Mechanism-based modelling of clinical and preclinical studies of glucose homeostasis

OSKAR ALSKÄR
Abstract


Glucose is an important nutrient and energy source in the body. However, too high concentration in the blood is harmful and may lead to several complications developing over time. It was estimated that 5 million people in the world died from complications related to diabetes during 2015. Several hormones and physiological factors are involved in the regulation of glucose homeostasis. To evaluate different aspects of glucose homeostasis and the effect of interventions, such as pharmacological treatment, glucose tolerance tests can be performed. In a glucose tolerance test glucose is administered either orally or intravenously, blood is sampled frequently and analyzed for different biomarkers. Mechanism-based pharmacometric models is a valuable tool in drug development, which can be applied to increase the knowledge about complex systems such as glucose homeostasis, quantify the effects of drugs, generate more information from clinical trials and contribute to more efficient study design. In this thesis, a new comprehensive mechanism-based pharmacometric model was developed. The model is capable of describing the most important aspects of glucose homeostasis during glucose tolerance test in healthy individuals and patients with type 2 diabetes, over a wide range of oral and intravenous glucose doses. Moreover, it can simultaneously describe regulation of gastric emptying and glucose absorption, regulation of the incretin hormones GLP-1 and GIP, hepatic extraction of insulin and the incretin effect, regulation of glucagon synthesis and regulation of endogenous glucose production. In addition, an interspecies scaling approach was developed by scaling a previously developed clinical glucose insulin model to describe intravenous glucose tolerance tests performed in mice, rats, dogs, pigs and monkeys. In conclusion, the developed mechanism-based models in this thesis increases the knowledge about short term regulation of glucose homeostasis and can be used to investigate combination treatments, drugs with multiple effects, and translation of drug effects between species, leading to improved drug development of new antidiabetic compounds.

*Keywords*: glucose homeostasis, pharmacometrics
To my family
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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## Abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CL</td>
<td>Clearance</td>
</tr>
<tr>
<td>EGP</td>
<td>Endogenous glucose production</td>
</tr>
<tr>
<td>FOCE</td>
<td>First-order conditional estimation</td>
</tr>
<tr>
<td>GIG</td>
<td>Glucose insulin glucagon</td>
</tr>
<tr>
<td>GIP</td>
<td>Gastric inhibitory polypeptide</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
<tr>
<td>HC</td>
<td>Healthy controls</td>
</tr>
<tr>
<td>HE</td>
<td>Hepatic extraction</td>
</tr>
<tr>
<td>IGI</td>
<td>Integrated glucose insulin</td>
</tr>
<tr>
<td>IIGI</td>
<td>Isoglycemic intravenous glucose infusion</td>
</tr>
<tr>
<td>IVGTT</td>
<td>Intravenous glucose tolerance test</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>MID3</td>
<td>Model informed drug discovery and development</td>
</tr>
<tr>
<td>MTT</td>
<td>Meal tolerance test</td>
</tr>
<tr>
<td>NLME</td>
<td>Nonlinear mixed effects</td>
</tr>
<tr>
<td>OFV</td>
<td>Objective function value</td>
</tr>
<tr>
<td>OGT1</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>pcVPC</td>
<td>Prediction corrected visual predictive check</td>
</tr>
<tr>
<td>PD</td>
<td>Pharmacodynamics</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetics</td>
</tr>
<tr>
<td>PsN</td>
<td>Pearl-speaks-NONMEM</td>
</tr>
<tr>
<td>SIR</td>
<td>Sampling importance resampling</td>
</tr>
<tr>
<td>T1D</td>
<td>Type 1 diabetes</td>
</tr>
<tr>
<td>T2D</td>
<td>Type 2 diabetes</td>
</tr>
<tr>
<td>V</td>
<td>Volume</td>
</tr>
<tr>
<td>WT</td>
<td>Weight</td>
</tr>
</tbody>
</table>
Introduction

Glucose homeostasis
Glucose is an important nutrient and energy source in the body. Glucose gets broken down into energy by two pathways, glycolysis and the citric acid cycle. In the cell glycolysis takes place in the cytosol whereas the enzymes of the citric acid cycle are active in the mitochondria. Protein and lipids can also be catabolized and enter into glycolysis and the citric acid cycle. However, using glucose is the most efficient way to produce energy, hence; glucose is the primary substrate for energy production in all cells. Under normal circumstances, glucose is the only substrate used for energy production in neural tissue. If the brain is deprived of glucose for any period of time, its cells will begin to die. For this reason, the body regulates the concentration of glucose though a complex system known as glucose homeostasis. Several different hormones, peptides and neural factors interact to keep plasma glucose concentrations in a narrow range between 4.4-6.4 mM. The interactions of some of the more important factors determining short term glucose homeostasis will be covered in this thesis1, 2, 3.

Insulin
Insulin is a peptide hormone (51 amino acids) that is secreted into the blood from beta cells in the pancreas. Insulin concentration increase when glucose concentrations are high. Glucose concentration in blood is reduced by insulin binding to the insulin receptor initiating cascades, which influence transport and cellular metabolism. The primary target tissues for insulin are the liver, adipose tissue and skeletal muscles where insulin either directly or indirectly facilitate the transport of glucose into the cell. Insulin also activates enzymes for glucose utilization (glycolysis) and for storage of glucose, as either glycogen or fat. Additionally insulin inhibits enzymes for glucose synthesis (gluconeogenesis), glycogen breakdown (glycogenolysis) and fat breakdown (lipolysis) to ensure anabolic metabolism7.
C-peptide
The prohormone proinsulin is cleaved into insulin and an inactive fragment known as C-peptide (31 amino acids). The two fragments are co-secreted from the beta cells of the pancreas in equimolar amounts. Insulin undergoes extensive hepatic extraction after release into blood while C-peptide does not. Hence, measurement of C-peptide can be used to assess beta cell secretion of insulin4.

Glucagon
Glucagon is a peptide hormone (29 amino acids) which is secreted from alpha cells in the pancreas. It is generally antagonistic to insulins effects on metabolism. Glucagon concentrations increase when glucose concentrations are low to prevent hypoglycemia. The liver is the primary target tissue for glucagon. Glucagon stimulates gluconeogenesis and glycogenolysis to increase circulating glucose concentrations2, 5.

Glucagon-like peptide-1
Glucagon-like peptide-1 (GLP-1) is a peptide hormone (30 amino acids) secreted from the intestinal enteroendocrine L-cells located in both the small and large intestine. GLP-1 is secreted in response to meal intake and rapidly degraded within minutes after secretion. Oral ingestion of glucose gives higher insulin secretion compared to the same glucose profile obtained by intravenous administration. This phenomenon is called the incretin effect and is the main action of GLP-1 on glucose homeostasis in addition to inhibition of glucagon secretion and gastric emptying6, 7.

Gastric inhibitory polypeptide
Gastric inhibitory polypeptide (GIP) also known as glucose-dependent insulinotropic peptide is a peptide hormone (42 amino acids). GIP is secreted from intestinal enteroendocrine K-cells, located in the upper part of the small intestine, in response to meal intake and is thereafter rapidly deactivated. GIP stimulates both insulin and glucagon secretion. Together GLP-1 and GIP constitute the two “incretin hormones”8.

Gastric emptying
To limit the amount of glucose in the small intestine ready for absorption and thereby limiting the glucose excursions in blood, the body control the rate of gastric emptying by neural regulatory mechanisms and hormones such as ghrelin an GLP-1. Caloric contents are delivered more slowly to the small
intestine than a non-caloric liquid due to negative feedback mediated by receptors in the small intestine, so that a constant rate of delivery of nutrients is maintained.\textsuperscript{9,10}

Assessments of glucose homoeostasis

To evaluate different aspects of glucose homeostasis and the potential effect of interventions different glucose tolerance tests can be performed. The tests are usually performed after an overnight fast (\(>8\) hours). In general, baseline concentrations are measured followed by administration of glucose either intravenously or orally. Blood is sampled and the concentration of glucose and other relevant biomarkers are measured to assess the impact of the intervention on glucose homeostasis. Glucose tolerance tests can also be used to test for diabetes, insulin resistance and impaired beta cell function. Many different glucose doses, sampling schedules and routes of administration are applied for various purposes. Time profiles for some of the different types of glucose tolerance tests in a healthy individual can be seen in figure 1.

Intravenous glucose tolerance test

A bolus dose (\(\sim 0.3\) g/kg) of glucose is administered after an overnight fast. In an insulin modified intravenous glucose tolerance test (IVGTT), insulin is administered, usually 20 minutes after the intravenous glucose dose, as a 5 min infusion (\(\sim 4\) mU/kg/min). Frequent sampling is performed during 3-4 hours for analysis of glucose and insulin. In healthy individuals insulin concentration increase rapidly after glucose administration, while patients with T2D have much lower stimulation of endogenous insulin secretion. Information about glucose effectiveness, insulin sensitivity and beta cell function can be derived from the test\textsuperscript{11}.

Oral glucose tolerance test

The efficiency of the body to dispose of glucose after an oral glucose dose is reflected in oral glucose tolerance. After a nights fast, an oral glucose solution (\(\sim 300\) ml) is drunk over 5 minutes. The standard glucose dose is 75 g but other doses may also be used. Sampling is often less frequent compared to an IVGTT. An oral glucose tolerance test (OGTT) corresponds more to the natural physiology compared to the IVGTT. In addition, to assess the effect of insulin and insulin secretion the OGTT also provides insight into factors that contribute to glucose tolerance, such as gastric emptying and incretins\textsuperscript{11,12}. The OGTT is also used to diagnose diabetes, where a two hour sample above 11.1 mM is a criteria for the diagnosis of diabetes\textsuperscript{13}. 
Isoglycaemic intravenous glucose infusion

In an isoglycaemic intravenous glucose infusion (IIGI) glucose is infused to mimic the glucose concentration time profile obtained in an OGTT. Any differences between the IIGI and OGTT in hormone secretion can thus not be caused by glucose since the profiles are the same. The IIGI is used to evaluate the incretin effect\textsuperscript{14,15}.

Meal tolerance test

A meal tolerance test (MTT) is similar to an OGTT but instead of administering only carbohydrate, protein and fat are also included. Meals with different composition and size are used, and they can be either liquid or solid. An example of nutrient composition used in an MTT is 75 g carbohydrates, 50 g fat and 36 g protein. MTT is the most physiologically relevant challenge to assess metabolic response\textsuperscript{16}.

Glucose clamps

In glucose clamps, glucose is “clamped” at a constant concentration by infusing glucose at varying rate. The clamp can be euglycemic, hyperglycemic or hypoglycemic referring to glucose being at, above or below the basal concentration. The glucose clamp is often combined with an insulin clamp to drive the glucose concentration in the desired direction. A hyperinsulinemic euglycemic glucose clamp is used to measure insulin sensitivity. When steady state conditions are achieved the glucose infusion rate is equal to the glucose disposal rate, this gives an estimate of insulin sensitivity\textsuperscript{11,17}.
Diabetes mellitus

Diabetes is not one but several diseases with different causes. The common denominator is that blood sugar concentration (glucose) is too high. High glucose concentration increase oxidative stress, damages blood vessels and nerves leading to complications developing over time\textsuperscript{18, 19, 20}. Some of the many complications related to diabetes are nephropathy, retinopathy and cardiomyopathy. The global prevalence of diabetes was estimated to 415 million in 2015, corresponding to 8.8% of the world population. Out of these, about 89% are having T2D, 9% having type 1 diabetes (T1D) and 2% having other types of diabetes\textsuperscript{21}. The total number of deaths in 2015 due to diabetes is estimated to be 5 million. T1D is characterized by a loss of insulin production due to autoimmune destruction of pancreatic beta cells. The other types of diabetes include gestational diabetes mellitus and several others with varying causes related to loss of insulin production and insulin resistance. T2D is catheterized by insulin resistance leading to progressive loss of beta cell insulin secretion\textsuperscript{13}. In this thesis only T2D will be covered.
Type 2 diabetes

The most abundant and most rapidly increasing type of diabetes in the world is T2D. The risk of developing T2D is determined by genetics and metabolic factors. The modern sedentary lifestyle combined with abundant food high in energy content can lead to obesity, which is the primary risk factor of developing T2D. Ethnicity, family history of diabetes and many other factors also contribute to increased risk.\textsuperscript{22, 23}

T2D starts with cells losing sensitivity to insulin, known as insulin resistance. Initially the body compensates by producing more insulin but after a while acute glucose stimulated insulin secretion is lost and only second phase insulin secretion is partially preserved. If the disease progresses further beta cell mass is gradually lost and less insulin can be produced, this leads to higher glucose concentrations in the blood.\textsuperscript{24, 25} Other T2D pathophysiological defects are reduced incretin effect, increased glucagon secretion, increased hepatic glucose output and increased visceral adipose tissue.\textsuperscript{26, 27}

Pharmacometrics

Pharmacometrics has been defined as “the science of developing and applying mathematical and statistical methods to characterize, understand, and predict a drug’s pharmacokinetic, pharmacodynamic, and biomarker-outcomes behavior”\textsuperscript{28}. Pharmacometrics can be used to give insights into complex physiological systems and to rationalize knowledge-driven decision making in the drug development process, a concept referred to as model-informed drug discovery and development (MID3).\textsuperscript{29} Further, pharmacometrics can be applied to individualize drug therapy and improve dosing strategies.

Nonlinear mixed effects models

In pharmacometrics nonlinear mixed-effects (NLME) models are often used, allowing recognition of the many levels of variability that are present in preclinical and clinical trials. Data from multiple individuals are used to fit one model, which describes the data best. A structural component, usually defined by differential equations, describes the dynamic changes over time for the typical individual (fixed effects). The variability of the data is described by random effects. Mixed effects refer to the use of both fixed and random effects. The random effects can be categorized in three types, inter-individual variability, inter-occasion variability and residual unexplained variability. Residual unexplained variability is the difference between an observation and an individual prediction and can be caused by model misspecification, errors in data recording, measurement error etc. In this thesis continuous data has been used and the general structure of an NLME model can be described by equation 1.
\[ y_{ij} = f(t_{ij}, g(\theta, \eta_i, x_i, z_i)) + h(t_{ij}, g(\theta, \eta_i, x_i, z_i), \epsilon_{ij}) \]

\( y_{ij} \) is the dependent variable, often a concentration, (e.g. \( j^{th} \) observation in individual \( i \) to be described by the model), \( f \) is the function of the structural model and \( h \) is the function of the residual error model. \( t_{ij} \) is the independent variable (e.g. time of observation), \( g \) is a vector function defining individual parameters based on the vectors of population parameters \( \theta \), \( \eta_i \) individual random effects, \( x_i \) the discrete design components (such as dose) and \( z_i \) covariates. The residual error model also includes the vector \( \epsilon_{ij} \) which describes the deviation between the model prediction and the observation. The random effects (\( \eta_i \) and \( \epsilon_{ij} \)) are generally assumed to be normally distributed with mean zero, and a variance defined by their respective covariance matrix \( \Omega \) and \( \Sigma \).

**Mechanism-based models**

Many mathematical functions can describe a set of data, a distinction between empirical and mechanistic models is often made. An empirical model is generally viewed as being less based on the current understanding about physiology and pharmacology, while a mechanistic model strives to represent the current understanding about the system of interest. Fully mechanistic models are often too complex to develop with preclinical/clinical data. Hence, the terminology, mechanism-based or semi-mechanistic is used to acknowledge the simplifications of the system that are made.

**Glucose homeostasis models**

To describe the regulation of glucose homeostasis many mathematical models have been developed\(^{30, 31}\). The model complexity varies as well as their intended purpose of use: diagnostic tests, PK/PD of different drugs, diabetes disease progression, organ level glucose homeostasis to mention a few examples. A model used in drug development is the integrated glucose insulin (IGI) model.

**The integrated glucose insulin model**

The IGI model was developed by Silber et al. in 2007\(^{32}\) to simultaneously describe glucose and insulin concentrations during IVGTTs in healthy individuals and patients with T2D. The model has since been extended to describe OGTTs in healthy individuals\(^{33}\) and patients with T2D\(^{34}\) as well as 24 hour profiles with multiple meal tests\(^{35}\). The IGI model consists of a glucose submodel with distribution into peripheral tissue and elimination from the central
compartment by two pathways, an insulin-independent and an insulin-dependent route. Glucose concentration above baseline stimulate second-phase insulin secretion and inhibit endogenous glucose production (EGP). Insulin has a basal secretion and is distributed in one compartment and eliminated by one first order route. A rapid first-phase secretion of insulin is triggered in response to an intravenous bolus dose of glucose. To be able to describe data from patients with T2D, some parameters have different values for patients with T2D and healthy individuals, such as the insulin dependent glucose clearance. Different glucose absorption models have been developed for different purposes. To describe glucose absorption in a 75 g OGTT in healthy individuals, an empirical flexible input model was used. MTT and OGTT in patients with T2D have been described by different transit absorption models. In figure 2 a graphical representation of the model for IVGTTs can be seen.

Figure 2. Schematic representation of the IGI model for IVGTTs. Broken arrows indicate control mechanisms and full arrows indicate mass flow. Gp and Gc, peripheral and central compartments of glucose; GE2 and GE1, delay compartments for control of insulin secretion and endogenous glucose production; IEPS, delay compartment for first-phase insulin secretion; I, insulin disposition compartment; IE delay compartment for insulin stimulation of glucose elimination; CLGl, CLG and Q, insulin-dependent, insulin-independent and intercompartmental glucose clearance; EGP, endogenous glucose production; CLI, insulin clearance; kIS, rate constant of first-phase insulin; kIE, kGE1 and kGE2, delay rate constants.

The model can describe the glucose doses that were used in the development (0.3 g/kg IVGTT, 75 g OGTT and 62.5 g of carbohydrate in meals). However, it cannot accurately describe multiple doses outside of this range, possibly due
to several empirical elements describing important regulatory mechanisms of glucose homeostasis. In the IGI model, the incretin effect is approximated by a function driven by the absorption rate of glucose. Moreover, the glucose absorption is described by a transit compartment model or as time dependent rates; thus ignoring regulation of gastric emptying. The first-phase insulin secretion is approximated by a dose of 704 mU of insulin entering into the central compartment, irrespectively of the amount of glucose in the bolus dose. The IGI model does not describe hepatic extraction of insulin and insulins and glucagons effect on EGP. In addition, the model does not provide predictions of GIP, GLP-1, C-peptide and glucagon during glucose tests.
The overall aim with this thesis was to improve the usefulness of mechanism-based pharmacometric models describing glucose homeostasis.

The specific aims were to:

- Develop a scaling model to describe glucose and insulin concentrations obtained during IVGTT in commonly used preclinical species such as mouse, rat, pig, dog and monkey.

- Develop a comprehensive mechanism-based model that describes:
  - The regulation of gastric emptying and glucose absorption.
  - The regulation of GIP and GLP-1 synthesis.
  - The incretin effect and hepatic extraction of insulin.
  - The regulation of glucagon synthesis.

After oral and intravenous glucose administration in both healthy controls and patients with T2D over a wide dose range.
Methods

Analysis data

Animal data (paper I)

The data included in the analysis was gathered from previously published studies\textsuperscript{36, 37, 38, 39, 40, 41}, in which IVGTTs were performed. In total data from 72 individuals were available for analysis, dogs (n=11), humans (n=24), mice (n=10), pigs (n=11) and rats (n=16). The studies are summarized in table 1.

Table 1. Summary of preclinical study designs

<table>
<thead>
<tr>
<th>Species</th>
<th>Subspecies</th>
<th>No.</th>
<th>Mean BW (g/kg)</th>
<th>Glucose dose (g/kg)</th>
<th>Insulin dose (U/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analysis data, individual level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog\textsuperscript{39}</td>
<td>Mongrel</td>
<td>11</td>
<td>28.7</td>
<td>0.3</td>
<td>0.03 at 20 min</td>
</tr>
<tr>
<td>Human\textsuperscript{40}</td>
<td>-</td>
<td>14</td>
<td>66.5</td>
<td>0.25-0.33</td>
<td>-</td>
</tr>
<tr>
<td>Human\textsuperscript{41}</td>
<td>-</td>
<td>10</td>
<td>70.0</td>
<td>0.33</td>
<td>0.03 at 20 min</td>
</tr>
<tr>
<td>Mouse\textsuperscript{36}</td>
<td>C57BL6</td>
<td>10</td>
<td>0.03</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>Pig\textsuperscript{38}</td>
<td>Large white</td>
<td>11</td>
<td>21.6</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Rat\textsuperscript{37}</td>
<td>Wistar</td>
<td>16</td>
<td>0.29</td>
<td>0.2, 0.5, 1.0</td>
<td>-</td>
</tr>
<tr>
<td><strong>External validation data, summary level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog\textsuperscript{42}</td>
<td>Beagle</td>
<td>6</td>
<td>10\textsuperscript{a}</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Human\textsuperscript{43}</td>
<td>-</td>
<td>8</td>
<td>70\textsuperscript{a}</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td>Monkey\textsuperscript{44}</td>
<td>Cynomolgus</td>
<td>7</td>
<td>3.6</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Pig\textsuperscript{45}</td>
<td>Ossabaw</td>
<td>7</td>
<td>29.4</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Rat\textsuperscript{46}</td>
<td>Sprague Dawley</td>
<td>7</td>
<td>0.25\textsuperscript{a}</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

BW, body weight
\textsuperscript{a}Imputed weight

In one of the studies performed in humans and the study in dogs, a bolus dose of insulin was administered 20 minutes after the glucose dose; so-called insulin-modified IVGTT. The inclusion criteria for included studies were; recorded body weight, repeated insulin and glucose concentrations sampled, normal body weight, normal diet, healthy untreated glucose homeostasis and IVGTT performed in a conscious state. Body weights range from 20 g to 70.3 kg from the lightest mouse to the heaviest human.

To validate the models ability to describe data from other studies, the average and standard deviation of glucose and inulin concentrations from published IVGTT studies performed in rats, pigs, monkeys, humans and dogs
were digitalized\textsuperscript{42, 43, 44, 45, 46}. The studies are summarized in Table 1. Monkeys, pigs and dogs were administered an intravenous glucose dose of 0.5 g/kg, rats 1 g/kg and humans 0.3 g/kg. The reported average body weight of monkeys and pigs were 3.6 kg and 29.4 kg respectively. For humans, rats and dogs the body weights were not reported. However, they were known to be lean, hence, 70 kg, 0.25 kg and 10 kg were used in the simulations. Glucose and insulin were simulated for 1000 subjects of each species using the reported insulin and glucose baselines with 25\% and 10\% interindividual variability, respectively. The mean insulin and glucose concentrations of the simulated data was calculated and overlaid on the digitalized summary measurements to assess model prediction accuracy.

Clinical data (paper II-V)

The data used in this analysis originates from a study by Bagger et al.\textsuperscript{47, 48}. The study included 8 patients with T2D and 8 sex-, BMI- and age-matched healthy individuals. The participants were studied at 6 different occasions; first 3 OGTTs with doses of 25, 75, and 125 g of glucose were performed. On the following occasions 3 IIGIs were performed, that mimicked the glucose profile from each of the OGTTs. Blood was frequently sampled for 240 minutes (20 samples of glucose, 15 samples of C-peptide and insulin, 10 samples of acetaminophen, GIP, GLP-1 and glucagon). The lower limit of detection (LOD) for glucagon was 1 or 2 pM, 76 observations were below LOD, which corresponds to 10.2\% of the observations in healthy controls and 5.6\% of the observations in patients with T2D. For the other measured biomarkers, the number of samples below LOD or the limit of quantification were minor.

To obtain more information on insulin secretion, insulin data from four previously published IVGTT studies were also included in the analysis\textsuperscript{32, 40, 41, 49}. Three studies included healthy individuals (totaling 64 individuals) and one including patients with T2D (42 individuals). A bolus dose of 0.25-0.33 g/kg was administered and blood was frequently sampled up to 180-240 minutes for determination of glucose and insulin concentrations. In one study of healthy individuals and the study with patients with T2D insulin was infused (0.03 U/kg for healthy and 0.05 U/kg for patients with T2D) over five minutes, twenty minutes after the glucose dose. The studies are summarized in table 2.
Table 2. Summary of clinical study designs

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Population</th>
<th>No.</th>
<th>Glucose dose (g)</th>
<th>Insulin dose (U/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a OGTT</td>
<td>HC + T2D</td>
<td>8+8</td>
<td>25 – 125</td>
<td>-</td>
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<tr>
<td>1b IIGI</td>
<td>HC + T2D</td>
<td>8+8</td>
<td>Corresponding to 1a</td>
<td>-</td>
</tr>
<tr>
<td>2 IVGTT</td>
<td>HC</td>
<td>40</td>
<td>0.3 g/kg</td>
<td>-</td>
</tr>
<tr>
<td>3 IVGTT</td>
<td>HC</td>
<td>14</td>
<td>0.33 g/kg</td>
<td>-</td>
</tr>
<tr>
<td>4 IVGTT+insulin</td>
<td>HC</td>
<td>10</td>
<td>0.25-0.33 g/kg</td>
<td>0.03 at 20 min, 5 min inf</td>
</tr>
<tr>
<td>5 IVGTT+insulin</td>
<td>T2D</td>
<td>42</td>
<td>0.3 g/kg</td>
<td>0.05 at 20 min, 5 min inf</td>
</tr>
</tbody>
</table>

Allometric scaling

Several methods of scaling between species exist. To scale pharmacokinetic parameters between different species, allometric scaling has been widely used. The method is based on the observation that many physiological factors such as blood flow and basal metabolic rate are related to the size of the animal50. One easily obtainable size descriptor is body weight. The relationship is described by the allometric equation, equation 2.

\[ Y = a \times WT^b \]

where \( b \) is the slope of the regression line on the log-log scale, \( a \) is the intercept and \( WT \) is body weight. The allometric equation is used to scale a pharmacokinetic parameter determined in humans to another animal through equation 3

\[ P_A = P_H \times \left(\frac{WT_A}{WT_H}\right)^b \]

where \( WT_H \) is the weight of humans (70 kg in this analysis), \( WT_A \) is the weight of the animal of interest, \( b \) is the allometric exponent, \( P_H \) is the parameter value in humans, \( P_A \) is the parameter value in the animal. It has been shown that the allometric exponent for volume (V) and clearance (CL) often takes the values of 1 and 0.75, respectively51, 52. The values of the exponent have been justified by transport of materials through space-filling fractal networks of branching tubes53.

Software

Non-Linear Mixed Effect Models version 7.3 (NONMEM), with the first order conditional estimation (FOCE) method and the differential equation solver ADVAN13 was used for the population data analysis54. Data management,
plotting, and post processing of NONMEM output was conducted in R\textsuperscript{55}. The R packages Xpose\textsuperscript{56} and ggplot2\textsuperscript{57} was used to create plots. Perl-speaks-NONMEM (PsN) was used as a tool to facilitate modelling with NONMEM and for model evaluation\textsuperscript{58}. Pirana was used as runrecord\textsuperscript{59}.

Parameter estimation and model selection (paper I)

Model selection was primarily guided by simulation-based diagnostics, such as prediction corrected visual predictive checks (pcVPCs)\textsuperscript{60} to assess the models’ predictive performance. In addition, model selection was guided by physiological plausibility, changes in objective function value (OFV), goodness-of-fit plots and parameter uncertainty. A difference of at least 6.63 in OFV was considered statistically significant for hierarchical models with one parameter difference. This corresponds to a significance level of 0.01 when comparing nested models with one degree of freedom. The sampling importance resampling (SIR) method was used to obtain parameter uncertainty for the final model\textsuperscript{61}.

Parameter estimation and model selection (paper II-V)

During the development of the models describing acetaminophen and glucose, GIP and GLP-1, C-peptide and insulin and glucagon, the observed concentrations of the relevant regulators were used as time varying covariates to reduce runtime and complexity of the model. The model development was guided primarily by mechanistic considerations, OFV, plausibility of parameter estimates, parameter uncertainty and graphical assessment. To assess the models predictive performance pcVPCs were used. A significance level of $\alpha=0.001$ was used in paper II, III and IV and $\alpha=0.01$ was used in paper V, which corresponds to a difference in OFV of 10.83 and 6.63 respectively for nested modes differing by one parameter. The differences between individual parameters were described using random effects, which were assumed to be normally distributed with mean zero. The distribution of the individual parameters around the typical population value was assumed to be log-normal or logit transformed for parameters bound between 0 and 1. An additive model was applied to describe the residual error of the log-transformed data. The residual error was assumed to arise from a distribution with mean zero and a variance, which was estimated. NONMEM covariance step and SIR were used to determine parameter uncertainty\textsuperscript{61}. 
Turnover models

Endogenous biomarkers can be described by turnover models where the net baseline effect is a balance between the rate of elimination and the rate of production. Production and elimination can be complex but in general one step is rate-limiting, making simplification to first-order rates descriptive of the mechanisms. A turnover model is described by the differential equation

\[ \frac{dA}{dt} = k_{in} - k_{out} \times A \]

where \( k_{in} \) is the production rate, \( k_{out} \) is the elimination rate constant and \( A \) is the concentrations of biomarker. The baseline concentration (\( A_{ss} \)) of the biomarker is given by equation 5

\[ A_{ss} = \frac{k_{in}}{k_{out}} \]

The biomarker concentration can be affected by four different mechanisms: stimulating elimination, inhibiting elimination, stimulating production or inhibiting production. For biomarkers involved in glucose homeostasis the effects are in general on the production.

Modelling of endogenous feedbacks systems

Modelling of endogenously produced compounds requires definition of the basal production. In this work all individuals were in fasting state when the sampling started. The basal production is generally defined as the rate that upholds steady state by multiplying the fasting concentration with the clearance of the compound as described in equation 6

\[ EP = C_{ss} \times CL \]

EP is the endogenous production, \( C_{ss} \) is the fasting concentration and CL is the clearance of the compound. Modelling of feedback systems requires that no stimulation or inhibition occurs at the basal state, hence, the ratio or the difference of the effector concentration to its baseline concentration is used. In this work the functions describing the regulatory systems are linear (equation 7), power (equation 8) and \( E_{\text{max}} \) (equation 9) functions.
EFF is the effect, α is the slope of the linear relationship, C is the concentration, P is the power controlling the shape of the effect, E_{\text{max}} is the maximum effect, EC_{50} is the concentration giving 50% of the effect. A linear model will increase or decrease infinitely which makes extrapolation more difficult. A power function where the power is between 0 and 1 will have less of an increase at higher concentration and an E_{\text{max}} function will have a maximal effect at high concentrations. The effect of the compound can be modeled as either direct or delayed, reflecting distribution from blood to the tissue or initiation of cascade reactions.

**Literature values (paper II-V)**

With the current dataset of GIP, GLP-1 and glucagon it is not possible to identify changes in input from output. Hence the elimination half-life of GIP, GLP-1 and glucagon was set to previously reported values of 6, 4 and 7.5 minutes. Glucose disposition parameters were set to the values of the IGI model, apart from insulin dependent clearance that was estimated. To be able to describe glucose movement through the small intestine it was assumed that the total transit time was 240 minutes and that the duodenum, jejunum and ileum comprised 8%, 37% and 55% of the total length respectively. To separate gastric emptying from absorption of acetaminophen, the absorption half-life of acetaminophen was set to previously reported value of 5 minutes. The first order emptying rate of a non-caloric liquid was also set to a half-life of 5 minutes.
Results

Interspecies scaling (paper I)

Scaling the first-phase insulin secretion using an exponent of 0.75, corresponding to allometric exponent of clearance, gave a better fit to the data compared to using an exponent of 1. Estimation of the allometric exponents gave values of -0.26 for rate constants, 0.92 for glucose volumes, 1.05 for insulin volume and 0.89 for clearances. The power functions describing glucose effect on glucose production and insulin secretion were determined to be unrelated to size of the animals. Thus, the values were kept fixed to the human values. With the allometric relationships determined dogs showed slower first-phase secretion than the model predicted and the corresponding parameter (k_{IS}) was estimated to be 0.093 min^{-1} (19\% of the allometrically scaled value). The model also predicted an earlier insulin peak for pigs and k_{IS} was estimated to be 0.085 min^{-1} (16\% of the allometrically scaled value). There was no additional benefit of estimating the magnitude of the first-phase secretion when the difference in rate was accounted for. This result can be interpreted as the same weight-adjusted amount of insulin is secreted as for the other species, however with slower release. Insulin-dependent glucose clearance (CL_{GI}) was estimated to be three times higher than the allometrically scaled value since the model predicted slower glucose elimination in pigs than indicated by data. Studies suggests that glucose effectiveness (insulin-independent glucose disposal) is the main determinant of intravenous glucose tolerance in rodents\textsuperscript{70,71}. A proportionality factor was estimated for the two glucose clearances (CL_{G} and CL_{GI}) to investigate this. The estimated values of the factors on were 1.66 and 0.28 for CL_{G} and CL_{GI} respectively. Using the theoretical allometric exponents with estimated proportionality factors instead of the estimated allometric exponents gave an increase in OFV to 34.7 with two fewer estimated parameters. A simpler model that incorporates current knowledge of rodent glucose metabolism was considered preferable over the model with estimated exponents. Figure 3 shows an overall good description of the data for all different species using the final model.
Figure 3. Internal model evaluation of the final model. In the VPCs the observations are compared with simulated glucose and insulin data for all included species. The solid line is the median based on the observed data. The shaded area is the 95% confidence interval around the median based on the simulated data.

Simulation of published IVGTT studies in dogs, humans, monkeys, pigs and rats (figure 4) show that the proposed model as well as a model without estimated species specific parameters can predict glucose and insulin concentrations in other studies of the investigated species. As well as in one other species than the model was developed on.
Figure 4. External model evaluation of a basic allometric and the final model. Simulations of published intravenous glucose tolerance test studies in rats, pigs, dogs, humans and monkeys. The black dots and error bars are the observed means and standard deviations. Lines are the mean of 1000 simulated animals. The dashed and solid lines are the simulated mean of the model without species adaptations and the final model including species adaptations, respectively.

Gastric emptying and glucose absorption (paper II)

Inhibition of gastric emptying was best described by inhibition of duodenal glucose as described by equation 10

\[ k_{SD} = k_w \ast \left( 1 - \frac{G_D(t)^\gamma}{IG_{D50}^\gamma + G_D(t)^\gamma} \right) \]

where \( k_{SD} \) is the rate constant of gastric emptying, \( k_w \) is the emptying rate constant of a noncaloric liquid, \( IG_{D50} \) is the amount of glucose in the duodenum that gives 50\% of the maximal inhibition of gastric emptying. The Hill factor \( \gamma \) describes the shape of the function. And \( G_D \) is the amount of glucose in the duodenum. A small but statistically significant effect of GLP-1 on gastric emptying was found, due to high parameter uncertainty this relationship was not included in the final model. The first-pass effect of acetaminophen was found to be saturable and therefore modeled as an amount of acetaminophen disappearing from the small intestine. Allowing a short lag period of 5 minutes representing the time after dose before gastric emptying starts improved the description of the data substantially.
One $E_{\text{max}}$ function for each intestinal segment described the oral glucose data well. The same transporter affinity for glucose was used for all three segments since the transporter was expected to be the same across the entire small intestine. To reflect differences in density of transporters and surface area in the segments segment-specific maximum rates of absorption were used. The first-pass effect of glucose was modeled as a proportionality factor ranging from 0 to 1. The rate of glucose appearance in plasma was described by equation 11

$$RA = \text{FP}_G \left( \frac{RA_{\text{maxD}} \cdot G_D(t)}{K_{MG} + G_D(t)} + \frac{RA_{\text{maxJ}} \cdot G_J(t)}{K_{MG} + G_J(t)} + \frac{RA_{\text{maxI}} \cdot G_I(t)}{K_{MG} + G_I(t)} \right)$$

where $\text{FP}_G$ is the first-pass effect on glucose, $K_{MG}$ is the amount of glucose giving 50% of the maximum rate of absorption. $RA_{\text{maxD}}, RA_{\text{maxJ}}, RA_{\text{maxI}}$ represents the maximum rates of absorption from the duodenum, jejunum and ileum, respectively. $G_D, G_I, G_J$ represent the glucose amount in each intestinal segment. The only absorption parameter that was significantly different between healthy subjects and patients with T2D was the first pass effect; healthy having a value of 0.91 while patients with T2D was estimated to 1. The predicted rate of glucose absorption for patients with T2D as well as healthy controls for the three different glucose doses are shown in figure 5.

![Figure 5](image)

*Figure 5.* Predicted glucose absorption rate over time for the three different glucose doses (25, 75 and 125 g). Each line represents one individual. Solid lines indicate healthy controls and dashed lines indicate patients with T2D.

Higher glucose doses gave higher absorption rates as well as increased variability in absorption rate. For the highest glucose dose, some subjects did not absorb the entire glucose dose within the time frame of the experiment, whereas others completed absorption 120 minutes after administration.
Secretion of GIP and GLP-1 (paper III)

GIP was described by a turnover model with the elimination rate constant fixed corresponding to a half-life of 6 minutes. Secretion of GIP was stimulated by duodenal glucose as described by the stimulation function ($F_{GIP}$) in equation 12

$$F_{GIP}(G_D) = 1 + \alpha_{GIP} \cdot G_D(t)$$  \hspace{1cm} 12

where $\alpha_{GIP}$ is the slope of the linear relationship between duodenal glucose ($G_D$) and basal secretion of GIP. More complex models were not statistically significant and were determined with higher uncertainty. A model with duodenal glucose driving the stimulation of GIP secretion gave the lowest OFV compared to jejunal and ileal glucose (41 and 164 points higher respectively). The result can be explained by that K cells are primarily found in the proximal small intestine. Inclusion of additional stimulatory effects of either jejunal or ileal glucose gave no further improvement in model fit.

GLP-1 was also described by a turnover model with the elimination rate constant fixed to a half-life of 4 minutes. Secretion of GLP-1 was stimulated by jejunal glucose as described by the below function ($F_{GLP-1}$), equation 13

$$F_{GLP1}(G_J) = 1 + \alpha_{GLP1} \cdot G_J(t)$$  \hspace{1cm} 13

where $\alpha_{GLP1}$ is the slope of the linear relationship between jejunal glucose ($G_J$) and basal secretion of GLP-1. A sigmoidal $E_{max}$ model described the data better than the linear model (dOFV = -23.4, df = 2). However, the parameter uncertainty was high, hence the less complex linear model was preferred. A model with jejunal glucose driving the stimulation of GLP-1 secretion gave the lowest OFV compared to duodenal and ileal glucose (13 and 26 points higher respectively). Inclusion of additional stimulatory effect of either duodenal and ileal glucose gave no further improvement in model fit.

Correlations between residuals (14.5%) of GIP and GLP-1 were included since the concentrations were assayed in the same sample. Even though the correlation was small, this addition decreased the uncertainty of the other parameters. Baseline GIP and GLP-1 was also negatively correlated with their respective slope of stimulation (-85.2% and -51.5 %). No differences between healthy individuals and patients with T2D were found.
Beta cell function (paper IV)

Secretion of insulin and C-peptide from the beta-cells was described using the mathematical beta cell model developed by Overgaard et al. The model was based on the distributed threshold hypothesis assuming that insulin/C-peptide is stored in pools of either active or passive vesicles. The two pools show different thresholds for secretion. Changes in plasma glucose concentrations will change the distribution between pools. Provision of new insulin/C-peptide to the different pools is also regulated by glucose concentrations. The passive pool also responds to rapidly increasing concentrations of glucose and is transferred to the active pool.

By coupling the shared secretion model with disposition models for C-peptide and insulin it was possible to characterize the hepatic extraction of insulin. C-peptide disposition has previously been described using a two-compartment model with first order elimination implemented with rate constants. This model was used in the current work with re-estimation of parameters. Insulin disposition has previously been described by a one-compartment model with first order elimination.

The model by Overgaard et al. did not describe beta cell secretion for patients with T2D. Hence, two structural modifications were made to be able to describe the data for this group. 1) The first-phase secretion of insulin/C-peptide from the passive to the active vesicles was excluded from the model. 2) The fraction of active vesicles was found to be independent of glucose and thus fixed to the fasting condition. The model performance was good for the different IVGTTs and is illustrated in figure 6.
Figure 6. Prediction corrected visual predictive check comparing observations with simulated data for all four IVGTTs performed in patients with T2D and healthy individuals. The solid line is the median based on the observed data, dashed lines are the 97.5\textsuperscript{th} and 2.5\textsuperscript{th} percentile of the observed data. Shaded areas are the 95\% confidence interval around the different percentiles based on the simulated data.

Hepatic extraction of insulin

The hepatic extraction of insulin was described by an inhibitory Emax function, equation 14

$$HE = HE_b \left(1 - \frac{\Delta SR^{hHE}}{HE_{50}^{hHE} + \Delta SR^{hHE}}\right)$$  

where HE\textsubscript{b} (estimated to be 57\%) is the fasting hepatic extraction for the baseline secretion rate. $\Delta SR$ is the difference from basal secretion rate. This function provides a continuous reduction to 0\% that occurs in some individuals at maximum secretion in the IVGTTs. A slope model was also investigated, however when the secretion rate was high this function gave a nonphysiological negative hepatic extraction, the $E_{\text{max}}$ model was selected since it also provided a better description of the data ($dOFV = -39$). The time courses of hepatic extraction for the OGTTs are shown in figure 7.
Figure 7. Time course for the hepatic extraction during the oral glucose tolerance tests (OGTTs) in patients with T2D (right) and healthy individuals (left). Light, medium and dark blue represents the average hepatic extraction for the 25 g, 75 g and 125 g OGTT respectively.

No significant differences in hepatic extraction were found between healthy individuals and patients with T2D. The differences seen in figure 7 are due to differences in secretion rate. The average hepatic extraction in the three different OGTTs (25 g, 75 g, 125 g) were 52%, 45%, and 38% for healthy individuals and 54%, 49% and 44% for patients with T2D.

Incretin effect

Effects of GIP and GLP-1 were included on relevant parts of the mathematical beta cell model to describe the increased secretion of C-peptide and insulin during OGTTs compared with the IIGIs. In healthy individuals, both hormones were found to have a stimulatory effect on the maximum steady state provision of new insulin/C-peptide and the redistribution rate constant from passive to active vesicles. The effects of GIP were determined with high uncertainty and were simplified to linear relationships. Patients with T2D are known to have severely decreased insulinotropic effect of GIP\textsuperscript{74, 75, 76}. Hence, no effect of GIP was included for patients with T2D. The effect of GLP-1 on the steady state provision was not significant for patients with T2D (dOFV = -5.7). The stimulatory effects of GIP and GLP-1 on steady state provision were described by equation 15

\[
P(G, \infty) = \begin{cases} 
\frac{E_{\text{max}} \cdot G^h}{E_{\text{IC}50} + G^h} \cdot \left(1 + \frac{E_{\text{max}, \text{GLP1,Emax}} \cdot \Delta \text{GLP1}}{E_{\text{IC}50, \text{GLP1,Emax}} + \Delta \text{GLP1}}\right) \cdot \left(1 + S_{\text{GLP1,Emax}} \cdot \Delta \text{GIP}\right) & \text{if HC} \\
\frac{E_{\text{max}} \cdot G^h}{E_{\text{IC}50} + G^h} & \text{if T2D}
\end{cases}
\]
where ΔGIP and ΔGLP-1 is the change from baseline concentration. To avoid a negative feedback of the incretin hormones when concentrations were below baseline the differences were set to zero. E_{max,GLP1,Emax} is the maximal effect of ΔGLP1 on steady state provision. EC\text{50,GLP1,Emax} is the concentration of ΔGLP-1 that produces 50% of the maximal effect. SL_{GIP,Emax} is the slope of the linear relationship with ΔGIP. The stimulatory effect of incretin hormones on the rate of vesicle activation was described by equation 16

\[
k_{rd} = \begin{cases} 
    k_{rd,0} \left( 1 + \frac{E_{max,GLP1,krd} \cdot \Delta GLP1}{EC_{50,GLP1,krd} + \Delta GLP1} \right) \left( 1 + \frac{SL_{GIP,krd} \cdot \Delta GIP}{EC_{50,GIP,krd} + \Delta GIP} \right) & \text{if HC} \\
    k_{rd,0} \left( 1 + \frac{E_{max,GLP1,krd} \cdot \Delta GLP1}{EC_{50,GLP1,krd} + \Delta GLP1} \right) & \text{if T2D} 
\end{cases}
\]

where \(k_{rd,0}\) is the distribution rate constant from passive to active vesicles. \(E_{max,GLP1,krd}\) is the maximal effect of ΔGLP-1 on packet distribution. \(EC_{50,GLP1,krd}\) is the concentration of ΔGLP-1 that gives 50% of the maximal effect. \(SL_{GIP,krd}\) is the slope of the linear relationship with ΔGIP. No significant differences between healthy individuals and patients with T2D was found in the parameters related to the effect of GLP-1 on packet distribution.

In general, the model was able to capture both C-peptide and insulin concentrations in all glucose challenges in both patients with T2D and healthy individuals. Figure 8 show that for healthy individuals the beta cell secretion for the 125 g OGTT was under-predicted, while for patients with T2D the dose response in study 1 was well captured (figure 9).

![Figure 8](image_url)

*Figure 8.* Time courses of C-peptide concentrations for healthy individuals stratified on study type and glucose dose. The dot is the median of the data, the error bars show the 95% confidence interval around the median. The red line is the median of the individual predictions; blue line is the median of the population predictions.

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Figure 9. Time courses of C-peptide concentrations for patients with T2D stratified on study type and glucose dose. The dot is the median of the data, the error bars show the 95% confidence interval around the median. The red line is the median of the individual predictions; blue line is the median of the population predictions.

Regulation of glucagon secretion (paper V)

During both OGTT and IIGI, glucagon concentrations decrease quickly and stay suppressed under baseline throughout the length of the study, even though insulin and glucose have returned to baseline, this can be seen in figure 10.

Figure 10. Observed median time courses of glucagon (gray), insulin (red) and glucose (blue) as difference from baseline. Solid lines represent healthy individuals, dashed lines represent patients with T2D.
The magnitude and time course of the initial suppression could be captured with a turnover model, where the production was inhibited by glucose and insulin concentrations above baseline in a power model. However, glucagon concentration increased too rapidly when insulin and glucose returned to baseline. A model where glucose potentiates the power model (more negative power) over time through a series of transit compartments was estimated to capture the prolonged suppression. This model allows for initial rapid suppression when glucose and insulin concentrations are high as well as sustained inhibition after the concentrations have returned to baseline. Two transit compartments gave a sufficient description of the data while keeping the model complexity low. Different baseline concentrations of glucagon were estimated for healthy individuals and patients with T2D, patients having higher concentrations. No additional significant parameter differences were found between healthy individuals and patients with T2D. Considerable variability was seen in the baseline glucagon concentration between both subjects and occasions. Hence, between subject variability was estimated (49%) as well as between occasion variability (23%). The glucagon baseline was also found to be negatively correlated (-50%) with the magnitude of inhibition of glucagon secretion.

The increased secretion during OGTT was described by stimulation of GIP, equation 17

\[ GIP_{eff} = \left( \frac{GIP}{BGIP} \right)^{GIPW} \]  

where GIPW is the power describing the magnitude of GIP stimulation on glucagon synthesis, BGIP is the baseline concentration of GIP and GIP is the time varying concentration of GIP. Patients with T2D were found to have stronger GIP stimulation compared to healthy individuals (0.279 and 0.169 respectively). Effect of GLP-1 that is known to have inhibitory effect on glucagon secretion was not identified.

A comprehensive glucose homeostasis model (paper V)

Connecting glucagon with the regulation of EGP was done through the GIG index introduced by Schneck et al.\textsuperscript{77}. This allowed for incorporation of glucose, insulin and glucagon to exert effect on EGP, equation 18

\[ GIG = \left( \frac{G_E}{G_{ss}} \times \frac{I_E}{I_{ss}} \times \frac{G_{Nss}}{G_{NE}} \right)^{EGPW} \]
GNe, Ie and Ge are the delayed glucagon, insulin and glucose concentration respectively. GNss, Iss, Gss are the baseline concentrations of glucagon, insulin and glucagon respectively. EGPPW controls the magnitude of effect on EGP.

With a link between glucagon and EGP the developed sub models for glucagon, Insulin and C-peptide, gastric emptying, glucose, GIP and GLP-1 were connected in one mechanistic model describing the regulation of glucose homeostasis during glucose tests. A schematic illustration of the full model can be seen in figure 11. Overall, the model describes the dose response well for all dependent variables for both patients with T2D and healthy individuals. The predictive performance of the model is show in figure 12-25.
Figure 11. Schematic illustration of the comprehensive glucose homeostasis model. Small dashed circles represent effect delay, dashed lines represent control mechanisms, full lines represent mass transfer, and colored circles represent compartments with observations. P; provision of new insulin/C-peptide. I\textsubscript{Passive}; compartment for passive packets. I\textsubscript{Active}; compartment for active packets. f(G); fraction of active packets at the glucose concentration G. Ph1; distribution of insulin/C-peptide from passive to active packets as consequence of rising glucose concentration. k\textsubscript{rd}; redistribution rate constant from passive to active packets. SR; secretion rate. C\textsubscript{pepC}; central compartment for C-peptide. C\textsubscript{pepP}; peripheral compartment for C-peptide. k\textsubscript{1} and k\textsubscript{2}; intercompartmental rate constants of C-peptide. k\textsubscript{3}; elimination rate constant of C-peptide. Insulin; distribution compartment for insulin. CL\textsubscript{I}; clearance of insulin. HE; hepatic extraction. k\textsubscript{in,GLP-1}; GLP-1 synthesis rate. k\textsubscript{out,GLP-1}; GLP-1 elimination rate constant. k\textsubscript{in,GIP}; GIP synthesis rate. k\textsubscript{out,GIP}; GIP elimination rate constant. k\textsubscript{SD}; gastric emptying rate constant. k\textsubscript{DJ}; transfer rate constant from duodenum to jejunum. k\textsubscript{JI}; transfer rate constant from jejunum to ileum. Q; intercompartmental clearance of glucose. CL\textsubscript{G}; insulin independent glucose clearance. GL\textsubscript{G}; insulin dependent glucose clearance. EGP; endogenous glucose production. k\textsubscript{in}; rate of glucagon synthesis. k\textsubscript{out}; is the elimination rate constant of glucagon. k\textsubscript{kin,pot}; is the rate for the baseline power of glucose and insulin inhibition. k\textsubscript{out,pot}; is the rate constant of transfer between transit compartments.
Figure 12. Prediction corrected visual predictive check comparing observations with simulated acetaminophen data for healthy stratified on glucose dose. The solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data.

Figure 13. Prediction corrected visual predictive check comparing observations with simulated GIP data for patients with T2D stratified on glucose dose. The solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data.
Figure 14. Prediction corrected visual predictive check comparing observations with simulated GIP data for healthy stratified on glucose dose. The solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data.

Figure 15. Prediction corrected visual predictive check comparing observations with simulated GIP data for patients with T2D stratified on glucose dose. The solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data.
Figure 16. Prediction corrected visual predictive check comparing observations with simulated GLP-1 data for healthy stratified on glucose dose. The solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data.

Figure 17. Prediction corrected visual predictive check comparing observations with simulated GLP-1 data for patients with T2D stratified on glucose dose. The solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data.
Figure 18. Prediction corrected visual predictive check comparing observations with simulated C-peptide data for healthy stratified on glucose dose and study type. The solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data.

Figure 19. Prediction corrected visual predictive check comparing observations with simulated C-peptide data for patients with T2D stratified on glucose dose and study type. The solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data.
Figure 20. Prediction corrected visual predictive check comparing observations with simulated insulin data for healthy stratified on glucose dose and study type. The solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data.

Figure 21. Prediction corrected visual predictive check comparing observations with simulated insulin data for patients with T2D stratified on glucose dose and study type. The solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data.
Figure 22. Prediction corrected visual predictive check comparing observations with simulated glucagon data for healthy stratified on glucose dose and study type. The solid black line is the median based on the observed data. Shaded grey area is the 95% confidence interval around the median based on the simulated data. The lower panels show 95% confidence intervals around the simulated median of the fraction below LOD of 1 pM. The blue line is the proportion of the data that is below LOD of 1 pM.
Figure 23. Prediction corrected visual predictive check comparing observations with simulated glucagon data for patients with T2D stratified on glucose dose and study type. The solid black line is the median based on the observed data. Shaded grey area is the 95% confidence interval around the median based on the simulated data. The lower panels show 95% confidence intervals around the simulated median of the fraction below LOD of 1 pM. The blue line is the proportion of the data that is below LOD of 1 pM.
Figure 24. Prediction corrected visual predictive check comparing observations with simulated glucose data for healthy stratified on glucose dose and study type. The solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data.

Figure 25. Prediction corrected visual predictive check comparing observations with simulated glucose data for patients with T2D stratified on glucose dose and study type. The solid line is the median based on the observed data. Shaded area is the 95% confidence interval around the median based on the simulated data.
Discussion

Interspecies scaling (paper I)

It was shown in paper I that the basic principles of allometric scaling can be applied to a complex homeostatic system to enable translational scaling between species. Suitable scaling for parameters that are not rate constants, volumes nor clearance was investigated. It was shown that the first-phase insulin secretion scaled better with an allometric exponent closer to 0.75 than 1, which supports the observation that metabolic processes generally scale well with an allometric exponent of 0.75\(^{50}\). Allometric scaling of the control mechanisms in the IGI model (GPRG and IPRG) did not improve the fit. This indicates that the longitudinal glucose in relation to the baseline for control mechanisms is independent of species and weight. Whether this is unidentifiable with the current data or indeed a way to scale control mechanisms remains to be further investigated.

Pigs and dogs showed slower first-phase insulin secretion. Differences in diet may affect insulin secretion. To avoid variability due to this, only omnivores with similar diet to humans were selected in this study. Despite this, differences were present. The first-phase insulin secretion is only occurring with brisk increases in glucose concentration such as an IVGTT. These unusually rapid increases in glucose concentration may highlight species differences, although only omnivores were investigated. In OGTTs or meal tolerance tests that are more frequently used in drug development, first-phase secretion of insulin is not present. Hence, accurately predicting first-phase insulin secretion may be less relevant.

The insulin-dependent glucose clearance was shown to be three times higher than the allometrically scaled value for pigs. This indicates that when performing extrapolations of insulin sensitivity to humans, the size of healthy pigs should not be considered, but rather as a one-to-one comparison.

It has been proposed that insulin-independent glucose disposal is the major determinant of glucose tolerance in rodents\(^{70, 71}\). This was supported in our analysis where the insulin-dependent glucose clearance and the insulin-independent glucose clearance was estimated to be 28% and 166% of the allometrically scaled values respectively, leading to a change in the ratio between the two pathways by a factor of 6.
Gastric emptying and glucose absorption (paper II)

A saturable rate of absorption of glucose is likely considering the high concentrations in the glucose solutions, the highest glucose dose of 125 g corresponds to a concentration of 2.3 M, about 4 times more sugar than in a can of coca cola. Since the subjects were fasted, we assume that the solution does not undergo further dilution in the gastrointestinal tract during the OGTTs. Thus, this concentration is 8 times higher than the highest concentration reported in a study\(^7\)\(^8\), where a curvature is present in the glucose absorption at higher concentrations. Glucose absorption profiles for a 75 g OGTT determined using double-tracer techniques are similar to the model predicted absorption profiles in terms of maximum rate and time to maximum rate\(^7\)\(^9\). When the maximum absorption rates are normalized with their respective length, the rates of absorption in the duodenum, jejunum and ileum are 0.073, 0.056 and 0.024 respectively, indicating that glucose transporter density declines along the small intestine, which has been observed by Yoshikawa et al.\(^8\)\(^0\) in mice.

Duodenal glucose and GLP-1 are highly correlated since glucose in the small intestine stimulates GLP-1 secretion. Thus, it was difficult to separate the effects of duodenal glucose and GLP-1 on gastric emptying from each other and only the effect of duodenal glucose was retained in the model. To describe the inhibition by GLP-1 a different study design would be needed, one that decorrelates duodenal glucose from GLP-1. Inclusion of a lag-time of gastric emptying improved the model fit. Solid meals often display a lag phase before gastric emptying starts, which increases with increased complexity of the ingested food, reflecting the milling process. A reason that a lag phase is not observed for liquids in some studies could be that the first sample is taken after the short lag phase has ended\(^8\)\(^1\). The rate constant of gastric emptying was set corresponding to a half-life of 5 minutes, adding the 5 minute lag phase gives a time to 50% of gastric emptying of 10 minutes. This is similar to previously reported values of 6 to 15 minutes for volumes ranging from 150 to 750 mL\(^8\)\(^2\),\(^8\)\(^3\),\(^8\)\(^4\).

Secretion of GIP and GLP-1 (paper III)

Glucose in the small intestine stimulates secretion of GIP and GLP-1. However, no data on glucose in the small intestine was available. Hence, the model developed in paper II was applied to predict the expected glucose in the duodenum, jejunum and ileum. This gives stimulation of GLP-1 and GIP secretion being dependent on the amount of glucose in different parts of the small intestine. If the glucose dilution is assumed to be constant and only that of starting dilution (300 ml), the slope of stimulation would correspond to 0.064 mM\(^{-1}\) and 0.081 mM\(^{-1}\) for GLP-1 and GIP respectively. Studies investigating the relationship between intestinal glucose concentration and GLP-1 secretion
have shown that the glucose concentration that trigger GLP-1 secretion range from 5 mM to 1 M\textsuperscript{85, 86, 87, 88}. These glucose concentrations correspond to model-predicted GLP-1 of 22 and 1090 pM respectively. The GLP-1 predictions in the high end of the interval are highly uncertain since the jejunal concentration in this work ranged from 0 to 80 mM. Linear stimulation was identified for both GLP-1 and GIP. However, many physiological processes have a natural maximum. An OGTT may not be the ideal way to identify the maximum secretory response of the incretin hormones, since the inhibition of gastric emptying by glucose limits the amount of glucose in the small intestine and thereby the secretion. It is plausible that other designs such as meal tolerance test could make it possible to identify a maximum through increased secretion by lipids.

**Beta cell function (paper IV)**

The model by Overgaard et al. was extended to describe beta cell secretion for patients with T2D. Increased activation of C-peptide/insulin with increasing glucose concentration could not be identified for patients with T2D, indicating that the beta cell is less sensitive to glucose concentrations compared to healthy individuals. This is in agreement with the current knowledge that patients with T2D have either lost or greatly impaired first phase response compared to healthy individuals\textsuperscript{89, 90}.

**Hepatic extraction of insulin**

Distribution models for insulin and C-peptide were coupled with the beta cell model. By inclusion of both insulin and C-peptide concentrations in the model we were able to characterize the dynamics of hepatic extraction of insulin. Indirect methods based on mathematical models are often used to assess hepatic extraction since direct measurement requires invasive procedures with measurements from the portal vein and artery\textsuperscript{91, 92}. It has been suggested that hepatic extraction is saturable and decreases with increasing insulin exposure\textsuperscript{93}. This was implemented in the model as a decreasing hepatic extraction with increasing beta cell secretion rate. The estimated mean values for the hepatic extraction were in accordance with previously reported values\textsuperscript{91, 93}.

**Incretin effect**

In healthy individuals both GIP and GLP-1 were found to stimulate the production of new C-peptide/insulin. In addition, both incretin hormones were found to enhance the rate of recruitment of C-peptide/insulin from passive to active vesicles. These results correspond well with present knowledge about
the properties of GLP-1 and GIP in normal physiology. Both incretin hormones facilitate the secretion of insulin by recruiting vesicles for exocytosis and thereby enhancing the beta cell response to glucose\textsuperscript{94, 95}. The incretin hormones also act to replenish the insulin hormone during secretion\textsuperscript{94, 96}.

It was assumed that GIP has no effects on C-peptide/insulin secretion for patients with T2D, hence GLP-1 was only included to describe the increased vesicle activation. This assumption was supported by findings of the reduced incretin effect seen in T2D. Our analysis was performed on endogenously secreted GIP, which is less likely to amplify beta cell secretion compared to the rather high bolus doses of GIP that have been seen to increase insulin secretion\textsuperscript{74, 76}. However, exogenous GLP-1 and GIP are unexplored and the model may not describe the insulinotropic effect in such scenarios. The effect of GLP-1 on provision was small (estimated to 23% of healthy value) and non-significant (at $\alpha=0.001$) in patients with T2D, hence it was not included in the model. However, data from more patients with T2D or higher exogenous concentrations of GLP-1 may give rise to a significant effect.

Regulation of glucagon secretion (paper V)

The mechanism behind the prolonged suppression of glucagon secretion is currently unknown. In the glucagon model we have hypothesized that the effect is triggered by glucose with a sustained average effect of 197 minutes after glucose and insulin are back at baseline. Glucose and insulin concentrations below baseline will reduce the time. Simulations of a 75 g OGTT show that samples should be collected for at least 265 and 555 minutes for healthy individuals and patients with T2D respectively. Several other studies have also shown this prolonged suppression\textsuperscript{97, 98}. However, for healthy individuals there are also studies where glucagon concentrations return to baseline within 4 hours after glucose ingestion\textsuperscript{99}. Allowing stimulation of glucagon secretion due to glucose concentration being below baseline caused a too rapid return to glucagon baseline for patients with T2D. Hence, a glucose concentration below baseline was not allowed to stimulate glucagon synthesis. This corresponds with current knowledge that patients with T2D have impaired hypoglycemic response of glucagon secretion\textsuperscript{100}. Different glucagon baselines and the effect of GIP were the only parameter differences detected between healthy individuals and patients with T2D.

The stimulation of GIP in the model should be interpreted as the net effect of inhibition by GLP-1 and stimulation of GIP. Inhibition by GLP-1 did not contribute significantly to the model fit, likely due to the GLP-1 and GIP profiles being highly correlated.

Considerable variability was estimated for glucagon which is reflected in the large confidence interval around the simulated median in figure 22 and 23 for healthy individuals and patients with T2D respectively. The residual error
was estimated to be 44%. In addition to model misspecifications a contributing factor is the assay variability which in general is large for glucagon assays\textsuperscript{101, 102}. The selectivity is also poor making comparisons between studies difficult. Hence, the baseline glucagon concentration needs to be appropriately handled in relation to the assay that will be used when performing simulations of other studies using the herein developed model.

A comprehensive glucose homeostasis model (paper V)

The IGI model, developed by Silber et al. did not include inhibition of EGP for patients with T2D, however using the same assumption with the current model and data led to overprediction of late glucose samples. A significant improvement in model fit was achieved when inhibition of EGP was included for patients with T2D. It has previously been shown that inhibition of EGP is similar for patients with T2D as for healthy individuals\textsuperscript{103, 104}.

The models describing the dynamics of gastric emptying and glucose absorption (paper II), GIP and GLP-1 (paper III), C-peptide and insulin (paper IV) and glucagon (paper V) were connected into one comprehensive model. This model describes glucose homeostasis during OGTT and corresponding IIGI for healthy individuals and patients with T2D. Moreover, the model is capable of simultaneously describe the dose response for all biomarkers. However, the GLP-1 concentration in the 25 g OGTT are over predicted for healthy individuals. The approach of developing each sub model conditioned on biomarker observations and then combining the sub models, was here showcased to work well and reduced the complexity of model development considerably. A large complex model with many interactions such as the comprehensive glucose homeostasis model requires considerable computational power. Hence, in its full form it is mainly appropriate for simulation studies, however each sub model may be used separately for estimation.

In this work many empirical elements were replaced with more mechanistic descriptions. However, the potentiation of the suppressive effect of glucose and insulin on glucagon secretion must be considered and empirical element. It is currently unknown what the underlying mechanism is and future research may reveal the mechanism behind.
Conclusions and future perspectives

The pharmacometric models developed in this thesis provide a more detailed description of glucose homeostasis during glucose tests than the ones previously available. In addition, this thesis provides a simple scaling procedure for preclinical investigations into glucose homeostasis.

In specific:

- A simple interspecies scaling procedure was developed, allowing model based investigation of drug effects in several preclinical species. As well as enabling translation of drug effects from animals to humans.
- A comprehensive glucose homeostasis model was developed covering the most important aspects of glucose homeostasis. The model is capable of simultaneously describing the regulation of gastric emptying, glucose absorption, incretin hormones, C-peptide and insulin, hepatic extraction of insulin, glucagon and EGP in both healthy individuals and patients with T2D after oral and intravenous glucose administration over a wide dose range.

To further increase the models predictive performance covariates can be included. Disease descriptors, such as duration of diabetes and size descriptors such as BMI may be predictive of glucose homeostasis. The new comprehensive glucose homeostasis model has the possibility to be used to investigate combination treatments and drugs with multiple effects. Further, the interspecies scaling procedure developed in this thesis may be applied to the comprehensive glucose homeostasis model, leading to improved drug development of new antidiabetic compounds.
Populärvetenskaplig sammanfattning


Matematiska modeller är värdefulla verktyg i läkemedelsutveckling och kan användas för att öka förståelsen för komplexa system såsom glukoshomeostasen, kvantifiera läkemedelseffekter och göra läkemedelsutvecklingsprocessen mer effektiv. I denna avhandling har en ny omfattande matematisk modell utvecklats som beskriver de viktigaste aspekterna av glukoshomeostas under glukostoleranstest i båda friska individer och patienter med typ 2 diabetes. Modellen kan simultant beskriva reglering av magtömning och glukosabsorption, reglering av två hormoner som ökar utsöndringen av insulin efter oralt intag av glukos, den ökade insulinsekretionen efter oralt intag av glukos, leverns upptag av nyligen framställt insulin, regleringen av hormonet glukagon som i motsats till insulin ökar blodsockernivåerna, samt regleringen av kroppseget bildande av blodsocker. Därutöver beskriver denna avhandling hur man kan skala en tidigare utvecklad modell för människa så den kan beskriva glukoshomeostas även i mus, rätta, gris, hund och apa.

Sammanfattningsvis så ökar de utvecklade modellerna i denna avhandling kunskapen om regleringen av glukoshomeostas under glukostoleranstest. De kan användas för att undersöka kombinationsbehandlingar och läkemedel med flera olika effekter, samt att de öppnar upp möjligheten att effektivt skala läkemedelseffekter mellan arter, vilket kan leda till förbättrad läkemedelsutveckling och i förlängningen nya läkemedel mot typ 2 diabetes.
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References


A doctoral dissertation from the Faculty of Pharmacy, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy”.)