 experiments per panel to obtain predefined ratios of insulin and glucagon at PG level of 3.9 mmol/l. The optimum glucagon dose to restore plasma glucose for each insulin level was chosen as the lowest glucagon dose yielding the maximal weighted success rate of the three treatment criteria 1) to increase PG above 5 mmol/l, 2) to have a peak PG below 10 mmol/l, and 3) to keep PG above 3.9 mmol/l for 120 min after the glucagon bolus.

Supplemental table 1: Table overview of treatment assessment in an in vivo dose finding study (Ranjan et al. Diabetes Obes Metab 2016). For each patient, insulin action time (IAT) and total daily insulin dose (TDD) was known and four study visits were performed. An insulin dose was given to induce mild hypoglycaemia of PG below 3.9 mmol/l. When the PG was 3.9 mmol/l, either a saline or glucagon bolus (100, 200, 300 µg) was given. The table shows the calculated insulin on board (IOB) and the normalized IOB (IOB/TDD) at the moment when glucagon was administered. Further, the right side of the table shows how many patients with the given glucagon dose could increase PG from 3.9 mmol/l to peak between 5 mmol/l (PG≥5) and 10 mmol/l (PG≤10), and keep PG above 3.9 mmol/l for 120 min after glucagon bolus (PG120≥3.9). The H-index for the overall success rate is also shown at the far right panel. The H-index for 200 and 300 µg glucagon was similar, why 200 µg is regarded as optimum glucagon dose to treat mild hypoglycemia when IOB is 0.9 U and IOB/TDD is 2.5-2.6%. 
<table>
<thead>
<tr>
<th>PTID</th>
<th>TDD, U</th>
<th>IAT, hrs</th>
<th>Glucagon, µg</th>
<th>Insulin, Unit</th>
<th>Time to PG=3.9, min</th>
<th>Se/ba-insulin</th>
<th>IOB,U</th>
<th>IOB/TDD &gt;5</th>
<th>PG &lt;10</th>
<th>PG &gt;3.9</th>
<th>All criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.3</td>
<td>5</td>
<td>0</td>
<td>1.60</td>
<td>180</td>
<td>1.5</td>
<td>0.64</td>
<td>3.2%</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>1.55</td>
<td>150</td>
<td>1.9</td>
<td>0.78</td>
<td>3.8%</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>2.40</td>
<td>150</td>
<td>2.1</td>
<td>1.20</td>
<td>5.9%</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>300</td>
<td>1.40</td>
<td>210</td>
<td>1.6</td>
<td>0.42</td>
<td>2.1%</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>16.6</td>
<td>5</td>
<td>0</td>
<td>0.30</td>
<td>60</td>
<td>1.9</td>
<td>0.24</td>
<td>1.4%</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>1.20</td>
<td>210</td>
<td>2.0</td>
<td>0.36</td>
<td>2.2%</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>0.45</td>
<td>90</td>
<td>2.3</td>
<td>0.32</td>
<td>1.9%</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>300</td>
<td>0.50</td>
<td>180</td>
<td>1.4</td>
<td>0.20</td>
<td>1.2%</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>34.5</td>
<td>5</td>
<td>0</td>
<td>3.00</td>
<td>90</td>
<td>2.2</td>
<td>2.10</td>
<td>6.1%</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>2.70</td>
<td>120</td>
<td>1.3</td>
<td>1.62</td>
<td>4.7%</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>2.40</td>
<td>90</td>
<td>2.1</td>
<td>1.68</td>
<td>4.9%</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>300</td>
<td>3.20</td>
<td>210</td>
<td>2.5</td>
<td>0.96</td>
<td>2.8%</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>4</td>
<td>37.0</td>
<td>4</td>
<td>0</td>
<td>3.10</td>
<td>180</td>
<td>1.5</td>
<td>0.78</td>
<td>2.1%</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>3.55</td>
<td>210</td>
<td>1.2</td>
<td>0.44</td>
<td>1.2%</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>1.30</td>
<td>180</td>
<td>1.4</td>
<td>0.33</td>
<td>0.9%</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>300</td>
<td>3.45</td>
<td>150</td>
<td>1.4</td>
<td>1.29</td>
<td>3.5%</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>54.7</td>
<td>4</td>
<td>0</td>
<td>1.80</td>
<td>90</td>
<td>1.1</td>
<td>1.13</td>
<td>2.1%</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>5.00</td>
<td>210</td>
<td>1.5</td>
<td>0.63</td>
<td>1.1%</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>2.75</td>
<td>150</td>
<td>1.4</td>
<td>1.03</td>
<td>1.9%</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>300</td>
<td>3.45</td>
<td>120</td>
<td>1.2</td>
<td>1.73</td>
<td>3.2%</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>29.5</td>
<td>4</td>
<td>0</td>
<td>4.50</td>
<td>210</td>
<td>1.9</td>
<td>0.56</td>
<td>1.9%</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>3.40</td>
<td>150</td>
<td>2.3</td>
<td>1.28</td>
<td>4.3%</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>3.00</td>
<td>210</td>
<td>1.3</td>
<td>0.38</td>
<td>1.3%</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>300</td>
<td>3.00</td>
<td>150</td>
<td>2.4</td>
<td>1.13</td>
<td>3.8%</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>58.3</td>
<td>4</td>
<td>0</td>
<td>4.60</td>
<td>120</td>
<td>2.4</td>
<td>2.30</td>
<td>3.9%</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>1.05</td>
<td>60</td>
<td>3.8</td>
<td>0.79</td>
<td>1.4%</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>4.60</td>
<td>180</td>
<td>2.9</td>
<td>1.15</td>
<td>2.0%</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>300</td>
<td>1.60</td>
<td>120</td>
<td>2.0</td>
<td>0.80</td>
<td>1.4%</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>2.60</td>
<td>180</td>
<td>1.7</td>
<td>0.65</td>
<td>2.4%</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>27.0</td>
<td>4</td>
<td>0</td>
<td>1.50</td>
<td>210</td>
<td>1.6</td>
<td>0.19</td>
<td>0.7%</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>2.40</td>
<td>210</td>
<td>1.7</td>
<td>0.30</td>
<td>1.1%</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>1.40</td>
<td>120</td>
<td>1.5</td>
<td>0.70</td>
<td>2.6%</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>

| Mean | 34.7  | 4.4      | 0            | 2.69          | 138.7             | 1.8           | 1.05  | 2.9%       | 0      | 8      | 0           |
|      |        |          | 100          | 2.49          | 165.0             | 1.9           | 0.76  | 2.4%       | 7      | 8      | 2           |
|      |        |          | 200          | 2.41          | 157.5             | 1.9           | 0.80  | 2.5%       | 8      | 8      | 4           |
|      |        |          | 300          | 2.25          | 157.5             | 1.8           | 0.90  | 2.6%       | 8      | 7      | 5           |

H-index
Bibliography


