Mechanism-Based Modeling of the Glucose-Insulin Regulation during Clinical Provocation Experiments

PETRA JAUSLIN-STETINA
Dissertation presented at Uppsala University to be publicly examined in B21, BMC, Husargatan 3, Uppsala, Friday, May 23, 2008 at 13:00 for the degree of Doctor of Philosophy (Faculty of Pharmacy). The examination will be conducted in English.

Abstract

Type 2 diabetes is a complex chronic metabolic disorder characterized by hyperglycemia associated with a relative deficiency of insulin secretion and a reduced response of target tissues to insulin. Considerable efforts have been put into the development of models describing the glucose-insulin system. The best known is Bergman’s “minimal” model for glucose, which is estimating glucose concentrations using fixed insulin concentrations as input. However, due to the involved feedback mechanisms, simultaneous modeling of both entities would be advantageous. This is particularly relevant if the model is intended to be used as a predictive tool. The mechanism-based glucose-insulin model presented in this thesis is able to simultaneously describe glucose and insulin profiles following a wide variety of clinical provocation experiments, such as intravenous and oral glucose tolerance tests, clamp studies and sequential meal tests over 24 hours. It consists of sub-models for glucose, labeled glucose and insulin kinetics. It also incorporates control mechanisms for the regulation of glucose production, insulin secretion, and glucose uptake. Simultaneous analysis of all data by nonlinear mixed effect modeling was performed in NONMEM.

Even if this model is a crude representation of a complex physiological system, its ability to represent the main processes of this system was established by identifying: 1) the difference in insulin secretion and insulin sensitivity between healthy volunteers and type 2 diabetics, 2) the action of incretin hormones after oral administration of glucose, 3) the circadian variation of insulin secretion and 4) the correct mechanism of action of a glucokinase activator, a new oral antidiabetic compound acting on both the pancreas and the liver.

These promising results represent a proof of concept of a mechanistic drug-disease model that could play an important role in the clinical development of anti-diabetic drugs.

Keywords: Glucose homeostasis, Type 2 diabetes, IVGTT, OGTT, Meal test, Circadian variation, Mechanism-based, NONMEM

Petra Jauslin-Stetina, Department of Pharmaceutical Biosciences, Box 591, Uppsala University, SE-75124 Uppsala, Sweden

© Petra Jauslin-Stetina 2008

ISSN 1651-6192
ISBN 978-91-554-7195-8
urn:nbn:se:uu:diva-8719 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8719)
To Matthias
Papers discussed

This thesis is based on the following papers:


III Jauslin PM, Frey N, Karlsson MO. Modeling of 24-hour glucose and insulin profiles of type 2 diabetics. (In manuscript.)

IV Jauslin PM, Karlsson MO, Frey N. Identification of the mechanism of action of a glucokinase activator from OGTT data in type 2 diabetics using and integrated glucose-insulin model. (In manuscript.)

Reprints were made with permission from the publisher.
Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>13</td>
</tr>
<tr>
<td>Regulation of glucose homeostasis in healthy subjects</td>
<td>13</td>
</tr>
<tr>
<td>Insulin</td>
<td>13</td>
</tr>
<tr>
<td>Glucagon</td>
<td>14</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus</td>
<td>14</td>
</tr>
<tr>
<td>Present treatment options for type 2 diabetes</td>
<td>15</td>
</tr>
<tr>
<td>Selected new targets for pharmacologic therapy</td>
<td>15</td>
</tr>
<tr>
<td>Experimental techniques for the assessment of glucose regulation</td>
<td>16</td>
</tr>
<tr>
<td>Clamp studies</td>
<td>17</td>
</tr>
<tr>
<td>Glucose Tolerance Tests</td>
<td>18</td>
</tr>
<tr>
<td>Non-linear mixed effect (NLME) modeling</td>
<td>20</td>
</tr>
<tr>
<td>Mechanism-based models</td>
<td>21</td>
</tr>
<tr>
<td>Rationales for the modeling of glucose homeostasis</td>
<td>22</td>
</tr>
<tr>
<td>Milestones in previous glucose-insulin modeling</td>
<td>23</td>
</tr>
<tr>
<td>Minimal model</td>
<td>23</td>
</tr>
<tr>
<td>Closed-loop glucose-insulin models</td>
<td>24</td>
</tr>
<tr>
<td>Drug effect models</td>
<td>24</td>
</tr>
<tr>
<td>Models incorporating disease progression</td>
<td>25</td>
</tr>
<tr>
<td>Aims</td>
<td>26</td>
</tr>
<tr>
<td>Methods</td>
<td>27</td>
</tr>
<tr>
<td>Data</td>
<td>27</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>27</td>
</tr>
<tr>
<td>Type 2 diabetic patients</td>
<td>28</td>
</tr>
<tr>
<td>Model development</td>
<td>30</td>
</tr>
<tr>
<td>Structural model</td>
<td>30</td>
</tr>
<tr>
<td>Glucose and insulin baselines</td>
<td>31</td>
</tr>
<tr>
<td>Parameter differences between healthy volunteers and diabetic patients</td>
<td>31</td>
</tr>
<tr>
<td>Absorption</td>
<td>32</td>
</tr>
<tr>
<td>Modeling of circadian variation</td>
<td>33</td>
</tr>
<tr>
<td>Drug effect model</td>
<td>33</td>
</tr>
<tr>
<td>Inter-individual, intra-individual and residual variability</td>
<td>34</td>
</tr>
<tr>
<td>Data analysis</td>
<td>35</td>
</tr>
<tr>
<td>Software</td>
<td>35</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Model selection</td>
<td>35</td>
</tr>
<tr>
<td>Model validation</td>
<td>36</td>
</tr>
<tr>
<td>Results</td>
<td>38</td>
</tr>
<tr>
<td>The glucose sub-model</td>
<td>42</td>
</tr>
<tr>
<td>The hot glucose sub-model</td>
<td>45</td>
</tr>
<tr>
<td>The insulin sub-model</td>
<td>45</td>
</tr>
<tr>
<td>Population and study-specific parameters (papers I&amp;II)</td>
<td>47</td>
</tr>
<tr>
<td>Circadian effects (paper III)</td>
<td>47</td>
</tr>
<tr>
<td>Drug Effects (paper IV)</td>
<td>50</td>
</tr>
<tr>
<td>Model validation</td>
<td>52</td>
</tr>
<tr>
<td>Internal validation</td>
<td>52</td>
</tr>
<tr>
<td>External validation</td>
<td>52</td>
</tr>
<tr>
<td>Discussion</td>
<td>53</td>
</tr>
<tr>
<td>Parameter estimates</td>
<td>53</td>
</tr>
<tr>
<td>Patient-specific parameters</td>
<td>54</td>
</tr>
<tr>
<td>OGTT-specific parameters</td>
<td>54</td>
</tr>
<tr>
<td>Circadian variation</td>
<td>55</td>
</tr>
<tr>
<td>Drug effects</td>
<td>56</td>
</tr>
<tr>
<td>Possible applications and limitations of the model</td>
<td>56</td>
</tr>
<tr>
<td>Conclusions</td>
<td>59</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>60</td>
</tr>
<tr>
<td>References</td>
<td>62</td>
</tr>
</tbody>
</table>
Abbreviations

ABSG  rate of glucose absorption
ABSG_50  glucose absorption rate producing 50% of the maximal incretin effect
AMP  amplitude of cosine function
BIOG  bioavailability of glucose
CL_G  insulin-independent glucose clearance
CL_GI  insulin-dependent glucose clearance
CL_I  insulin clearance
Corr  correlation between individual estimates
DA_50  amount of drug in the biophase compartment producing 50% of the maximal drug effect
D_max  maximal drug effect
DPP-IV  dipeptidyl peptidase IV
DV  dependent variable (observation)
E_max  maximal effect
FOCE  first order conditional estimation
FPG  fasting plasma glucose
FPS  amount of insulin secreted during first-phase secretion
FSI  fasting serum insulin
FSIGT  frequently sampled intravenous glucose tolerance test
G_A  absorption compartment for glucose
G_C  central compartment for glucose
G_CM1  control of glucose production by plasma glucose
G_CM2  control of insulin secretion by plasma glucose
G_E1  effect compartment for glucose effect on glucose production
G_E2  effect compartment for glucose effect on insulin secretion
GIP  gastric inhibitory protein
GK  glucokinase
GKA  glucokinase activator
GLP-1  glucagon-like peptide 1
GLUT-1  glucose transporter 1
Gp  peripheral compartment for glucose
GPRG  control parameter for the glucose effect on glucose production
G_PROD  endogenous glucose production
G_PROD_0  baseline endogenous glucose production
G_scale  scaling parameter for the glucose baseline
GSS  steady state (baseline) glucose concentration
GT  transit compartment for glucose
HbA$_{1C}$  glycosylated hemoglobin
HC  central compartment for hot glucose
HGP  hepatic glucose production
Hp  peripheral compartment for hot glucose
HV  healthy volunteer
I  disposition compartment for insulin
IE  effect compartment for insulin effect on glucose clearance
IFS  delay compartment for first-phase insulin secretion
IIV  inter-individual variability
IOV  inter-occasion variability
IPRED  individual prediction
IPRG  control parameter for the glucose effect on insulin production
Iscale  scaling parameter for the insulin baseline
ISEC  total insulin secretion
ISEC,0  baseline insulin secretion
ISS  steady state (baseline) insulin concentration
IV  intravenous
IVGTT  intravenous glucose tolerance test
ka  glucose absorption rate constant
kCA  rate constant for glucose transport into the central compartment
kDE  rate constant of drug entering a biophase compartment from a depot compartment (K-PD model)
kDEI  rate constant for the biophase compartment for the drug effect on the liver
kDEp  rate constant for the biophase compartment for the pancreatic drug effect
kGE1  rate constant for the glucose effect compartment controlling insulin secretion
kGE2  rate constant for the glucose effect compartment controlling glucose production
kIE  rate constant for the effect compartment for insulin
kIS  rate constant for insulin entering the central compartment following first-phase secretion
K-PD  kinetic-pharmacodynamic
MTT  mean transit time of glucose absorption
n  number of transit compartments
N  shape-defining parameter of the suppressor function
NLME  non-linear mixed effects
OFV  objective function value
OGTT  oral glucose tolerance test
PAR  parameter
PAR(av) 24-hour mean of a parameter that is subject to circadian variation
PAT patient
PD pharmacodynamic
\( P_i \) individual parameter
PK pharmacokinetic
PK-PD pharmacokinetic-pharmacodynamic
PO oral
PRED population prediction
PsN Pearl-speaks-NONMEM
PT suppression peak time
Q inter-compartmental clearance of glucose
RESE multiplying error factor for early time points (<2 minutes)
RESH residual error for total glucose
RESI residual error for insulin
RSE relative standard error
s Hill coefficient (sigmoidicity factor of an \( E_{\text{max}} \) function)
S slope of the linear relationship between amount of drug in the biophase and the pancreatic drug effect
SA suppression amplitude
SGLT sodium-glucose linked transporter
\( S_{\text{incr}} \) slope of the linear link between the absorption rate of glucose and insulin secretion (incretin effect)
SUP suppression (as described by suppressor function)
SW suppression width
t time
t\(_{\text{max}}\) time of maximal plasma concentration
Tmax phase shift of a cosine function (time at which the amplitude reaches the maximum)
\( V_G \) volume of distribution of the central glucose compartment
\( V_I \) volume of distribution of the insulin disposition compartment
\( V_P \) volume of distribution of the peripheral glucose compartment
VPC visual predictive check
\( \alpha \) type I error (false positive results)
\( \varepsilon \) difference between individual prediction and observation (residual error)
\( \eta \) difference between population and individual parameter estimate
\( \theta \) fixed-effect parameter (typical value)
\( \kappa \) difference in individual parameter estimates between occasions
\( \pi \) standard deviation of \( \kappa \)
\( \sigma \) standard deviation of \( \varepsilon \)
\[ \Omega \] variance-covariance matrix of \( \eta \)
\[ \omega \] standard deviation of \( \eta \)
Introduction

Regulation of glucose homeostasis in healthy subjects

In healthy individuals, plasma glucose levels are maintained within relatively narrow limits by a complex regulatory system; both elevated glucose concentrations (hyperglycemia) and too low glucose concentrations (hypoglycemia) have serious consequences, reaching from tissue damage up to coma and death. Normal concentrations are 70–110 mg/dl (approximately 6–8 mmol/l) before meals and below 140 mg/dl (7.8 mmol/l) after meals. The term “glucose tolerance” describes how quickly glucose is cleared from the blood after ingestion of carbohydrates, until the fasting level of plasma glucose is restored. This ability to dispose of plasma glucose varies over the course of a day. In healthy subjects, glucose tolerance is highest in the morning. It is decreased in the afternoon and throughout most of the night.

The two main organs involved in the maintenance of glucose homeostasis are the pancreas and the liver. The pancreas releases the two most important hormones that control glucose homeostasis: insulin, produced in pancreatic β-cells, and glucagon, produced in α-cells. The action of these two hormones will be discussed below. The role of the liver is that of a storage organ of excess blood glucose. When blood glucose levels are high, it takes up glucose and converts it into glycogen. When glucose levels are low, it releases glucose by either re-converting stored glycogen (glycogenolysis) or synthesizing new glucose (gluconeogenesis).

Besides insulin and glucagon, many other substances influence this complex system, including hormones (somatostatin, growth hormone, cortisol, adrenalin, estrogen and progesterone, as well as gastrointestinal hormones), amino acids and fatty acids, among others. The autonomous nervous system also affects the pancreas and thus, insulin and glucagon secretion.

Insulin

Insulin plays an important role for the storage of excess energy in the body, which includes the storage of glucose as glycogen in liver and muscles, its conversion into fat and the storage of the latter in adipose tissue. Thus, insulin very effectively promotes the clearance of plasma glucose through uptake into various tissues, supplying tissue cells with energy. The only cells that do not depend on insulin in order to take up glucose are the brain cells and the...
erythrocytes. In the fasting state, the brain consumes about 80% of the glucose utilized by the whole body\(^3\). Another important action of insulin in the context of glucose regulation is the suppression of hepatic glucose production.

At normal fasting levels of plasma glucose, the rate of insulin secretion is minimal (approximately 25 ng/min/kg body weight). When plasma glucose rises after a meal, insulin secretion is stimulated. Two phases of insulin secretion can be distinguished. The first-phase secretion leads to an approximately 10-fold rise of basic insulin secretion within less than five minutes. It is caused by a release of pre-formed insulin from pancreatic \(\beta\)-cells. As \(\beta\)-cells become depleted of insulin, this first-phase secretion ceases after 5–10 minutes and is replaced by the slower but long-lasting second-phase insulin secretion. This second-phase secretion involves both the synthesis and the subsequent secretion of insulin.

Apart from glucose, insulin secretion is also stimulated by amino acids, gastrointestinal hormones, glucagon, growth hormone, cortisol, and to a lesser extent also by progesterone and estrogen. Gastrointestinal hormones such as glucagon-like peptide 1 (GLP-1) and gastric inhibitory protein (GIP) are of particular interest. These hormones are released after ingestion of a meal and lead to an “anticipatory” insulin response before a rise in plasma glucose can be detected.

**Glucagon**

Glucagon is secreted by pancreatic \(\alpha\)-cells when blood glucose levels fall below normal, particularly during exercise\(^2\). It acts as an antagonist of insulin by causing hepatic glucose output to rise. This is either achieved by glycogen breakdown or increased gluconeogenesis. Increased blood glucose levels inhibit glucagon secretion. The effects of glucagon mainly become apparent in prolonged hypoglycemia.

**Type 2 diabetes mellitus**

Type 2 diabetes is a complex metabolic disorder characterized by hyperglycemia associated with an absolute or relative deficiency of insulin secretion, excessive hepatic glucose production (HGP) and a reduced response of target tissues to insulin (frequently called “insulin resistance”), or a combination of the above\(^4\). Insulin is either produced by the pancreas in insufficient quantities, or the tissue cells’ response to insulin is impaired. Its metabolic and clinical features are heterogeneous. Type 2 diabetic patients range from normal or underweight persons with a predominant deficiency of insulin secretion to the more common obese persons with substantial insulin resistance\(^5\). About 80% of type 2 diabetic patients are obese.
In contrast to type 1 diabetes, which is usually diagnosed in childhood and is caused by an autoimmune destruction of the pancreatic β-cells, type 2 diabetes has long been regarded as a disease of the middle-aged or elderly. However, recently it has also become a problem in children.

Of the 150 million diabetic patients worldwide, at least 85% suffer from type 2 diabetes. Its incidence is increasing dramatically in both industrialized and developing countries: it is predicted to rise to 300 million by 2025. While there is good evidence for a strong genetic contribution to both obesity and diabetes, the increase in these conditions appears to be due to a changing balance between energy intake and energy expenditure through physical activity.

Present treatment options for type 2 diabetes

Even though treatment of newly diagnosed type 2 diabetes might be limited to diet and other lifestyle modifications, the vast majority of patients will eventually require pharmacologic treatment with oral antidiabetic compounds and/or insulin. The major classes of oral antidiabetic drugs to treat this disorder include thiazolidinediones (improving insulin sensitivity), biguanides (reducing HGP in addition to the enhancement of insulin sensitivity), α-glucosidase inhibitors (delaying digestion and absorption of intestinal carbohydrates), sulfonylureas and meglitinides (insulin secretagogues). Obese patients with predominant insulin resistance are likely to benefit from metformin or thiazolidinediones. In slim patients who generally have substantial pancreatic β-cell failure, sulphonylureas or meglitinides might be more effective. However, all of these classes of oral antidiabetics have documented limitations.

At present, no single agent is capable of achieving acceptable, long-lasting blood glucose control in the majority of patients. The abundant use of combination therapy indicates that it is difficult to control the disease by attacking only one pathway, addressing only one of the several underlying pathophysiological defects. Although combinations of available drugs can result in superior glycemic control, they also tend to lose efficacy over time. About 50% of type 2 diabetic patients need insulin treatment within 6 years from diagnosis. Thus, there is a pressing need for safe, novel drugs with improved efficacy.

Selected new targets for pharmacologic therapy

Glucagon-like peptide-1 (GLP-1) analogues and dipeptidyl peptidase IV (DPP-IV) inhibitors

GLP-1 regulates blood glucose via stimulation of glucose-dependent insulin secretion, inhibition of gastric emptying, and inhibition of glucagon secretion.
GLP-1 may also regulate glycogen synthesis in adipose tissue and muscle; however, the mechanism for these peripheral effects remains unclear. The main problem with this molecule is its short duration of action. This is partly caused by the enzyme dipeptidyl peptidase IV (DPP-IV); hence GLP-1 analogs that are resistant to DPP-IV cleavage may be more potent. Alternatively, DPP-IV inhibitors may be used to prolong the action of endogenous GLP-1. DPP-IV inhibitors and GLP-1 agonists are expected to restore β-cell mass and thus offer a great potential in the prevention, or even cure, of diabetes.

Inhibition of Na\(^+\)-glucose co-transporter (sodium-glucose linked transporter; SGLT) proteins

SGLTs represent a novel approach to the treatment of diabetes. They block renal glucose re-absorption from urine, thus maintaining blood glucose control. There are at least 3 forms of SGLT. SGLT1 is found in the epithelial cells of the intestine and kidney, while SGLT2 is found only in renal epithelium cells. SGLT3 is reported to exist in several tissues including intestine, spleen, liver, muscle, and in lesser amounts in the kidney. Thus, SGLT's may show a variety of mechanisms to maintain normal glycemia, but their main activity is in the early proximal tubule segment of the kidneys.

Glucokinase activators (GKA)

Glucokinase (GK) is an enzyme that plays a central role in whole body glucose homeostasis. It mainly acts in the pancreas and in the liver, although it is expressed in numerous other cell types. It catalyzes the first and rate-determining step of glucose metabolism, the phosphorylation of glucose to glucose-6-phosphate. Thus, it acts as a glucose sensor both in hepatocytes and in pancreatic β-cells. An increase in glucose-6-phosphate triggers the release of insulin and inhibits hepatic glucose production. The dual mechanism of action of GKA's in β-cells and liver suggest that they will exert their biological effects in type 2 diabetic patients by improving overall β-cell function coupled with a suppression of hepatic glucose output.

Experimental techniques for the assessment of glucose regulation

To study the regulation of glucose homeostasis and its pathological changes in diabetes, different provocations of the glucose-insulin system are employed. These experiments are designed to obtain informative data that allow for calculation of parameters quantifying an individual’s or a population’s health or disease state. Such parameters typically include the ability to dispose of blood glucose independently of insulin (“glucose effectiveness”), the
sensitivity of tissue cells to insulin (“insulin sensitivity”) or the responsiveness of β-cells to a glucose stimulus. For example, high plasma glucose levels are needed to explore β-cell function in its whole range, and high plasma insulin levels facilitate the detection of the insulin effect on glucose elimination. All tests are usually started in the morning after an overnight fast. Sometimes, a certain percentage of radioactively or stable isotope labeled (“hot”) glucose is used in glucose provocation experiments. This is done in order to be able to distinguish between the endogenous glucose produced by the liver and the exogenously administered glucose.

Clamp studies
The interactions between glucose and insulin are complex; to facilitate their study the clamp technique was developed. Its principle is to keep one entity (either glucose or insulin) constant in order to obtain a clearer picture of the dynamics of the other entity.

Euglycemic hyperinsulinemic clamp
The euglycemic hyperinsulinemic clamp is often referred to in the literature as the gold standard for assessing insulin sensitivity . An insulin infusion is targeted at maintaining the plasma insulin concentration at approximately 100 mU/l over a period of 2–4 hours. The objective of this procedure is to raise insulin to approximate usual postprandial levels, which will suppress hepatic glucose production. To maintain this plateau and to keep the individual’s plasma glucose levels within a physiological range, variable amounts of a glucose solution must be infused. As the plasma insulin level is fixed, the amount of glucose infused will depend on the subject’s insulin sensitivity. Adapting glucose rates of infusion to maintain euglycemia (normal plasma glucose concentrations at approximately 90 mg/dl) requires frequent blood sampling (in 5–15 min intervals) for the determination of plasma glucose concentrations. Samples for insulin levels are generally obtained every 30 to 60 min throughout the clamp procedure.

Hyperglycemic clamp
This type of clamp experiment is less common than the euglycemic hyperinsulinemic clamp. The hyperglycemic clamp technique evaluates the insulin response to sustained hyperglycemia. With this technique, the β-cells of all subjects are stimulated with the same glucose concentration. Thus, β-cell sensitivity as well as peripheral tissue sensitivity can be assessed. Moreover, a measure of non-insulin-mediated glucose uptake can also be obtained.

Hyperinsulinemic hypoglycemic Clamp
The hyperinsulinemic hypoglycemic clamp technique is very similar to that of the euglycemic insulinemic clamp. However, the hypoglycemia caused by
elevated insulin levels is not corrected by a variable glucose infusion. This type of clamp is particularly useful if the research question involves hypoglycemia and counterregulatory responses\textsuperscript{25}.

Variations of the above named clamp techniques that involve the infusion of isotope-labeled glucose allow the researcher to evaluate the contribution of hepatic glucose output to insulin resistance\textsuperscript{26}. Drawbacks of all clamp tests are that they are labor intensive, technically difficult to perform and expensive. They also involve some inconvenience for the test subjects, as two intravenous lines need to be inserted, one for glucose and insulin infusions and the other one for blood sampling. Another potential limitation of clamp techniques is that these tests are performed under steady state conditions. Therefore, they do not realistically portray dynamic conditions such as those occurring after normal meals\textsuperscript{23}.

**Glucose Tolerance Tests**

All variants of glucose tolerance tests have in common that a relatively high glucose load is administered as single dose. The compensatory responses to this challenge of the glucose regulatory system, and in particular the dynamics of blood glucose disposal, are assessed over a certain period of time (usually 2–5 hours).

**Intravenous glucose tolerance test (IVGTT)**

At the start of the experiment, a glucose solution is usually injected as a bolus dose or infused over a 1 to 2-minute period\textsuperscript{23}. The typical dosage amounts to 300 mg/kg of body weight. When using this test in diabetic patients, intravenous insulin administration may be necessary, as the subjects’ own endogenous insulin secretion would be too low to appropriately counteract the sudden rise in plasma glucose levels. Intravenous insulin is typically injected 20 minutes after the start of the experiment at a dosage of 0.02 to 0.05 U/kg body weight. The blood sampling protocols vary. In the so-called frequently sampled IVGTT (FSIGT), a baseline blood sample plus approximately 30 post-dose samples are obtained.

The following phases can be distinguished during the experiment: the first 7 to 10 minutes after the glucose injection constitute the initial distribution phase of glucose in the circulation. Insulin secretion is stimulated as soon as the elevated glucose levels are detected in the pancreatic \( \beta \)-cells. In healthy persons, a peak of glucose-stimulated endogenous insulin secretion is observed. However, in a patient with substantial \( \beta \)-cell impairment, this peak might be missing. At approximately the same time, glucose production by the liver ceases. If an exogenous insulin infusion is administered during the IVGTT, an additional insulin peak originating from this infusion can be observed. After the insulin peak(s), a pronounced increase in glucose clearance can be detected.
Advantages of the IVGTT are its high reliability and reproducibility. It is not as labor intensive or as expensive to perform as a clamp study. However, it is still a more complex experiment than the commonly used oral glucose tolerance test (OGTT, see below). The ability to identify and separate glucose-mediated and insulin-mediated glucose disposal, particularly if a certain percentage of hot glucose is used, is another significant advantage of the FSIGT.

Oral glucose tolerance test (OGTT)
Besides the determination of simple fasting plasma glucose values, the OGTT is the method most frequently used by clinicians to establish the diagnosis of diabetes\textsuperscript{27,26}. It is also commonly used in the clinical development of antidiabetic drugs\textsuperscript{28}.

After collecting a blood sample for the determination of fasting glucose, and sometimes fasting insulin, the patient drinks a standard amount of a glucose solution within 5 minutes. There are several variations of this test in terms of the oral glucose dose and sampling schedule. Usually, glucose doses are 50, 75 or 100 g. A more individualized approach is the administration of 1.75 g glucose per kg body weight, up to a maximum dose of 75 g. Post-dose sampling is usually performed in 30–60 min intervals during 2–5 hours for measurement of glucose and sometimes insulin levels. However, for simple diabetes screening, only the pre-dose and the 2 hour samples may be collected. During an OGTT, glucose levels increase after a variable lag period, then reach a peak before falling again and eventually returning to baseline. The responses show a high variability even in the same subject upon repeated testing\textsuperscript{24}.

The OGTT is technically quite simple to perform and certainly lower in cost than the IVGTT or a clamp study, which enables its use even in large epidemiological studies. In addition, oral glucose provocations reproduce physiological conditions more closely because they mimic glucose absorption after a meal. However, the OGTT might be less appropriate in some research situations. The variability in the rate of gastric emptying and glucose absorption from the gastrointestinal tract may negatively affect the reproducibility of the results\textsuperscript{29}. Furthermore, the OGTT does not always provide adequate information regarding the dynamics of glucose and insulin action, particularly in diabetics with impaired insulin secretion. In this case, a modeling approach can be helpful for gaining more information from OGTT data.

Meal tests
Of all glucose provocation experiments, meal tests come closest to normal physiological behavior. They enable investigators to study the effect of an antidiabetic drug or any other intervention under “real-life” conditions by providing a measure of the response of blood glucose levels to normal die-
tary intake. Instead of a glucose dose, a standardized meal containing defined amounts of nutrients (carbohydrates, protein and fat) is provided and followed by blood sampling during the subsequent hours. Standardized meals need to be consumed within a defined time frame. They can either be liquid (e.g. soup, milk shakes) or solid, the latter being more common. Repeated solid meal tests over the course of a day for the study of circadian variation in glucose tolerance are common.

Disadvantages of meal tests comprise the high variability in glucose absorption, depending on gastric emptying and many other factors. These problems are partly avoided by the administration of liquid meal tests that are faster absorbed and associated with less variability. Meal tests are more complex to perform than oral glucose tolerance tests. Their strengths lie in the close resemblance of real-life conditions.

Non-linear mixed effect (NLME) modeling

The aim of clinical trials is to learn about the physiological and pathophysiological properties of the study population and the pharmacological properties of the study drug acting in this population. However, the processes taking place when a drug (or an endogenous substance) interacts with a biological system do not operate at the population level, but at the individual level. In NLME analyses, this dual estimation problem is solved by constructing structural models for describing the events at the individual level. It is then assumed that individuals differ in the values of parameters that describe the system. These differences are divided into components that can be explained by observable factors (covariates) and an unexplained, but quantified, component.

In contrast to individual data analysis, the population approach offers the possibility of gaining integrated information from relatively sparse data or a combination of sparse and dense data. It allows for the analysis of data from unbalanced designs as well as data obtained from special patient populations, such as pediatric and elderly patients. The approach uses individual observations, which may be sparse, unbalanced, or fragmentary. Parameters of the population are estimated from the full set of these individual observations.

At least two hierarchical levels of variability (referred to as random effect parameters) are identified and separated. One level explains differences between the parameter values for the different subjects, while the other level handles the residual unexplained variability, i.e. the discrepancy between the individual prediction and the observation. This residual variability may be due to measurement errors, assay imprecision, erroneous time or dose recording or model misspecification. A third level of variability may account
for differences between study occasions within the same subject, if applicable.

Usually, both the typical parameter values of a population and the inter- and intra-individual variability of these values are of interest. The constants representing the fixed effects are denoted as $\theta$. An individual model parameter $P_i$ can be estimated for normally distributed parameters as

$$ P_i = \theta^p + \eta_i^p $$

(1)

where $\eta_i^p$ is the normally-distributed, zero-mean, difference between the population mean parameter and that of the $i^{th}$ individual. As most physiological parameters tend to be log-normally distributed (i.e. cannot be negative), a more common form of defining individual parameters is

$$ P_i = \theta^p \cdot e^{\eta_i^p} $$

(2)

The standard deviation of $\eta_i^p$ is denoted by the symbol $\omega_p$. Collectively, the set of variances and covariances among parameters is denoted as $\Omega$.

Estimation methods used for fitting population models to data are generally based on the statistical principle of maximum likelihood. Basically, the probability of the data under the model is written as a function of the model parameters, and parameter estimates are chosen to maximize this probability.

The $j^{th}$ observation in individual $i$ can be described as follows:

$$ y_{ij} = f(x_{ij}, P_i) + \varepsilon_{ij} $$

(3)

where $f(x_{ij}, P_i)$ is the individual prediction described by a function determined by the parameter vector $P_i$ (all parameters of an individual) and the independent variables $x_{ij}$ (e.g. time, dose). $\varepsilon_{ij}$ is a random effect termed residual error, describing differences between observations and individual predictions. The mean error is assumed to be zero, i.e. the model is adjusted so that, on average, deviations are neither systematically positive nor systematically negative. Epsilon values are assumed to be normally distributed with an estimated standard deviation $\sigma$.

The best parameter estimates are those that render the observed data more probable than they would be under any other set of parameters.

Mechanism-based models
Mechanism-based modeling is an approach in which the physiological, pathological and pharmacological processes of relevance to a given problem
are represented as directly as possible\textsuperscript{32}. Formalizing biological knowledge into mechanism-based models has important advantages over merely empiric data description. As a mechanism-based model is determined by the underlying physiology, it should be able to describe all the available data across different clinical trials, to interpolate between and extrapolate beyond the range of observed data\textsuperscript{33} and to be used as a predictive tool\textsuperscript{34}. This also implies the possibility of bridging between different populations such as healthy volunteers and patients, given the knowledge of the pathophysiology of the disease.

Since mechanism-based models consist of components that can be directly related to biological observations, they also allow for testing whether assumed hypotheses are consistent with observed behavior\textsuperscript{32}. This might lead to a better understanding of the biological systems in question. These models make it possible to better understand processes not directly amenable to experiments, and to predict system behavior under conditions not previously experienced. They also offer the possibility of examining the sensitivity of a system to parameter variation. This is particularly true for whole body biosimulation models. Different experiments probe the behavior of each tissue or organ under a range of conditions. Therefore, combining the results from various experiments and using them for calibration of a predictive model can yield valuable insights about gaps in biological understanding\textsuperscript{35}. Hypotheses that explain the experimental disagreement can be proposed and tested by incorporating them into the model.

However, mechanistic models tend to be high dimensional\textsuperscript{33}, which can easily lead to non-identifiability. In the case of the above-named whole-body simulation models, the only solution is to resort to fixing parameters to published values instead of estimating them from available clinical data. A pragmatic middle course is represented by semi-mechanistic models that are based on physiology but kept to a complexity that can be derived from clinical data.

**Rationales for the modeling of glucose homeostasis**

Endocrinology is a field in which mechanism-based modeling is particularly appropriate, as negative feedback regulation and other control mechanisms influencing hormone and metabolite concentrations play a crucial role\textsuperscript{36}. Model-based approaches, in combination with innovative experiments that generate informative data, can thus lead to a better understanding of the complex control relationships between glucose and insulin. They also offer the possibility to calculate clinically relevant parameters that are used for gaining information on the disease state of diabetic patients and for understanding sources of variability in the patient population.
In the development of type 2 antidiabetic drugs, models representing the glucose-insulin homeostasis play an important role in assessing or overcoming uncertainties in treatment response arising from the complexity of the disease, its chronic evolution and a high inter-patient variability. They are particularly relevant for making informed decisions in the early development stages, when only limited information is available from glucose provocation experiments that are often difficult to interpret. In this context, prediction of drug effects in the target population when only healthy volunteer data are available would be particularly valuable.

Possible applications of mechanistic models representing the glucose-insulin system include the interpretation of clinical data, the extrapolation beyond the range of observations in a particular clinical study and the optimization of subsequent study designs. They could allow for gaining more insight into the mechanisms of action of novel antidiabetic drug classes and facilitate the investigation of the interaction between food intake and drug effects. Ultimately, they could be used as tools for simulation of clinical trial outcomes of novel antidiabetic drugs as single substances or in combination therapy.

**Milestones in previous glucose-insulin modeling**

**Minimal model**

In the last three decades, much effort has been put into the development of models of the glucose-insulin system. Probably the best known model is Bergman’s “minimal” model for glucose\(^{37}\), published in 1979. Its main intention was to gain a better understanding of the insulin action to clear glucose from circulation. The model output is summarized in two main parameters, insulin sensitivity and glucose effectiveness. Insulin sensitivity is a measure of the sensitivity of glucose clearance to insulin concentrations and glucose effectiveness represents the ability to clear excess glucose independently of insulin action. These parameters have ever since been extensively used to assess the metabolic status of individual patients both in research and clinic. In the minimal model, the complexity of the glucose-insulin interaction was handled by using insulin concentrations as known fixed input while modeling parameters related to glucose concentrations. The model was fitted to individual data. Similar models have been created for insulin\(^{38}\), c-peptide\(^{39,40}\) and other entities involved in the glucose-insulin regulation.

The description of glucose profiles by the original minimal model was improved by Caumo and Cobelli, who developed the “hot two-compartment minimal model”\(^{41}\) in 1993. They introduced a peripheral glucose compartment into Bergman’s minimal model and used hot glucose to distinguish between administered and endogenously produced glucose. Both additions
lead to considerable improvements of the description of the system. However, problems with this model comprised sometimes unsatisfactory precision of parameter estimates and the fact that in some situations the model predicted physiologically implausible negative values. In addition, it shared the problem of all other models using individual estimation techniques that the data from one individual are often not enough to identify the entire model\textsuperscript{28}. This problem was overcome by Vicini and Cobelli, as they applied a population approach to minimal modeling and thus obtained improved parameter estimate precision despite reduced sampling during the IVGTT\textsuperscript{42}.

From 2000 on, considerable efforts have been devoted to the development of models for OGTT or meal test data, one important reason being the need for experimentally simple methods for large studies\textsuperscript{43-45}. These models use the minimal model description of insulin effects on glucose clearance, but add additional model components to extract information on glucose rate of appearance from the plasma glucose profile.

Closed-loop glucose-insulin models

All models derived from Bergman’s minimal model share the drawback that each one focuses on a separate entity, usually glucose or insulin. As these models only represent one part of the glucose-insulin system and require the other part as independent variable, they cannot be used for simulation.

Glucose and insulin interact in the same system and at the same time; therefore it would be preferable to analyze data collected on the kinetics of both substances simultaneously. In a theoretical work, DeGaetano and Arino showed that the two parts of the minimal model cannot be combined and used for simultaneous fitting of glucose and insulin data\textsuperscript{46}. Hence, they proposed an alternative model combining glucose and insulin kinetics and allowing for single-step parameter fitting. It is a one-compartment model for both glucose and insulin, relying on individual parameter fitting, and it does not make use of tracer glucose data. Thus, this innovative approach still leaves room for refinement. Other integrated glucose-insulin models have been proposed previously\textsuperscript{47,48}, but have not shown satisfactory simulation properties\textsuperscript{48}.

Drug effect models

With the exception of models for insulin therapy\textsuperscript{47-49}, only few drug-disease models have been published so far. However, the minimal model has been extensively used to analyze drug effects. This was typically done by correlating a summary parameter of drug exposure (concentration at a certain point in time, maximal concentration or area under the concentration-time curve) to a minimal model parameter. Agersø and Vicini\textsuperscript{50} presented a minimal model-derived approach that could reproduce a dose-dependent effect of
GLP-1 on insulin secretion. The model was based on observations in healthy volunteers and required the input of data obtained by an IVGTT. The parameters affected by the drug were determined by plotting drug plasma concentrations at a time point close to $t_{\text{max}}$ against individual parameter estimates. Significant covariate regression relationships were included in the model. Models like this are valuable tools for descriptive data analysis. However, as the time aspect is not taken into account and dynamic control mechanisms are not reflected by this approach, it cannot be used for predictive purposes.

Models incorporating disease progression

An interesting new approach trying to elucidate the mechanisms leading to the development of diabetes was proposed by Topp et al\textsuperscript{51}. The authors developed a model which adds the dynamics of $\beta$-cell mass as a third major factor determining glycemic control besides glucose and insulin kinetics. Starting from a single-compartment minimal modeling approach, a component representing a slow, glucose-dependent change in $\beta$-cell mass was added. Under the assumption that a gradual increase in plasma glucose causes an increase in $\beta$-cell mass (compensation), while a large increase in glucose causes a decrease in $\beta$-cell mass (pancreatic exhaustion), three distinct pathways of $\beta$-cell failure leading to hyperglycemia and diabetes were described by the model.

While Topp et al focused on the deterioration of glycemic control in untreated individuals, Frey et al\textsuperscript{52} developed a model to study the long-term effect of gliclazide on FPG in type 2 diabetic patients. A population PK-PD model incorporating an empirical linear model for disease progression was used to quantify the effect of gliclazide over time based on repeated FPG determinations. With this model it was possible to estimate a mean rate of disease progression and the associated variability.

A mechanism-based model for comparison of the long-term effects of three antidiabetic compounds with different mechanisms of action (pioglitazone, metformin and gliclazide) in type 2 diabetics was published by De Winter et al in 2006\textsuperscript{53}. It describes time courses of FPG, fasting serum insulin (FSI) and glycosylated hemoglobin (HbA$_{1C}$). This model is a tool for the evaluation of disease modifying properties of antidiabetic drugs by discriminating disease progression, short-term symptomatic effects and long-term protective effects. However, precise knowledge of the mechanisms of action of the tested drugs is required to be incorporated into the model. It is therefore anticipated that the DeWinter-model will be most valuable in late-stage drug development or evaluation of drugs already on the market.
Aims

The aim of this thesis was to develop a mechanistic model for the regulation of glucose homeostasis simultaneously incorporating the information of both glucose and insulin observations. This model should be able to describe and predict:

- data obtained from all types of clinical provocation experiments, with and without the use of labeled glucose. This explicitly includes clamp studies, IVGTTs, OGTTs and meal tests,

- glucose and insulin profiles from multiple successive challenges, in particular from multiple meals,

- data in healthy volunteers and type 2 diabetic patients, characterizing the differences between these two populations,

- 24-hour glucose and insulin profiles under consideration of circadian variations, and

- effects of oral antidiabetic drugs on the glucose-insulin system according to their mechanism of action.
Methods

Data
The data used for model development and validation were obtained from different glucose provocation experiments, including studies in healthy volunteers and in type 2 diabetic patients. The majority of the data sets is comprised of glucose, insulin and in some cases labeled glucose measurements in untreated subjects. One data set contains measurements of glucose and insulin levels in diabetic patients on active treatment. A summary of all data sets used for model development is presented in table 1.

Healthy volunteers

Clamp study
Observations from a euglycemic clamp experiment were part of the data set used for the development of the model for intravenous (IV) glucose provocations (paper I). The clamp study was performed in six healthy volunteers. The experiment was conducted in two parts. The first part was performed in the fasting state. A tracer dose of hot glucose was administered intravenously, and frequent blood samples were drawn for 150 minutes. After 150 minutes, a euglycemic hyperinsulinemic clamp was begun and continued for 180 minutes (i.e up to 330 minutes after the start of the experiment). Glucose concentrations were held constant at 87 mg/dl by a variable glucose infusion. After 240 minutes, a second tracer dose of hot glucose was given, and frequent blood samples were drawn for another 90 minutes, until the end of the experiment. The total number of blood samples amounted to 66 per individual. Hot glucose concentrations and the target concentrations of glucose and insulin were available for analysis from the experiment.

IVGTT
Two stable labeled IVGTT studies in healthy volunteers were included into the database for the IV model (paper I). The first study was performed in 14, the second one in 10 individuals. An intravenous bolus dose of glucose, enriched with hot glucose, was administered and frequent blood samples were drawn pre-dose and for 240 minutes following the glucose dose. The second study also included a 5-minute insulin infusion which was started after 20 minutes. A total of 30 blood samples per individual were
analyzed to determine the concentration of total glucose, hot glucose and insulin.

**Type 2 diabetic patients**

**IVGTT**

IVGTT data in type 2 diabetic patients were used both for the development of the IV model (paper I) and the subsequent development of the OGTT model (paper II). An insulin-modified stable isotopically labeled IVGTT was performed in 42 type 2 diabetic patients, who had undergone a three-week washout period. An intravenous bolus dose enriched with stable labeled glucose was administered at the start of the experiment. After 20 minutes, a 5-minute insulin infusion was started. A total of 34 blood samples per individual were drawn pre-dose and during 240 minutes following the glucose dose. Plasma samples were analyzed to determine the concentration of glucose, hot glucose and insulin.

**OGTT**

*Placebo only*

An OGTT was performed in the same 42 patients mentioned above, one day prior to the IVGTT. The subjects drank a solution of 75g glucose within 5 minutes. Six blood samples for the determination of plasma glucose and insulin were collected pre-dose and up to 240 minutes after the glucose load. These data were the basis for the development of the OGTT model (paper II).

*Placebo and active drug*

A study investigating the effect of a new antidiabetic compound was used for the development of the drug-effect model (paper IV). This study was a single-center, randomized, double-blind, placebo-controlled, three-period cross-over study. Each of the 15 enrolled patients received placebo and 2 different doses of the study drug. The treatment periods were separated by wash-out periods of 14 days.

The study drug or placebo was administered at approximately 6 AM. Two hours later, coinciding with the anticipated maximal plasma concentration ($t_{\text{max}}$) of the study drug, an OGTT was performed. An oral glucose load of 75 g was administered over 5 minutes. Blood sampling lasted for 7 hours (2 hours under fasting conditions and 5 hours after the start of the OGTT), resulting in a total of 25 measurements of glucose and insulin concentrations per patient and occasion.
Table 1. Summary of glucose provocation experiments

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Population</th>
<th>Number of individuals</th>
<th>Number of samples per individual</th>
<th>Glucose/CHO dose</th>
<th>Tracer dose, type of tracer</th>
<th>Insulin dose</th>
<th>Total duration of experiment</th>
<th>Used for development of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clamp</td>
<td>Healthy</td>
<td>6</td>
<td>66</td>
<td>Variable infusion at 150 minutes¹</td>
<td>68 and 94 μCi², [3-3H]glucose</td>
<td>Constant 1 mU/min/kg infusion starting at 150 min</td>
<td>5.5 h</td>
<td>IV model (paper I)</td>
</tr>
<tr>
<td>IVGTT</td>
<td>Healthy</td>
<td>14</td>
<td>30</td>
<td>0.25-0.33 g/kg</td>
<td>10% of total glucose, [6,6-2H₂]glucose</td>
<td>-</td>
<td>4 h</td>
<td>IV model (paper I)</td>
</tr>
<tr>
<td>IVGTT + insulin</td>
<td>Healthy</td>
<td>10</td>
<td>30</td>
<td>0.33 g/kg</td>
<td>10% of total glucose, [6,6-2H₂]glucose</td>
<td>0.03 U/kg at 20 minutes, 5-min infusion</td>
<td>4 h</td>
<td>IV model (paper I)</td>
</tr>
<tr>
<td>IVGTT + insulin</td>
<td>Type 2 diabetic</td>
<td>42</td>
<td>34</td>
<td>0.3 g/kg</td>
<td>13% of total glucose, [6,6-2H₂]glucose</td>
<td>0.05 U/kg at 20 minutes, 5-min infusion</td>
<td>4 h</td>
<td>IV and OGTT model (paper I&amp;II)</td>
</tr>
<tr>
<td>OGTT</td>
<td>Type 2 diabetic</td>
<td>42 (same as the above)</td>
<td>6</td>
<td>75 g</td>
<td>-</td>
<td>-</td>
<td>4 h</td>
<td>OGTT model (paper II)</td>
</tr>
<tr>
<td>OGTT</td>
<td>Type 2 diabetic</td>
<td>15</td>
<td>25 x 3 occasions</td>
<td>75g</td>
<td>-</td>
<td>-</td>
<td>7 h</td>
<td>Drug effect model (paper IV)</td>
</tr>
<tr>
<td>Meal test</td>
<td>Type 2 diabetic</td>
<td>59</td>
<td>38</td>
<td>62.5g (meals) 12.5g (snacks)</td>
<td>-</td>
<td>-</td>
<td>24 h</td>
<td>24-hour meal test model (paper III)</td>
</tr>
<tr>
<td>Meal test</td>
<td>Type 2 diabetic</td>
<td>18</td>
<td>27</td>
<td>90.7 - 92.7g per meal</td>
<td>-</td>
<td>-</td>
<td>14 h</td>
<td>24-hour meal test model (paper III)</td>
</tr>
</tbody>
</table>

¹ Variable glucose infusion starting at 150 minutes to balance the constant insulin infusion
² At time 0 and 240 minutes, respectively
Meal tests
A multiple meal test study, performed in 59 type 2 diabetic patients, provided the basis for the development of the 24-hour meal test model (paper III). The patients received three major meals at approximately 8 AM, 2 PM and 8 PM and three snacks at 11 AM, 5 PM and 11 PM. The carbohydrate input was calculated to 62.5 g for meals and 12.5 g for snacks. Blood samples for the determination of plasma glucose and insulin were taken every 0.5 hours for 16 hours, followed by bi-hourly collection up to 24 hours, resulting in a total of 38 blood samples.

Data from a second meal test study were used for external validation of the 24-hour meal test model. This data set contained glucose- and insulin observations obtained from 18 placebo-treated type 2 diabetic patients during 14 hours. These patients received 3 standardized meals at approximately 8 AM, 1 PM and 6 PM. The carbohydrate intake per meal ranged from 90.7 to 92.7 g. The data set contained 27 glucose and insulin measurements per patient. Samples were taken every 20 minutes up to two hours after a meal, and every hour thereafter.

Model development
Structural model
Aiming at the development of an integrated glucose-insulin model able to simultaneously analyze glucose and insulin concentration-time data, a glucose sub-model and an insulin sub-model needed to be combined. One- and two-compartment pharmacokinetic models for both sub-models were tested. Labeled glucose was assumed to exhibit the same pharmacokinetic properties as “normal” glucose.

While insulin degradation was assumed to be linear, different pathways of glucose elimination and their combinations were evaluated. Among these were insulin-dependent and insulin independent components, both from the central and from the peripheral compartment, and linear as well as saturable processes. Baseline insulin secretion and endogenous glucose production were modeled as equal to insulin and glucose elimination, respectively, at steady state.

The identifiability of known physiological interactions between glucose and insulin based on available clinical data was explored by incorporating different types and combinations of control mechanisms. Among those were the stimulation of the second-phase insulin secretion by glucose, the inhibition of endogenous glucose production by glucose and/or insulin and the stimulation of glucose elimination by insulin. In case of oral ingestion of glucose or carbohydrates (papers II–IV), the insulin response triggered by
gastrointestinal peptides (the “incretin effect”)\textsuperscript{11,58,59} was considered as an additional control mechanism. These control mechanisms were modeled with linear functions, power functions or different types of $E_{\text{max}}$ functions. As their actions set in with some delay, the use of effect-delay compartments\textsuperscript{60} was evaluated.

Initially, it was assumed that glucose and insulin disposition parameters and their variances should not significantly differ between the various types of provocation experiments of the glucose-insulin system. Therefore, the parameters were estimated based on the IV data that were much richer in information (paper I). Subsequently, they were fixed to the previously determined values when analyzing less informative data obtained from OGTTs and meal tests (papers II–IV). Only the additional parameters related to absorption were estimated. To test the validity of this hypothesis, separate disposition parameters were estimated one at a time while simultaneously fitting the model to IVGTT and OGTT data (paper II). If a significant difference between corresponding IV and oral parameters was observed and the splitting of parameters lead to an improved model fit, this specific parameter for the OGTT was kept in the model.

Glucose and insulin baselines
Different models to describe glucose and insulin baseline concentrations were applied. At first, the observed baselines were used as covariates, afflicted with a random error similar to all subsequent measurements\textsuperscript{61} (paper I, II & IV). However, in order to be able to use the model for simulation, it appeared favorable to estimate glucose and insulin baselines as populations values and inter-individual variabilities, thus avoiding being dependent on baseline measurements (paper III).

Parameter differences between healthy volunteers and diabetic patients
Parameter differences between healthy volunteers and type 2 diabetic patients were explored by estimating a separate set of parameters for both populations in the IV model. If no significant difference in the corresponding parameter values between the two populations could be detected, the two parameters were merged. Thus, the differences in glucose and insulin profiles between the healthy and the diseased population could be attributed to certain parameters and quantified.
Absorption

The absorption of orally administered glucose was described by a chain of transit compartments through which the glucose dose entered the central compartment (papers II & IV). The concept of transit compartments for the modeling of absorption delay was introduced by Savic et al⁶². As all data were log-transformed, the transit compartment equation was expressed as follows:

\[ ABSG(t) = k_{CA} \cdot e^{\log(dose \cdot BIO_{G}) - n \cdot \log(k_{CA}) - t \cdot \log(n!)} \]  \hspace{1cm} (4)

where \( ABSG \) is the rate of glucose absorption, \( BIO_{G} \) is the oral bioavailability of glucose, \( n \) is the number of transit compartments, and \( t \) is the time after the start of the experiment.

The rate constant for glucose transport into the central compartment \( (k_{CA}) \) is calculated as follows:

\[ k_{CA} = \frac{n}{MTT} \]  \hspace{1cm} (5)

The mean transit time (MTT) and \( n \) were estimated. MTT characterizes the average time for an orally administered glucose molecule to be absorbed. To implement the term \( \log(n!) \) of the transit compartment equation into NONMEM, a version of the sterling approximation published by Savic et al⁶² was used in its logarithmized form:

\[ \log(n!) = \log(\sqrt{2 \cdot 3.1415}) + (n + 0.5) \cdot \log(n) - n + \log(1 + \frac{1}{12 \cdot n}) \]  \hspace{1cm} (6)

The absorption model described above was used for single glucose provocations (papers I, II & IV). However, this model requires one equation per glucose dose or meal, thus putting a constraint on the number of consecutive provocations to be analyzed. As a consequence, it was necessary to apply a simpler absorption model for describing the absorption profiles of multiple meals (paper III). Both a simple first-order absorption model with lag-time and a transit compartment in combination with first-order absorption were considered. In meal tests, the “glucose dose” was assumed to be equal to the meal’s carbohydrate content. Incorrectness of this assumption would be reflected in the estimated bioavailability.
Modeling of circadian variation

Based on the 24h data set, the presence of circadian variation was evaluated on selected model parameters (paper III). A review of the literature suggested four parameters being subject to variations during the course of a day: glucose production\textsuperscript{63,64}, insulin secretion \textsuperscript{65-67}, insulin sensitivity\textsuperscript{63,67,68} and insulin degradation\textsuperscript{69,70}.

The first method for modeling circadian variations that was evaluated was a sum of cosine functions\textsuperscript{71} according to the following equation:

\[
PAR(t) = PAR(\text{av}) \cdot (1 + \sum_{i=1}^{4} (AMP_i \cdot \cos((t - T_{\text{max}}_i) \cdot \frac{2 \cdot i \cdot \pi}{24})))
\]

with \(PAR(\text{av})\) denoting the 24-hour mean, \(AMP_i\) being the amplitude of each respective cosine function and \(T_{\text{max}}_i\) representing the phase shift (i.e. the time when the amplitude reaches its maximum). The period of the first cosine function (i=1) is 24 hours, of the second cosine (i=2) 12 hours, of the third cosine 8 hours and of the forth cosine 6 hours.

The second approach made use of a previously published “surge function”\textsuperscript{72,73}, which in this case was subtracted from baseline to describe the suppression of the respective parameter during night-time. This function, as outlined below, is characterized by three parameters that define the suppression (SUP): its amplitude (SA), its width (SW) and the time of the peak (PT).

\[
SUP = \frac{SA}{(\frac{t - TP}{SW})^N + 1}
\]

\[
PAR(t) = PAR(\text{av}) \cdot (1 - SUP)
\]

\(PAR(\text{av})\) denotes again the 24-hour mean, \(N\) is a parameter defining the shape of the suppression and can take on any even number. \(N=4\) has most often been found to result in the best description\textsuperscript{72,73}. To verify this assumption, values of \(N=2, 4\) and 6 were evaluated.

Drug effect model

**Placebo model**

Drug effects were estimated based on the OGTT model. After fitting the model to the placebo data, all parameters were fixed. Parameters for which variability was estimated were fixed to their individual posthoc estimates. The placebo model was then applied to all data, including the 25 and 100 mg
dose groups. This run served as a reference run and provided the basis for the estimation of the effects of the test drug.

**Drug effects**

The model parameters on which an antidiabetic drug could plausibly exert its action were identified as being insulin secretion, glucose production, glucose elimination and the insulin effect on it.

The relationship between dose and effect on the four tested sites of action was described by a kinetic-pharmacodynamic (K-PD) model\(^7^4\). The respective dose was administered into a depot compartment, from which it entered a biophase compartment at an estimated rate \((k_{DE})\). Drug elimination from the biophase compartment occurred at the same rate. The link between the amount of drug in the biophase and its effect was described by a sigmoid \(E_{\text{max}}\) function. If the data did not support a sigmoid \(E_{\text{max}}\) function, it was reduced to an \(E_{\text{max}}\) function or a linear function.

Drug effects on the different sites of action were first tested one by one, each run being compared to the reference run. After selection of the model including a drug effect at the most significant site of action, a second evaluation round was started. The remaining effect sites were tested one by one on top of the effect already selected. When combining drug effects on several sites, each effect was mediated by a separate biophase compartment. If the addition of any of the other effect sites resulted in a significant model improvement, the best combination of two drug effects was selected. Addition of a third and a fourth drug effect was tested in an analogous manner until no significant improvement could be obtained any more.

**Inter-individual, intra-individual and residual variability**

The differences between individual parameters were regarded as random and were modeled in terms of eta \((\eta)\) variables. Eta variables were assumed to be normally distributed with a mean of zero and an estimated variance of \(\omega^2\). The distribution of the individual parameters around the typical population value was assumed to be log-normal (except for the variability in amplitude of the suppression function (paper III), which was logit transformed to assure positive values). The need for inclusion of inter-individual variability (IIV) terms was evaluated in all parameters.

Correlations between individual parameter distributions were evaluated by means of graphical assessment and the output of the covariance step in the non-linear mixed effects modeling software NONMEM\(^7^5\).

The IVGTT and the OGTT used for the development of the OGTT model (paper II) had been performed in the same 42 subjects. Therefore, random effects for inter-occasion variability (IOV) were investigated as well. The variation of parameters between the two study occasions within an individual was regarded as random and log-normally distributed. It was modeled in
terms of kappa (κ) variables as previously described by Karlsson and Sheiner. In analogy to the η variables, each κ variable was assumed to have a mean of zero and an estimated variance (κ²).

The differences between the logarithm of the observed plasma concentrations and the logarithm of the predicted plasma concentrations were regarded as random and modeled in terms of epsilon (ε) variables. Each ε variable was assumed to have a mean of zero and an estimated variance (ε²). An additive error model was applied to the log-transformed data, which approximately corresponds to a multiplicative error in normal scale. Separate residual error terms were estimated for total glucose (RESG), insulin (RESI), as well as hot glucose (RESH) if applicable (paper I). In addition, a multiplicative down-weighing factor (RESE) was estimated to account for higher discrepancies between measurements and predictions during the first two minutes of IV provocation experiments (paper I & 2), where the error was expected to be larger due to rapid concentration changes. This multiplying factor was applied simultaneously to the residual error terms for glucose, insulin and hot glucose.

Data analysis

Software

The data were analyzed by non-linear mixed effects modeling, using the first order conditional estimation (FOCE) method of NONMEM version VI and the differential equation solver ADVAN6. Simulations were performed with the same software. NONMEM runs were automated with PsN versions 2.2.2–2.2.4. Data set creation for NONMEM was performed using the SAS System for Windows version 8.2 (SAS Institute Inc., Cary, NC, USA). Graphics were produced in S-PLUS versions 6.1–7 (Insightful Inc, Seattle, WA, USA) and Xpose version 3.1.

Model selection

Model selection was based on goodness-of-fit plots, the physiological plausibility and the precision of parameter estimates, posthoc distributions of individual parameter values and the objective function value (OFV) provided by NONMEM. Classical goodness-of-fit plots such as observed values (DV) versus population predictions (PRED), DV versus individual predictions (IPRED) as well as population conditional and individual weighted residual errors versus time or versus concentrations were used for graphical assessment of the quality of the model fit. A difference in the OFV of at least 10.83 per added parameter was considered significant for nested models, which corresponds to a level of significance of α = 0.001.
Model validation

**Internal validation**

*Bootstrap*

The parameter precision of the final IV and OGTT model (papers I & II) was further evaluated by bootstrap analyses. Due to long run times, the number of bootstrap samples was limited to thirty in both cases. In the case of the IV model (paper I), the data set was composed of four different glucose provocation experiments. Therefore, the bootstrap to evaluate IV parameters was stratified by study.

*Log-likelihood profiling*

Log-likelihood profiling was used to create 95% confidence intervals around parameter estimates that were difficult to obtain or unstable.

*Visual predictive check*

Visual predictive checks (VPCs) were performed to evaluate the predictive performance of the model at various steps of model development. Each time, one hundred data sets were simulated. The medians and 90% prediction intervals (5th – 95th percentile) of these simulated individual concentration–time profiles of glucose and insulin were calculated and superimposed on the respective observed data (papers I–III). Good simulation properties are shown if approximately 5% of the observations are below and 5% are above the prediction interval.

*Other internal validation methods*

The question whether the parameters estimated separately for healthy volunteers and diabetic patients by the IV model (paper I) reflected significant differences between the two populations, and whether no other significant differences had been missed, was systematically assessed. All merged parameters were split one by one and all split parameters were merged one by one, and the model was re-estimated each time. The results were then compared to the final IV model.

**External validation**

The final 24-hour meal test model (paper III) was applied to an external data set containing glucose- and insulin observations obtained from 18 diabetic patients over 14 hours. Its performance was first evaluated with all parameters fixed by examining goodness-of-fit plots. Thereafter, the model parameters were re-estimated, with the exception of the parameters associated with the circadian rhythm. These needed to remain fixed, as data over only 14-hours (i.e. not including the night) would not allow for their estimation. The drop in OFV between the first and the second run, as well as important pa-
parameter shifts, were used as criteria to judge the general validity of the model.
Results

An integrated mechanism-based model able to simultaneously describe total glucose, hot glucose and insulin profiles was developed. Input data from various types of clinical provocation experiments were used, including intravenous provocation experiments, some of them containing labeled glucose (paper I), OGTTs (paper II&IV) and meal tests (paper III). The final model structures for intravenous glucose challenges in healthy volunteers and for OGTTs in type 2 diabetic patients are graphically presented in figures 1 and 2.

**Figure 1.** Schematic representation of the model for intravenous glucose provocations in healthy volunteers. The model for hot glucose is identical to the model for total glucose in all parts except glucose production and therefore not shown. Full arrows indicate flows and broken arrows indicate control mechanisms. $G_C$ and $G_P$, central and peripheral compartments of glucose; $G_{E1}$ and $G_{E2}$, effect compartments for the glucose-mediated control of glucose production and insulin secretion; $I$, insulin disposition compartment; $I_{FPS}$, delay compartment for the first-phase insulin secretion; $I_E$, effect compartment for control of glucose elimination by insulin; $Q$, $CL_G$ and $CL_{Gt}$, clearance parameters of the glucose model; $CL_I$ and $k_{IS}$, parameters of the insulin model; $k_{GE1}$, $k_{GE2}$ and $k_{IE}$, rate constants for the effect compartments.
2. The final parameter estimates obtained in all different studies (paper I–IV) are reported in table II. To graphically assess the predictive performance of the model, VPCs were performed. The result of a VPC of the final run describing glucose and insulin profiles during an IVGTT and an OGTT in diabetic patients (paper II) is shown in figure 3.

![Figure 2](image)

**Figure 2.** Schematic representation of the OGTT model in type 2 diabetic patients. Full arrows indicate flows and broken arrows indicate control mechanisms. GC and GP, central and peripheral compartments of glucose; GE2, effect compartment for the glucose-mediated control of insulin secretion; I, insulin disposition compartment; Ie, effect compartment of insulin for the control of glucose elimination; Q, CLG, CLGI, kCA, n, kinetic parameters of the glucose sub-model; CLI, insulin clearance; kGE2 and kIE, rate constants for the effect compartments; Emax, maximal effect of the absorption rate of glucose on insulin secretion; ABSG50, glucose absorption rate producing 50% of Emax.
Table 2. Final model parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical value</th>
<th>RSE (%)(^a)</th>
<th>IIV (%)</th>
<th>RSE (%)(^a)</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_G) (L)</td>
<td>9.33</td>
<td>6 (4)</td>
<td>30</td>
<td>18 (18)</td>
<td>1-4</td>
</tr>
<tr>
<td>(V_P) (L)</td>
<td>8.56</td>
<td>5 (4)</td>
<td>30</td>
<td>28 (32)</td>
<td>1-4</td>
</tr>
<tr>
<td>(CL_{GI\ HV}) (L/min)</td>
<td>0.0894</td>
<td>4 (13)</td>
<td>59</td>
<td>35 (32)</td>
<td>1</td>
</tr>
<tr>
<td>(CL_{GI\ PAT}) (L/min)</td>
<td>0.0287</td>
<td>15 (14)</td>
<td>59</td>
<td>35 (32)</td>
<td>1-4</td>
</tr>
<tr>
<td>(CL_{GI\ HV}) (L/min/(mU/L))</td>
<td>0.00829</td>
<td>33 (11)</td>
<td>53</td>
<td>22 (20)</td>
<td>1</td>
</tr>
<tr>
<td>(CL_{GI\ PAT\ IV}) (L/min/(mU/L))</td>
<td>0.00297</td>
<td>9 (8)</td>
<td>53</td>
<td>22 (20)</td>
<td>1, 2</td>
</tr>
<tr>
<td>(CL_{GI\ PAT\ \text{PO}}) (L/min/(mU/L))</td>
<td>0.0059</td>
<td>14 (10)</td>
<td>46</td>
<td>23 (26)</td>
<td>2, 4</td>
</tr>
<tr>
<td>Q (L/min)</td>
<td>0.442</td>
<td>16 (20)</td>
<td>85</td>
<td>27 (30)</td>
<td>1-4</td>
</tr>
<tr>
<td>(k_{G1\ HV}) (/min)</td>
<td>0.0573</td>
<td>11 (10)</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>(k_{G2\ HV}) (/min)</td>
<td>0.0289</td>
<td>14 (21)</td>
<td>85</td>
<td>43 (47)</td>
<td>1, 2</td>
</tr>
<tr>
<td>(GPRG_{HV}) (-)</td>
<td>-2.79</td>
<td>14 (11)</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>(GPRG_{PAT}) (-)</td>
<td>0 FIX</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>(G_{scale}) (-)</td>
<td>0.891</td>
<td>5 (8)</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>(BIOG) (-)</td>
<td>0.811</td>
<td>5 (8)</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>(MTT) (min)</td>
<td>34.9</td>
<td>11 (5)</td>
<td>11</td>
<td>42 (45)</td>
<td>1, 4</td>
</tr>
<tr>
<td>(N) (-)</td>
<td>1.27</td>
<td>24 (12)</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>(E_{max}) (-)</td>
<td>1.47</td>
<td>27 (9)</td>
<td>55</td>
<td>40 (38)</td>
<td>2</td>
</tr>
<tr>
<td>(ABSG_{S50}) (mg/min)</td>
<td>14.8</td>
<td>21 (52)</td>
<td>114</td>
<td>41 (50)</td>
<td>2</td>
</tr>
<tr>
<td>(k_g) (/min)</td>
<td>0.0151</td>
<td>4</td>
<td>19</td>
<td>29</td>
<td>3</td>
</tr>
<tr>
<td>(S_{inr}) (/mg)</td>
<td>0.000994</td>
<td>7</td>
<td>35</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>(GSS) (mg/dL)</td>
<td>158</td>
<td>2</td>
<td>14</td>
<td>21</td>
<td>3</td>
</tr>
</tbody>
</table>

**Glucose**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical value</th>
<th>RSE (%)(^a)</th>
<th>IIV (%)</th>
<th>RSE (%)(^a)</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_G) (L)</td>
<td>6.09</td>
<td>9 (6)</td>
<td>41</td>
<td>27 (27)</td>
<td>1-4</td>
</tr>
<tr>
<td>(CL_{L\ HV}) (L/min)</td>
<td>1.22</td>
<td>5 (4)</td>
<td>29</td>
<td>24 (20)</td>
<td>1-4</td>
</tr>
<tr>
<td>(FPS_{HV}) (mU)</td>
<td>704</td>
<td>20 (18)</td>
<td>67</td>
<td>35 (41)</td>
<td>1</td>
</tr>
<tr>
<td>(FPS_{PAT}) (mU)</td>
<td>0 FIX</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>(k_{IS\ HV}) (/min)</td>
<td>0.384</td>
<td>23 (22)</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>(k_{IE}) (/min)</td>
<td>0.0213</td>
<td>13 (13)</td>
<td>58</td>
<td>20 (26)</td>
<td>1-4</td>
</tr>
<tr>
<td>(I_{max}) (-)</td>
<td>1.42</td>
<td>12 (14)</td>
<td>35</td>
<td>46 (64)</td>
<td>1-4</td>
</tr>
<tr>
<td>(I_{scale}) (-)</td>
<td>0.93</td>
<td>3 (4)</td>
<td>-</td>
<td>-</td>
<td>1, 2</td>
</tr>
<tr>
<td>(ISS) (mU/L)</td>
<td>10</td>
<td>6</td>
<td>49</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>(SA) (%)</td>
<td>0.24</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>(SW) (min)</td>
<td>138</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>(PT) (min; clock time)</td>
<td>1122</td>
<td>1:10 AM</td>
<td>1</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

**Insulin**
Table 2 (continued)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical value</th>
<th>RSE (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IIV (%)</th>
<th>RSE (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Residual</strong>&lt;br&gt;Error**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RESG&lt;sub&gt;IV&lt;/sub&gt; (%)</td>
<td>4.36</td>
<td>4 (4)</td>
<td>-</td>
<td>-</td>
<td>1, 2</td>
</tr>
<tr>
<td>RESG&lt;sub&gt;PO&lt;/sub&gt; (%)</td>
<td>7.32</td>
<td>10 (11)</td>
<td>4</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10.7</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>13.2</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>RES&lt;sub&gt;I&lt;/sub&gt; (%)</td>
<td>25.2</td>
<td>6 (7)</td>
<td>3</td>
<td>-</td>
<td>1, 2</td>
</tr>
<tr>
<td></td>
<td>25.8</td>
<td></td>
<td></td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>RESH&lt;sub&gt;IV&lt;/sub&gt; (%)</td>
<td>10.3</td>
<td>18 (20)</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>RESH&lt;sub&gt;PAT&lt;/sub&gt; (%)</td>
<td>5.12</td>
<td>8 (9)</td>
<td>-</td>
<td>-</td>
<td>1, 2</td>
</tr>
<tr>
<td>RESE (-)</td>
<td>3.31</td>
<td>21 (18)</td>
<td>-</td>
<td>-</td>
<td>1, 2</td>
</tr>
<tr>
<td><strong>Correlations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corr&lt;sub&gt;VG-Q&lt;/sub&gt; (-)</td>
<td>-0.75</td>
<td>23 (31)</td>
<td>-</td>
<td>-</td>
<td>1, 2</td>
</tr>
<tr>
<td>Corr&lt;sub&gt;VG-VI&lt;/sub&gt; (-)</td>
<td>0.71</td>
<td>27 (32)</td>
<td>-</td>
<td>-</td>
<td>1, 2</td>
</tr>
<tr>
<td>CorrQ-VI (-)</td>
<td>-0.35</td>
<td>50 (178)</td>
<td>-</td>
<td>-</td>
<td>1, 2</td>
</tr>
<tr>
<td>Corr CLGI-IV -&lt;br&gt; CLGI-PO (-)</td>
<td>0.77</td>
<td>27 (37)</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><strong>Drug</strong>&lt;br&gt;<strong>effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k&lt;sub&gt;Dep&lt;/sub&gt; (min)</td>
<td>0.00848</td>
<td>20</td>
<td>66</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>S (mg)</td>
<td>0.0544</td>
<td>20</td>
<td>73</td>
<td>45</td>
<td>4</td>
</tr>
<tr>
<td>K&lt;sub&gt;DE1&lt;/sub&gt; (min)</td>
<td>0.0158</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>DA&lt;sub&gt;50&lt;/sub&gt; (mg)</td>
<td>5.27</td>
<td>42</td>
<td>200</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>D&lt;sub&gt;max&lt;/sub&gt; (-)</td>
<td>0.282</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>s (-)</td>
<td>4.17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>

<sup>a</sup> The RSE% from the bootstrap is presented in parentheses.

IIV%, inter-individual variability in percent; RSE%, relative standard error in percent; IV, specific parameter for IV provocation experiments; PO, specific parameter for oral provocation experiments; HV specific parameter for healthy volunteers, PAT specific parameter for diabetic patients; Corr, correlation between individual estimates; V<sub>G</sub>, volume of distribution of the central glucose compartment; V<sub>P</sub>, volume of distribution of the peripheral glucose compartment; CL<sub>G1</sub>, insulin-independent glucose clearance; CL<sub>G2</sub>, insulin-dependent glucose clearance; Q, inter-compartmental clearance of glucose; k<sub>G1E1</sub>, rate constant for the glucose effect compartment controlling glucose production; k<sub>G2E2</sub>, rate constant for the glucose effect compartment controlling insulin secretion; GPRG, control parameter for the plasma glucose effect on glucose production; G<sub>scale</sub>, scaling parameter for the glucose baseline; BIOG, bioavailability of glucose; MTT, mean transit time of glucose absorption; n, number of transit compartments; E<sub>max</sub>, maximal effect of the absorption rate of glucose on insulin secretion; ABSG<sub>50</sub>, glucose absorption rate producing 50% of E<sub>max</sub>; k<sub>σ</sub>, glucose absorption rate constant; S<sub>incr</sub>, slope of the linear link between the absorption rate of glucose and insulin secretion; G<sub>SS</sub>, steady state glucose concentration; V<sub>h</sub>, volume of distribution of insulin; CL<sub>I</sub>, insulin clearance; k<sub>IE</sub>, rate constant for the effect compartment for insulin; IPRG, control parameter for the glucose effect on insulin secretion; I<sub>scale</sub>, scaling parameter for the insulin baseline; I<sub>SS</sub>, steady state insulin concentration; SA, suppression amplitude on baseline insulin secretion; SW, suppression width on baseline insulin secretion; PT, suppression peak time on baseline insulin secretion; RESG, residual error for total glucose; RESI, residual error for insulin; RESH, residual error for hot glucose; RESE, multiplying error factor for early time points (<2 minutes); k<sub>DEp</sub>, rate constant to and from the biophase compartment for the pancreatic drug effect; S, slope of the linear relationship between amount of drug in the biophase and the pancreatic drug effect; k<sub>DEI</sub>, rate constant to and from the biophase compartment for the drug effect on the liver; DA<sub>50</sub>, amount of drug in the biophase compartment producing 50% of D<sub>max</sub>; D<sub>max</sub>, maximal drug effect on the liver; s, Hill coefficient of the E<sub>max</sub> function defining the amount-effect relationship of the drug acting in the liver.
Figure 3. Visual predictive check. One hundred IVGTT and OGTT data sets in diabetic patients were simulated. Above, IVGTT profiles (left, glucose; right, insulin) and below, OGTT profiles (left, glucose; right, insulin) are displayed. Observations from the original data set are plotted as points. The black lines show the medians of the individual predictions of the one hundred simulations, and the gray lines indicate the 5th and 95th percentiles. The small insert in the insulin IVGTT concentration–time graph shows the same profile in log-scale.

The glucose sub-model

Glucose pharmacokinetics was described by a two-compartment model (central compartment, G_C, and peripheral compartment, G_P) with endogenous production of glucose (G_PROD) entering the central compartment. Glucose elimination occurred from the central compartment and was separated into an insulin-dependent and an insulin-independent component.

\[
\frac{dG_C(t)}{dt} = G_{\text{PROD}}(t) + \frac{Q}{V_P} \cdot G_p(t) - \frac{CL_G + CL_{GI} \cdot I_E(t) + Q}{V_G} \cdot G_C(t)
\]

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

(10)
\[
\frac{dG_P(t)}{dt} = Q \cdot \left( \frac{G_C(t)}{V_G} - \frac{G_P(t)}{V_P} \right), \quad G_P(0) = G_{SS} \cdot V_P
\]

The disposition parameters are estimated as clearances and volumes. \( V_G \) and \( V_P \) are the central and peripheral volumes of distribution for total glucose, respectively. \( V_G \) is proportional to weight which was incorporated as a covariate and normalized to 70 kg. \( Q \) is the inter-compartmental clearance of glucose, and \( CL_G \) and \( CL_{GI} \) are the insulin-independent and insulin-dependent clearances of glucose from the central compartment. \( I_E \) denotes the effect of insulin on glucose elimination.

In the case of oral glucose absorption modeled via a chain of transit compartments (papers II&IV), equation 10 gets extended by an absorption term:

\[
\frac{dG_C(t)}{dt} = \text{ABSG}(t) + G_{PROD}(t) + \frac{Q}{V_P} \cdot G_P(t) - \frac{CL_G + CL_{GI} \cdot I_E(t) + Q}{V_G} \cdot G_C(t)
\]

\[ G_C(0) = G_{SS} \cdot V_G \]  \quad (12)

However, for the description of several consecutive meals (paper III) the absorption model was changed to a first-order absorption model with one transit compartment for glucose. Thus, two compartments were added: one absorption compartment (\( G_A \)) and one transit compartment (\( G_T \)).

\[
\frac{dG_A(t)}{dt} = -G_A(t) \cdot k_a
\]

\[ \frac{dG_T(t)}{dt} = k_a \cdot (G_A(t) - G_T(t)) \]  \quad (14)

\( k_a \) denotes the glucose absorption rate constant.

In this case, the equation for the central compartment of glucose becomes

\[
\frac{dG_C(t)}{dt} = k_a \cdot G_T(t) + G_{PROD}(t) + \frac{Q}{V_P} \cdot G_P(t) - \frac{CL_G + CL_{GI} \cdot I_E(t) + Q}{V_G} \cdot G_C(t)
\]

\[ G_C(0) = G_{SS} \cdot V_G \]  \quad (15)

The glucose model also comprised two effect compartments (\( G_{E1} \) and \( G_{E2} \)), accounting for the time course of the control plasma glucose exerts on endogenous glucose production and on second-phase insulin secretion.
\[
\frac{d G_{E1}(t)}{dt} = k_{GE1} \cdot \left( \frac{G_e(t)}{V_G} - G_{E1}(t) \right), \quad G_{E1}(0) = G_{SS} \tag{16}
\]

\[
\frac{d G_{E2}(t)}{dt} = k_{GE2} \cdot \left( \frac{G_e(t)}{V_G} - G_{E2}(t) \right), \quad G_{E2}(0) = G_{SS} \tag{17}
\]

The baseline endogenous glucose production \( G_{\text{PROD,0}} \), described by equation 18, is modeled as replacing the eliminated glucose at steady state \( G_{SS} \).

\[
G_{\text{PROD,0}} = G_{SS} \cdot \left( CL_G + CL_GI \cdot I_{SS} \right) \tag{18}
\]

The negative feedback control of glucose production by plasma glucose \( G_{CM1} \) was described by a power function, the power \( GPRG \) being an estimated parameter (equation 19).

\[
G_{CM1}(t) = \left( \frac{G_{E1}(t)}{G_{SS}} \right)^{GPRG} \tag{19}
\]

Equation 20 describes the total endogenous glucose production.

\[
G_{\text{PROD}}(t) = G_{\text{PROD,0}} \cdot G_{CM1}(t) \tag{20}
\]

As the feedback of plasma glucose on glucose production is impaired in diabetic patients and \( GPRG \) was estimated to a negligible value, glucose production was modeled as constant in this population, as outlined in equation 21:

\[
G_{\text{PROD}} = G_{\text{PROD,0}} \tag{21}
\]

Alternative models with an insulin effect on glucose production alone or in addition to the negative glucose feedback resulted in either a significantly higher OFV (in the case of insulin control of glucose production) or parameter estimates indicating a negligible insulin effect on glucose production (in the case of a combination of both control mechanisms). The two alternative models were therefore rejected.
The hot glucose sub-model

The model for hot glucose was assumed to be identical to the model proposed for total glucose in all parts but glucose production. It was described by the following differential equations for the central, $H_C$, and peripheral, $H_P$, compartments:

$$\frac{dH_C(t)}{dt} = \frac{Q}{V_p} \cdot H_P(t) - \frac{CL_G + CL_{GI} \cdot I_E(t)}{V_G} \cdot H_C(t)$$

$H_C(0) = 0 \quad (22)$

$$\frac{dH_P(t)}{dt} = Q \cdot \left( \frac{H_C(t)}{V_G} - \frac{H_P(t)}{V_P} \right), \quad H_P(0) = 0 \quad (23)$$

The insulin sub-model

Insulin kinetics was characterized by a one-compartment model with first order elimination, as described by equation 24, where $I$ represents the insulin disposition compartment.

$$\frac{dI(t)}{dt} = I_{SEC}(t) - \frac{CL_I}{V_I} \cdot I(t), \quad I(0) = I_{SS} \cdot V_I \quad (24)$$

In addition, the insulin sub-model also contained one effect compartment for the regulation of insulin-dependent glucose elimination, $I_E$ (equation 25).

$$\frac{dI_E(t)}{dt} = k_{IE} \cdot \frac{I(t)}{V_I} - k_{IE} \cdot I_E(t), \quad I_E(0) = I_{SS} \quad (25)$$

$CL_I$ is the clearance of insulin, and $V_I$ is the volume of distribution of its disposition compartment. In analogy to $V_G$, $V_I$ is modeled as proportional to body weight.

Insulin secretion ($I_{SEC}$) was described in two separate parts representing first- and second-phase secretion. The first-phase secretion was modeled as an estimated bolus dose (FPS), entering the disposition compartment through a delay compartment, $I_{FPS}$, with the rate constant $k_{fS}$. 

45
\[
\frac{dI_{FPS}(t)}{dt} = -k_{IS} \cdot I_{FPS}(t), \quad I_{FPS}(0^+) = FPS
\]  \hspace{1cm} (26)

The baseline \((I_{SEC,0})\) and second-phase insulin secretion are described in analogy to endogenous glucose production. \(I_{SEC,0}\) is equal to the amount of insulin cleared from plasma per time unit at steady state \((I_{SS})\).

\[
I_{SEC,0} = I_{SS} \cdot CL_I
\]  \hspace{1cm} (27)

A power function with the estimated power IPRG was used to describe the stimulation of insulin secretion by plasma glucose at non-steady state \((G_{CM2})\).

\[
G_{CM2}(t) = \left( \frac{G_{E2}(t)}{G_{SS}} \right)^{IPRG}
\]  \hspace{1cm} (28)

The enhanced insulin secretion following the OGTT compared to an IV glucose provocation (“incretin effect”) was handled by establishing an \(E_{\text{max}}\)-relationship between the absorption rate of glucose and the secretion of insulin (paper II),

\[
I_{ABSG}(t) = 1 + \frac{E_{\text{max}} \cdot ABSG(t)}{ABSG(t) + ABSG_{50}}
\]  \hspace{1cm} (29)

where \(E_{\text{max}}\) is the maximal effect of the absorption rate of glucose on insulin secretion, and \(ABSG_{50}\) refers to the glucose absorption rate producing 50% of the maximal effect.

In situations with limited data in the non-linear part of effect-concentration curve because of slower absorption (paper III) or few subjects (paper IV), the \(E_{\text{max}}\) function had to be replaced by a slope \((S_{\text{incr}})\) for stability reasons (equations 30 and 31 for absorption via transit compartment equation (paper IV) or first order absorption plus transit compartment (paper III), respectively).

\[
I_{ABSG}(t) = 1 + S_{\text{incr}} \cdot ABSG(t)
\]  \hspace{1cm} (30)

\[
I_{ABSG}(t) = 1 + S_{\text{incr}} \cdot G_{T}(t)
\]  \hspace{1cm} (31)

Total insulin secretion is described in healthy volunteers (paper I) as the sum of first phase and second phase insulin secretion.
\[ I_{SEC}(t) = k_{IS} \cdot I_{FPS}(t) + I_{SEC,0} \cdot G_{CM2}(t) \]  

(32)

In diabetic patients, FPS was estimated to a negligible value. Equation 30 therefore reduces to

\[ I_{SEC}(t) = I_{SEC,0} \cdot G_{CM2}(t) \]  

(33)

However, for oral glucose provocations (papers II–IV), this term becomes

\[ I_{SEC}(t) = I_{SEC,0} \cdot G_{CM2}(t) \cdot I_{ABSG}(t) \]  

(34)

due to the incretin effect.

Population and study-specific parameters (papers I&II)

In the final IV model, four parameters differed between healthy volunteers and type 2 diabetic patients. These parameters included CL_G, CL_Gi, FPS and GPRG. The latter two were estimated at very small values for the patient population. Therefore, they were fixed to zero in the final model. As a consequence, the structural model was simplified as indicated above. Both CL_G and CL_Gi were significantly lower in patients than in healthy volunteers. To correct for the fact that the observed glucose and insulin concentrations in patients were below baseline at the end of the experiment, scaling factors were incorporated to improve the fit.

Only one parameter was found to differ significantly between the IV and oral glucose provocations: the estimated insulin-dependent clearance of glucose (CL_Gi) was approximately twice as high for the OGTT (paper II).

Circadian effects (paper III)

Estimating circadian changes of insulin secretion lead to a better model fit than attributing the variation to any other of the tested parameters.

The best model comprising cosine functions was composed of a sum of two cosines on the baseline insulin secretion (I_{SEC,0}). One cosine was not flexible enough to describe the asymmetric physiologic time-course of insulin secretion. On the other hand, a combination of three cosines provided too much flexibility, with the consequence of random fluctuations without physiological meaning appearing in the profiles.
\[ I_{SEC,0}(t) = I_{SEC,0}(av) \cdot \left( 1 + \sum_{i=1}^{2} (AMP_i \cdot \cos((t - T \max_i) \cdot \frac{2 \cdot i \cdot \pi}{24})) \right) \quad (35) \]

The suppressor function model delivered results very similar to the cosine model, both in terms of parameter values and OFV. This model was more parsimonious concerning the number of estimated parameters, ran with shorter run times, delivered more precise parameter estimates and showed overall improved model stability. Therefore, it was selected as the final model. The suppressor function exerted its effect on insulin secretion between approximately 9 PM and 5 AM, resulting in a maximal reduction of insulin secretion of 24%.

\[ I_{SEC,0}(t) = I_{SEC,0}(av) \cdot \left[ 1 - \frac{SA}{(\frac{t - TP}{SW})^{4} + 1} \right] \quad (36) \]

The effects of the suppressor function describing circadian variation of insulin secretion on glucose and insulin concentration-time profiles is shown in figure 4.
Figure 4. Population predictions by the final 24-hour meal test model showing plasma glucose concentration (top), plasma insulin concentration (middle) and baseline insulin secretion (bottom) over time. Full lines show population predictions with suppressor function and broken lines the same predictions without suppressor function. The population prediction for baseline insulin secretion without suppressor function would be a constant line at approximately 8 mU/min (not shown).
Drug Effects (paper IV)

A schematic representation of the pre-determined sites of drug action (insulin secretion, glucose production, insulin-independent glucose elimination and the insulin effect on glucose elimination) in the OGTT model for diabetic patients is shown in figure 5. Estimating a drug effect on insulin secretion led to the largest improvement in goodness-of-fit. Adding another effect on either glucose production or insulin-independent glucose elimination additionally improved the fit, whereof the combined effect on insulin secretion and glucose production performed best.

Graphical analysis revealed that the inclusion of two drug effects already corrected all the bias in glucose profiles observed with the placebo model (see paper IV) and resulted in an adequate model fit (figure 6). In addition, adding a third drug effect consistently lead to model failure because of nu-
merical difficulties or identifiability issues. Therefore, the run with a stimulating effect on insulin secretion and an inhibiting effect on glucose output was identified as the best and final run.

![Glucose and insulin concentration-time profiles for placebo and two active GKA dose groups. Dots represent observations and the grey area depicts the 90% individual prediction interval (5th-95th percentage of IPREDs) by the final model. The full lines show the medians of the observed data, the broken lines display the medians of individual model predictions.](image)

Figure 6. Glucose and insulin concentration-time profiles for placebo and two active GKA dose groups. Dots represent observations and the grey area depicts the 90% individual prediction interval (5th-95th percentage of IPREDs) by the final model. The full lines show the medians of the observed data, the broken lines display the medians of individual model predictions.

The potential application of this model to predict the effects of this compound on glucose and insulin levels is shown in figure 7. Ninety-percent prediction intervals of the active drug (100 mg) versus placebo illustrate the magnitude of drug effect that could be expected (first row: total drug effect on glucose and insulin profiles). The model also allowed for separating out the effects of the drug on the pancreas and on the liver by removing the respective other part before simulation. The relative contributions of the two sites of effect on glucose and insulin profiles are shown in the second and third row of figure 7. The effect on the liver did not seem to play any significant role on the insulin profiles and mainly influenced glucose levels during the fasting period. In contrast, the effect on insulin secretion had a major impact on glucose levels, particularly after the start of the OGTT.
Model validation

Internal validation

The results of the different methods of internal validation applied at the different stages of model development are reported in the respective papers.

External validation

The 24-hour meal test model was successfully validated with a different data set containing observations from 18 type 2 diabetic patients over 14 hours. The model adequately predicted the data. When applying the final model with all parameters fixed to the 14-hour data, goodness-of-fit plots looked satisfactorily. On re-estimating the 17 model parameters, the OFV dropped only 76 points, and no major parameter shifts were observed. However, it was not possible to evaluate the description of circadian variation based on the 14-hour data set, as no nighttime data were available.

Figure 7. Total GKA effect on glucose and insulin profiles as compared to placebo (top), and drug effects at specific sites of action (pancreas: middle and liver: bottom). Areas represent the 90% range of individual model predictions (5th-95th percentile) of 100 mg active drug (shaded area) versus placebo (full area).
Discussion

The model presented in this thesis has been shown to simultaneously describe glucose and insulin levels and their regulation. Its development was based on data originating from different kinds of intravenous (paper I) and oral (paper II) glucose provocation experiments in both healthy volunteers and type 2 diabetic patients. Its ability to describe data obtained from several consecutive meal tests has also been confirmed (paper III). The model has been shown to have good simulation properties, as illustrated by a visual predictive check (figure 3). This represents a major advantage in comparison to previously published models.

In addition to the estimation and prediction of placebo data, the model has also been applied for estimation of antidiabetic drug effects (paper IV). It has been shown to be able to determine the correct mechanisms of action of a GK agonist under development for the treatment of type 2 diabetes. Furthermore, it was possible to accurately quantify the effects of the test compound on glucose and insulin concentration-time profiles (figure 6). To the best of our knowledge, this is the first time this has been achieved with a mechanistic drug-disease model for type 2 diabetes.

Parameter estimates

The parameter estimates that were obtained for healthy volunteers (paper I) were overall consistent with the literature.\(^{83-85}\)

Endogenous glucose production ($G_{\text{PROD}}$) is known to be influenced by both plasma glucose and plasma insulin.\(^{85,86}\) However, it was not possible to separate out the effects of glucose and insulin on $G_{\text{PROD}}$ in the model. Additional data such as the glucose infusion rate of the hyperinsulinemic euglycemic clamp experiment might have been helpful in this context but unfortunately were not available. Incorporating the glucose effect on $G_{\text{PROD}}$ alone resulted in a better model fit than only incorporating the insulin effect on $G_{\text{PROD}}$.

Insulin secretion was modeled in two components: the first- and the second-phase secretion. The magnitude of the first-phase secretion was estimated independently of the glucose dose. This type of approach has been previously used.\(^{38}\) The description of the first-phase secretion as a function of dose was not possible because all individuals received almost the same
dose. Thus, the dose-response relationship was not explored. The insulin half-life was estimated to be 3.5 minutes in both healthy volunteers and patients, which is in line with reported literature values\textsuperscript{86,87}.

**Patient-specific parameters**

The model was able to identify differences in glucose kinetics between healthy volunteers and diabetic patients (paper I). The estimated parameter values of both $\text{CL}_{\text{GI}}$ and $\text{CL}_{\text{G}}$ were decreased in diabetic patients. The decrease in $\text{CL}_{\text{GI}}$ can be attributed to insulin resistance. The decrease in $\text{CL}_{\text{G}}$ as a result of decreased expression of glucose transporter 1 (GLUT-1) receptors in muscle tissue is also well supported in the literature\textsuperscript{88-90}.

Endogenous glucose production was expected to be higher in patients than in healthy volunteers\textsuperscript{91}, but was estimated at a similar value. This might be explained by a lack of information in the patient data, as the patients did not return to baseline during the experiment. The feedback control of glucose production was estimated to be close to zero, indicating a constant glucose production. It is known that the regulation of hepatic glucose production in type 2 diabetic patients is impaired, but generally not altogether lacking\textsuperscript{85}. Again, this inability to estimate the regulation of glucose production in diabetic patients is most likely due to lack of information in the data (i.e. in the designs of the experiments from which they were obtained).

In diabetic patients, the first-phase secretion was estimated at a very small value and was therefore fixed to zero in the final model. The absence of an early insulin response on a glucose stimulus is one of the characteristics of type 2 diabetes\textsuperscript{92,93}. No parameters involved in the estimation of the second-phase secretion were different between healthy volunteers and patients. However, the average baseline concentration of insulin was higher in patients than in healthy volunteers. This was probably attributable to compensatory insulin secretion, a common feature in type 2 diabetic patients\textsuperscript{91}.

**OGTT-specific parameters**

The action of insulinotropic hormones\textsuperscript{44,94}, most importantly GLP-1\textsuperscript{95}, which are secreted on stimulation by nutrients in the gut lumen, is reflected in the model by introduction of a link between the rate of glucose absorption (paper II & IV) or the amount of glucose in the transit compartment (paper III) and insulin secretion. In this way, the fact that the secretion of insulinotropic hormones takes place before glucose becomes apparent in plasma is taken into account. The amount of glucose taken up via the gastrointestinal tissues had a direct impact on insulin secretion, and no effect delay was observed. This is in accordance with current research on GLP-1 secretion\textsuperscript{96}.

The only parameter that needed to be estimated separately for orally and intravenously administered glucose was the insulin-dependent clearance of
glucose. CL_{Gl} was found to be twice as high during the OGTT than during the IVGTT (paper II). The same difference of a factor of 2 was observed in a previous study by Caumo et al^{43} comparing insulin-dependent glucose disposal derived from an IVGTT and a meal test. The proposed explanations included the action of gastrointestinal peptides and/or first-pass effects enhancing insulin sensitivity in the liver or the muscle tissue after oral glucose intake. Another reason might be the impact of the insulin infusion administered only during the IVGTT. This infusion may have led to a more prominent glucagon secretion during the IVGTT, which then counteracted the glucose-lowering effect of insulin.

The model was able to adequately describe the absorption of orally administered glucose by a chain of transit compartments. The bioavailability of glucose was estimated to be approximately 80% (paper II), which is within the range proposed in the literature^{43,97-100}. Even when administered as carbohydrates (paper III), the bioavailability remained in a comparable order of magnitude.

Circadian variation

Even though model performance of the base model describing the 24-hour meal test data without inclusion of circadian effects was already quite satisfactory, the fit was further improved by incorporation of a circadian effect leading to a night-time reduction of insulin secretion. A model-based analysis of insulin secretion performed by Toschi et al^{101} resulted in similar findings as the current study; their model also predicted lower insulin secretion during the night, even after accounting for the lower glucose levels.

The underlying causes of circadian variations in glucose homeostasis cannot be fully captured in their whole complexity, as the model does not consider the actions of other key players in the circadian regulation of the system, such as cortisol and growth hormone. Therefore, the description of the change in insulin secretion by empirical functions such as cosine or suppressor functions remained the only feasible option. The suppressor function performed equally well capturing circadian variations as the best combination of cosine functions in terms of OFV and goodness-of-fit diagnostics. However, it offered advantages concerning parameter precision and model stability. In contrast to the cosine functions, the suppressor function did not affect daytime insulin secretion, which is in closer agreement with prior expectations about physiological behavior.
Drug effects

A kinetic-pharmacodynamic (K-PD) approach was chosen to describe the pharmacodynamic (PD) effect of the study drug in a dose-response-time model. The modeling of PD data in the absence of pharmacokinetic information has been evaluated\textsuperscript{74,102,103} and applied\textsuperscript{104,105} several times in the literature. This approach does not rely on drug concentration measurements. The only required input are the PD data, which inherently contain information on the drug’s biophase kinetics\textsuperscript{103}. Even when pharmacokinetic information was available, such models have been successfully used in the past to streamline the analysis in the case of complex mechanism-based models and to reduce processing times. When the description of the pharmacokinetics (PK) of a drug or the link between the PK and the PD is complex, K-PD models require considerably fewer parameters and were shown to deliver very similar results to the corresponding pharmacokinetic-pharmacodynamic (PK-PD) models\textsuperscript{105}.

The K-PD approach was chosen to facilitate the analysis of drug effects on top of an already complex placebo model. The aim of this work was the proof of concept that the model could be used to estimate drug effects according to their mechanism of action. For this purpose, the PK of the study drug was of secondary importance, and the use of the K-PD approach seemed justified. If this model will be used for the development of a specific drug candidate, the relative un informativeness of the drug PK needs to be shown upfront. This might be possible for a drug with little pharmacokinetic variability. Otherwise, the K-PD model needs to be replaced by a PK-PD model.

The model was able to determine the correct dual mechanism of action of a GK agonist out of several plausible possibilities. It was also able to quantify the effects of the test compound on glucose and insulin concentration-time profiles. Both the magnitude of the drops in OFV (approximately 700 points for the effect on insulin secretion and 160 points for the effect on glucose production) and model simulations with one of the two drug effects set to zero indicated that the stimulating effect of the compound on insulin secretion was dominant. The inhibiting effect on glucose production was also significant but of secondary importance. This is in line with information on the action of glucokinase available in the literature, stating that impaired GK function in the β-cell has clearly more severe effects than in the liver\textsuperscript{106,107}.

Possible applications and limitations of the model

The aim of this work was to develop a tool for the analysis of clinical studies in the field of type 2 diabetes to be used in drug development. The integrated glucose-insulin model proposed in this thesis is able to simultaneously de-
scribe glucose and insulin data obtained by almost any type of experimental design. In particular, the extension to repeated meal tests (paper III) represents an important enlargement of the applicability of the model; the model thus has been shown to correctly handle not only data collected in controlled and rather artificial clinical glucose provocation experiments, but also in real-life situations with patients eating meals of varying nutrient compositions at different times of the day. The model has also been demonstrated to be capable of simulating these profiles over the whole course of a day and beyond.

The ability of the model to analyze the effects of antidiabetic drugs on parameters related to glucose metabolism according to their mechanism of action will potentially increase the understanding of drug influence on the whole physiological system. However, although these results of a mechanism-based drug-disease model predicting treatment outcome are promising, they can merely be regarded as a first proof of concept. The approach requires further validation by application to other compounds with different mechanisms of action. In a second step, the estimation of the effect of drug combinations will be attempted. As combination therapy is very common in the treatment of type 2 diabetes, the possibility to distinguish drug effects at different sites of action and to explore the influence of each of the contributors on the rest of the system might be a particular strength of this approach.

However, it has to be kept in mind that the model was developed under the assumption that glucose and insulin are the main drivers of glucose homeostasis, and that the influence of other components is negligible. This assumption holds true during glucose challenge experiments and particularly in untreated type 2 diabetic patients. However, the model is not expected to correctly deal with prolonged periods of hypoglycemia. Applying the 24-hour meal test model (paper III) to a healthy volunteer population might therefore be problematic. To be able to account for hypoglycemic periods the incorporation of a glucagon sub-model into the integrated glucose-insulin model would be necessary.

Next steps for the further development of this model will focus on disease progression and aim at incorporating the link between glucose homeostasis and HbA$_{1c}$. This will provide the basis for the analysis of long-term drug effects. Bridging between preclinical and clinical investigation by applying the model to animal data could also be a potential field of application. Another interesting aspect worth further investigation is the possibility of bridging between drug effects seen during an OGTT to effects and their variations over a whole day by means of the 24-hour glucose homeostasis model (paper III).

In summary, it is likely that the model proposed in this work will find its main applications in the earlier stages of drug development. In particular, it might prove useful for dose-finding, for gaining more insight into the
mechanism of action in humans and for bridging to potential back-up compounds.
Conclusions

The integrated model presented in this thesis is capable of describing and predicting data following different kinds of glucose provocation experiments in healthy volunteers as well as in type 2 diabetic patients. The ability of the model to handle input from multiple successive meals in a real-life setting over the course of a day and to account for circadian variations in model parameters has also been confirmed. Important differences between healthy volunteers and type 2 diabetic patients have been identified and quantified in accordance with established physiological knowledge.

Most importantly for its future applicability in drug development, the model’s ability to estimate the dual mechanism of action of a glucokinase activator was presented. The model was able to determine the compound’s correct mechanism of action out of several plausible possibilities. Furthermore, it could adequately predict glucose and insulin concentrations over time for placebo and the different dose groups, thus offering the new possibility of a longitudinal kinetic-pharmacodynamic analysis.

The proposed model might be useful for dose-finding and for gaining more insights into a drug’s mechanism of action in early drug development. It could also be used to test potential back-up compounds for desired pharmacologic properties. Clinical trial simulation might then be applied to assess short-term treatment outcome of potential drug candidates. For the simulation of longer studies, disease progression will need to be taken into account to accomplish this goal.

To the best of our knowledge, this is the first model able to simultaneously describe glucose and insulin profiles applicable to data from almost any type of provocation of the glucose-insulin system, including multiple meals over a 24-hour period. It is also the first model able to determine the correct mechanism of action of an antidiabetic compound and to accurately estimate its effects on glucose and insulin levels.
The work presented in this thesis was carried out in the Modeling and Simulation Group at the department of Clinical Pharmacology/Biomathematics at F. Hoffmann-La Roche, Basel, Switzerland, in collaboration with the Department of Pharmaceutical Biosciences, Division of Pharmacokinetics and Drug Therapy, at the University of Uppsala, Sweden. Roche is gratefully acknowledged for being the sponsor of this thesis.

I would like to express my sincere gratitude to all who have contributed to this thesis:

My supervisor, professor Mats Karlsson, for his support of this thesis, particularly for sharing his tremendous knowledge of pharmacometrics and inspiring the project with sparkling ideas.

My co-supervisor at Roche, Dr. Nicolas Frey, for his dedicated and continuous support over the last four years. You always encouraged me to have ambitious plans and to strive for the highest quality. Your tenacity was simply unique!

Dr. Karin Jorga, for making this thesis possible and accepting me as a PhD student in the Modeling and Simulation Group at Roche.

Dr. Ulrika Simonsson, for being my co-supervisor at the University of Uppsala during the first 2 years of my PhD studies.

Hanna Silber, for the good collaboration on the first two publications describing this model, and for the friendship which resulted out of this. Many thanks also for your help with the proof-reading of the thesis and for being my local Swedish contact for all arrangements for the dissertation that would have been difficult to make from Switzerland.

Dr. Ronald Gieschke, for vivid scientific discussions, for sharing your expert knowledge of NONMEM and for your perseverance in solving tricky programming problems.
My colleagues in Roche Modeling and Simulation for creating the collegial and friendly atmosphere in our group. Thanks to all of you that you are always willing to give advice despite your sometimes very high workload.

The members of the Division of Pharmacokinetics and Drug Therapy at Uppsala University, for always making me feel welcome during my visits at the University. Special thanks to Dr. Andrew Hooker, Dr. Lars Lindbom and Pontus Pihlgren for your help with methodological and IT issues. Both PsN and Xpose tremendously facilitated my work.

And finally my husband, Matthias, for your constant encouragement and care! Many thanks for taking over so many of my duties to free up more time for working on my thesis. Thank you also for proof-reading the thesis and for the helpful comments concerning comprehensibility of the text from a non-pharmacometrician point of view.
References


Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 73

Editor: The Dean of the Faculty of Pharmacy

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”.)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-8719