Safety and Efficacy Modelling in Anti-Diabetic Drug Development

BENGT HAMRÉN
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Abstract


A central aim in drug development is to ensure that the new drug is efficacious and safe in the intended patient population.

Mathematical models describing the pharmacokinetic-pharmacodynamic (PK-PD) properties of a drug are valuable to increase the knowledge about drug effects and disease and can be used to inform decisions. The aim of this thesis was to develop mechanism-based PK-PD-disease models for important safety and efficacy biomarkers used in anti-diabetic drug development.

Population PK, PK-PD and disease models were developed, based on data from clinical studies in subjects with varying degrees of renal function, non-diabetic subjects with insulin resistance and patients with type 2 diabetes mellitus (T2DM), receiving a peroxisome proliferator-activated receptor (PPAR) α/γ agonist, tesaglitazar.

The PK model showed that a decreased renal elimination of the metabolite in renally impaired subjects leads to increased levels of metabolite undergoing interconversion and subsequent accumulation of tesaglitazar. Tesaglitazar negatively affects the glomerular filtration rate (GFR), and since renal function affects tesaglitazar exposure, a PK-PD model was developed to simultaneously describe this interrelationship. The model and data showed that all patients had decreases in GFR, which were reversible when discontinuing treatment.

The PK-PD model described the interplay between fasting plasma glucose (FPG), glycosylated haemoglobin (HbA1c) and haemoglobin in T2DM patients. It provided a mechanistically plausible description of the release and aging of red blood cells (RBC), and the glucose dependent glycosylation of RBC to HbA1c. The PK-PD model for FPG and fasting insulin, incorporating components for β-cell mass, insulin sensitivity and impact of disease and drug treatment, realistically described the complex glucose homeostasis in the heterogeneous patient population.

The mechanism-based PK, PK-PD and disease models increase the understanding about T2DM and important biomarkers, and can be used to improve decision making in the development of future anti-diabetic drugs.

Keywords: pharmacokinetic, pharmacodynamic, mechanism-based, modelling, type 2 diabetes mellitus, tesaglitazar, PPAR, drug development, NONMEM

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Till min familj
Papers discussed

This thesis is based on the following papers, which will be referred to in the text by the Roman numerals assigned below.


II Hamrén B, Öhman KP, Svensson MK and Karlsson MO. Pharmacokinetic pharmacodynamic assessment of the interrelationships between tesaglitazar exposure and renal function in patients with type 2 diabetes mellitus. In manuscript

III Hamrén B, Björk E, Sunzel M and Karlsson MO. Models for plasma glucose, HbA1c and hemoglobin interrelationships in patients with type 2 diabetes following tesaglitazar treatment. Accepted for publication in Clinical Pharmacology and Therapeutics. Reprinted with permission from Nature Publishing Group

IV Ribbing J, Hamrén B, Svensson MK and Karlsson MO. A model for glucose, insulin, beta-cell and HbA1c dynamics in subjects with insulin resistance and patients with type 2 diabetes. Submitted
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### Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>Alpha</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the concentration time curve</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Beta</td>
</tr>
<tr>
<td>C</td>
<td>Concentration</td>
</tr>
<tr>
<td>CL</td>
<td>Clearance</td>
</tr>
<tr>
<td>CrCL</td>
<td>Creatinine clearance</td>
</tr>
<tr>
<td>Css</td>
<td>Average concentration at steady state</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DGR</td>
<td>Disease group</td>
</tr>
<tr>
<td>EC$_{50}$</td>
<td>Concentration achieving half of the maximum effect</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>Maximum effect</td>
</tr>
<tr>
<td>F</td>
<td>Bioavailability</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acid</td>
</tr>
<tr>
<td>FI</td>
<td>Fasting insulin</td>
</tr>
<tr>
<td>FPG</td>
<td>Fasting plasma glucose</td>
</tr>
<tr>
<td>FOCE</td>
<td>First order conditional estimation method</td>
</tr>
<tr>
<td>FOCE INTER</td>
<td>First order conditional estimation method with interaction</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Gamma</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycosylated haemoglobin</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>IS</td>
<td>Insulin sensitivity</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography-Mass spectrometry</td>
</tr>
<tr>
<td>MBDD</td>
<td>Model-based drug development</td>
</tr>
<tr>
<td>MDRD</td>
<td>Calculated GFR (Modified Diet in Renal Disease$^1$)</td>
</tr>
<tr>
<td>NPC</td>
<td>Numerical predictive check</td>
</tr>
<tr>
<td>OFV</td>
<td>Objective function value</td>
</tr>
<tr>
<td>$\Delta$OFV</td>
<td>Difference in objective function value</td>
</tr>
<tr>
<td>PD</td>
<td>Pharmacodynamic</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetic</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type two diabetes mellitus</td>
</tr>
<tr>
<td>VPC</td>
<td>Visual predictive check</td>
</tr>
<tr>
<td>V</td>
<td>Volume of distribution</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>Difference between observation and individual prediction</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Difference between population and individual parameter</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Typical value of a parameter, fixed effects parameter</td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>Variance of the $\epsilon$'s</td>
</tr>
<tr>
<td>$\omega^2$</td>
<td>Variance of the $\eta$'s</td>
</tr>
</tbody>
</table>
1 Introduction

A central aim in drug development is to ensure that the new drug is efficacious and safe in the patient population it is intended to be used in. Pharmacokinetic-pharmacodynamic (PK-PD) models describe the relationships between dose, drug exposure (in plasma), pharmacological response and clinical endpoint. These relationships are important to understand when selecting optimal doses and dosing regimen in the target patient population\(^2\), \(^3\).

Today, the pharmaceutical industry is struggling with low productivity and high costs, and less than 10% of new compounds entering clinical development makes it to the market\(^4\). In 2004, the Food and Drug Administration (FDA) in USA published the Critical Path initiative where they presented their view on how the pharmaceutical industry could improve and make drug development more productive\(^5\). FDA highlighted the need for more efficient use of the vast amount of data collected and emphasised the use of model-based drug development (MBDD). This approach, including pharmaco-statistical models for efficacy and safety integrating relevant (clinical) data, will increase the understanding of the drug and disease and consequently improve decision making\(^6\)–\(^8\). Model-based analysis is likely most beneficial during the early clinical development phases (phase I-II), as the data and models can be used to address important questions like: has the drug the desired efficacy-safety profile, or what is the likelihood that the phase III study will succeed to meet the objectives? FDA has implemented a interaction called the End of Phase Ila meeting, to motivate MBDD and to support more effective us of early clinical data with the purpose to help pharmaceutical industry in design of phase IIb and III studies\(^9\), \(^10\).

This thesis presents mechanistic PK-PD models for safety and efficacy, that were derived during the clinical development of tesaglitazar, a peroxisome proliferator-activated receptor (PPAR) \(\alpha/\gamma\) agonist, which was intended to be used for treatment of type 2 diabetes mellitus (T2DM).
1.1 PK-PD modelling

The reasons to model data can be summarised as: to describe, to understand, to predict and to support. The model can be used to describe data, which can be exemplified when using a PK model to characterise the pharmacokinetic properties of a drug in a specific study. This is often a demand from the regulatory authorities\textsuperscript{11, 12}. The model can be used to increase the understanding of a drug and disease. This is the area where most research is done and different types of models will be discussed below. The model can be used to predict and to support, which relates to when models are used to inform decisions, i.e. dose selection and optimisation of study design\textsuperscript{13-15}.

A PK model describes the relationship over time between dose and drug concentration. A PD model describes the pharmacological response to a drug. The combination of the two, a PK-PD model, can be used to characterise the relationship between dose, drug exposure and response. There are also models that describe disease and disease progression, which can be extended to include impact of drug treatment. Population PK-PD models include estimation of variability at two or more levels, i.e. within and between individuals, and can be used to quantify and explain sources of variability in a population.

PK-PD models can be divided into those describing direct relationships between drug exposure and effect, and models for delayed relationships. The latter models are applied when the pharmacological response is delayed in relation to changes in plasma drug concentrations. The majority of drug responses may be considered indirect by nature. There are many types of models for indirect PK-PD relationships of which some will be described below.

The effect-compartment model accounts for the lag-time in response by assuming a delayed distribution of drug into a hypothetical effect compartment where the drug acts\textsuperscript{16}. More commonly used PK-PD models for delayed effects are the so called indirect response models\textsuperscript{17}. They can be applied when the drug affects either the production or removal of response. The models assume that the response is produced and lost by zero- and first-order rate processes, respectively. These models have been used to describe drugs affecting endogenous substances\textsuperscript{18}. A similar type of model is the transit models, which consist of a number of, in series coupled, compartments. Examples of where transit models have been used are to describe signal transduction processes\textsuperscript{19} and effects of anti-cancer treatment on haematological cell maturation\textsuperscript{20}. Transit models allow the lifespan of a cell to vary between cells within an individual. A fourth type of indirect models are the lifespan models, and they have been applied for drugs that alter the generation of natural cells\textsuperscript{21}. These models differ from the indirect response models in the assumption that the elimination is not a first-order process. Here
the loss is assumed to be a consequence of aging or conversion to another cell type. All cells live for the same period of time (lifespan) before disappearing.

The science of PK-PD modelling is gradually evolving from empirical to mechanistic models. Mechanistic models are favourable since they aim to characterise physiology in a meaningful manner, as well as impact of disease and/or drug on the system. Thus, mechanistic models should separate biological system-specific parameters from drug-specific parameters. Mechanistic models allow merging of heterogeneous information from various sources into a single quantitative framework which enables in depth insight into physiology, disease and drug effects. They could also be of high value to support decision making in drug development by more accurate model extrapolation\textsuperscript{22}.

1.2 Type 2 Diabetes Mellitus

Diabetes mellitus is estimated to be the fifth leading cause of death globally and the risk of cardiovascular disease is higher in diabetic subjects compared to non-diabetics\textsuperscript{23, 24}. Diabetes is a growing problem, in 2003 it was estimated that approximately 194 million people had diabetes, in 2007 the total was 246 million. Of these, the majority (90\%) had type 2 diabetes mellitus (T2DM). By 2025, the number is expected to rise to 380 million\textsuperscript{25}.

T2DM is a progressive metabolic disorder characterised by continuous worsening of glycemic control. Figure 1 schematically describes the gradual process from normal glucose haemostasis to overt T2DM.

\textbf{Figure 1.} Gradual development from normal glucose haemostasis to overt type 2 diabetes mellitus (Adapted from Norberg\textsuperscript{26})
Major components of T2DM are reduced peripheral insulin sensitivity and decline in pancreatic β-cell function. This process is coupled with disturbances in fat and protein metabolism and is often accompanied by obesity, dyslipidemia and hypertension.

Blood glucose is normally maintained by a fine-tuned balance between carbohydrate ingestion, production by the liver, and uptake and utilisation as storage or energy in muscles, brain and other tissues. When blood glucose increases, β-cells in pancreas release insulin which reduces glucose levels through inhibition of endogenous hepatic glucose production, and stimulation of peripheral glucose uptake and storage. Insulin is also important in lipid homeostasis as it stimulates lipid storage and inhibits lipolysis.

Prior to developing T2DM, most pre-diabetic patients experience loss of insulin sensitivity, but have sufficient β-cell function to maintain glucose control. This state is often called the metabolic syndrome or the insulin resistance syndrome (IRS)\textsuperscript{27, 28}. Obesity is associated with increased levels of free fatty acid (FFA) and triglycerides. Increased FFAs are thought to be crucial for the development of peripheral insulin resistance\textsuperscript{29}.

The pancreatic β-cells overcompensate with increased insulin secretion to maintain normoglycaemia in insulin resistant subjects, but will eventually fail with hyperglycaemia as the result. The reason for the deteriorating β-cell function is poorly understood, but it is likely that several factors, including lipotoxicity, glucotoxicity, insulin resistance within the β-cells, inflammation and oxidative stress (Figure 2) are involved\textsuperscript{30}. In addition, elevated FFA levels can impair insulin elimination in the liver which contributes to increased levels of insulin in obese and insulin resistant patients\textsuperscript{31}.

![Figure 2](image.png)

**Figure 2.** The vicious circles linking insulin resistance and β-cell dysfunction with hyperglycaemia, hyperlipidaemia, glucotoxicity and lipotoxicity. (Adapted from Bonora\textsuperscript{30})
1.2.1 Biomarkers in Type 2 Diabetes Mellitus

There are a number of different biomarkers and methods to assess glycemic status. Fasting plasma glucose (FPG) is frequently used as a day-to-day biomarker of short-term glycemic control, but as values can vary considerably between days it is not reliable for assessing long-term status. Instead glycosylated haemoglobin (HbA1c), which is formed through a non-enzymatic and irreversible reaction between haemoglobin (Hb) and glucose, is the primary surrogate marker for long-term glycemic control and provides information on the average glucose levels during the past months. Fasting insulin (FI) and C-peptide levels are used to evaluate endogenous insulin production, assess insulin resistance and β-cell function, as well as determining when a patient needs to start using insulin medication.

1.2.2 Treatment of Type 2 Diabetes Mellitus

The results of the United Kingdom Prospective Diabetes Study (UKPDS), found that an intensive strategy using sulphonamides in newly diagnosed patients with T2DM was associated with stricter glycemic control compared to conventional care, as well as a 25% reduction in the risk for microvascular complications. In the same study, overweight and obese patients randomised to metformin experienced significant reduction in myocardial infarction and diabetes-related deaths. The results from the UKPDS study changed the view on how T2DM should be treated as it provided firm evidence of the benefits of intensified glycemic control on micro- (and macro-) vascular disease.

However, in the UKPDS study, none of the drugs showed ability to beneficially affect the natural disease progression of hyperglycaemia, most likely since these classes of agents do not seem to affect the progressive loss of β-cell function.

Drugs that simultaneously normalize the disturbed glucose and lipid metabolism, and in addition have protective effects on β-cell function would most likely have beneficial effects on the large and increasing population of patients with IRS and T2DM. PPAR γ and α/γ agonists, and incretin-mimetic agents such as dipeptidyl peptidase-IV inhibitor and glucagon-like peptide-I agonists may play a significant future role in this respect.

1.2.2.1 Sulphonamides

Sulphonamides (e.g. glibenclamide, glipizide) have been extensively used for almost 50 years. These agents lower blood glucose primarily by directly stimulating insulin secretion from the β-cells of the pancreatic islets. Sulphonamides do not appear to affect the continuous loss of β-cell function over time.
1.2.2 Biguanides

Metformin is another widely used anti-diabetic drug and is often called an insulin-sensitiser since it acts mainly by increasing insulin action in muscle and liver. Its primary effect is thought to be by decreasing hepatic glucose production, but its precise mechanism of action is still not completely understood. As with sulphonamides, metformin did not show beneficial effect on the rate of loss of β-cell function.

1.2.2.3 PPAR agonists

1.2.2.3.1 Thiazolidinediones (PPAR γ agonists)

Thiazolidinediones are selective ligands of the nuclear PPAR, subtype gamma (γ) receptor. This class of agents (rosiglitazone and pioglitazone) improves whole body insulin sensitivity via multiple actions on gene expression. PPAR γ agonists ameliorate glucose homeostasis by increasing glucose uptake in skeletal muscles, decreasing hepatic glucose production, and in addition have beneficial effects on lipid metabolism. The thiazolidinediones have demonstrated clinical improvements in indices of β-cell dysfunction and have potential to improve β-cell function. This could be a significant improvement compared to sulphonamides and metformin if it is proven that this class can slow down the rate of β-cell disease progression. Weight gain, oedema and decreased haemoglobin are known side effects with PPAR γ agonists, and their beneficial effects on long-term cardiovascular events are questioned.

1.2.2.3.2 Tesaglitazar (PPAR α/γ agonist)

Since this thesis focuses on the PK and PK-PD properties of tesaglitazar, a detailed description of the compound is presented here. Tesaglitazar is a dual PPAR α/γ agonist which combines the anti-hyperglycaemic and insulin-sensitising properties of PPAR γ agonism with triglyceride-lowering and HDL-raising effects of PPAR α agonism.

In an absorption, disposition, metabolism and excretion (ADME) study in healthy male subjects who were given either oral or intravenous single doses, tesaglitazar was found to be completely and rapidly absorbed. Tesaglitazar is a low clearance compound with a small volume of distribution resulting in an elimination half-life of approximately 45 h. In the same study it was reported that tesaglitazar was mainly metabolised by UGT1A3 and UGT2B7, and most radioactivity (91%) was found in the urine, predominantly as the acyl glucuronide of tesaglitazar. The molecular structure of tesaglitazar and its metabolite is found in Figure 3.
About 20% of the dose was recovered unchanged in the urine and plasma protein binding of tesaglitazar was high, approximately 99.9%. Furthermore, it was reported that tubular secretion was likely to contribute to renal clearance of tesaglitazar. The pharmacokinetic properties of tesaglitazar is similar in T2DM patients\textsuperscript{44}. Patients had slightly longer half-life (60-70 h), and apparent clearance (CL/F) was found to be dependent on renal function resulting in 2-3 higher average exposure (AUC) in patients with impaired renal function compared to those with normal renal function (Figure 4).

Two double-blind, placebo controlled, randomised dose-finding studies in non-diabetic subjects with insulin resistance or in patients with T2DM treated with tesaglitazar for 3 months showed dose and time dependent changes in FPG, TG and HDL\textsuperscript{45,46}. Two doses (0.5 and 1 mg) of tesaglitazar were selected to be tested in the phase III programme which consisted of 8 different studies of 6 or 12 months of duration in T2DM patients. Development of tesaglitazar was discontinued in 2006 when phase III studies indicated that the overall benefit-risk profile was unlikely to give patients an advantage over currently available anti-diabetic therapies\textsuperscript{47-50}. The main safety concerns were negative effects on renal function and on haematological variables (mainly haemoglobin).
Figure 4. Relationship between tesaglitazar plasma exposure (AUCτ) vs. GFR (assessed by iohexol clearance) in subjects with various renal function.

1.2.2.4 Other oral anti-diabetic agents

1.2.2.4.1 α-Glucosidase inhibitors
Inhibitors of intestinal α-glucosidase enzymes decrease the rate of carbohydrate digestion, thereby reducing post-prandial hyperglycaemia\textsuperscript{51}.

1.2.2.4.2 Dipeptidyl peptidase 4 (DPP-4) inhibitors
DPP-4 inhibitors (i.e. sitagliptin) are a new class of anti-diabetic drugs that potentially can restore β-cell function. They increase the levels of incretins which leads to increased secretion of insulin from beta-cells, decreased secretion of glucagon from pancreatic α-cells, and at the same time reduced glucose production in the liver\textsuperscript{52}.

1.2.2.5 Insulin
Due to the progressive degeneration of β-cell function in patients with T2DM, a majority of individuals with T2DM will eventually require exogenous insulin therapy to maintain glycemic control\textsuperscript{53}. Inhaled, rapid acting, insulin was approved in 2006 for use in patients not adequately controlled by oral anti-diabetic agents and may prove easier use compared to injected insulin\textsuperscript{54}. 
1.3 PK-PD modelling in Type 2 Diabetes Mellitus

There are several mathematical models describing the immediate interplay between glucose and insulin. The best known model for glucose regulation is the so called “minimal model”, which uses data from glucose provocation studies to assess insulin sensitivity and glucose effectiveness\textsuperscript{55}. Integrated, mechanism-based, models for glucose and insulin regulation have recently been presented\textsuperscript{56, 57}. These are preferable since they describe physiology in a more meaningful and interpretable manner.

There are other types of PK-PD models that describe relationships between the basal state (fasting) of different biomarkers, and the effect of drug treatment on these. Frey et al used an empirical effect compartment model for the delayed effect in FPG after treatment with gliclazide in T2DM patients\textsuperscript{58}. A more mechanistic approach has been presented by de Winter et al\textsuperscript{59}. They developed a population PD model with focus on disease progression, describing the interplay between fasting insulin, FPG and HbA1c, based on two large, one year long studies with pioglitazone, compared to either metformin or gliclazide in T2DM patients. Their model included components for β-cell function and insulin sensitivity, distinguishing immediate treatment effects from drug effects on long term disease progression. Another mechanistic model which integrates β-cell mass (BCM), insulin and glucose dynamics in the healthy state has been proposed by Topp et al\textsuperscript{60}. This model is derived from a mechanistic reasoning and model parameters are collected from various literature sources, thus the model has not been fitted to observed data. The model does not incorporate effects of disease or anti-diabetic treatment on the homeostasis relationships. This model was implemented and further developed in Paper IV.
2 Aim

The overall aim of this thesis was to develop mechanism-based pharmacokinetic (PK), pharmacokinetic-pharmacodynamic (PK-PD) and disease models that can support decision making in anti-diabetic clinical drug development by qualitatively and quantitatively describing relationships between important safety and efficacy biomarkers. The models were derived throughout the development of the peroxisome proliferator-activated receptor (PPAR) \( \alpha/\gamma \) agonist tesaglitazar and the specific aims were to:

- Describe the pharmacokinetic properties of tesaglitazar in subjects with various degrees of renal function and in particular gain insight into the likely mechanism of the increased tesaglitazar exposure in renally impaired subjects
- Evaluate and quantitate the interrelationships between tesaglitazar pharmacokinetics and renal function over time in patients with type 2 diabetic mellitus (T2DM) using different markers for assessing renal function
- Develop a PK-PD model for the interplay between tesaglitazar exposure, fasting plasma glucose (FPG) and glycosylated haemoglobin (HbA1c) in patients with T2DM with specific focus to describe, in a mechanistic reasonable manner, the release and aging of red blood cells (RBC) and the glycosylation of RBC to HbA1c
- Investigate plausible mechanisms for the tesaglitazar induced effect on haemoglobin (Hb)
- Implement and further develop a mechanistic model describing the dynamics of FPG, fasting insulin, insulin sensitivity and \( \beta \)-cell mass in a heterogeneous population, ranging from non-diabetic subjects with insulin resistance to patients with advanced T2DM
3 Methods

3.1 Clinical studies

Data from five clinical studies with tesaglitazar were included in this thesis\textsuperscript{45-47}. All studies were conducted according to the principles of the Declaration of Helsinki and in accordance with the Guidance of Good Clinical Practice. The studies were approved by independent ethics committees, and signed informed consent was received from all subjects/patients.

3.1.1 Study to investigate the impact of renal function on tesaglitazar PK (Paper I)

This was an open study (RENAL) with 23 subjects with varying degrees of renal impairment (mild, moderate and severe) and 18 subjects with normal renal function, matched on age and gender. All subjects received daily doses of tesaglitazar 1 mg for six weeks. Subject characteristics are found in Table 1.

Table 1. Subjects characteristics

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>GFR\textsuperscript{a} (mL/min/1.73 m\textsuperscript{2})</th>
<th>SCr\textsuperscript{b} (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with renal insufficiency (n=23)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>17/6</td>
<td>55</td>
<td>84</td>
<td>32</td>
</tr>
</tbody>
</table>

Healthy controls (n=18)

| Median  | 10/8        | 53          | 75     | 90     | 71                                       |
| Range   | 41–73       | 60–97       | 75–120 | 52–91  |                                          |

\textsuperscript{a} Assessed by plasma iohexol clearance

\textsuperscript{b} Serum creatinine
3.1.2 Study to investigate the impact of tesaglitazar on renal function (Paper II)

This was an open-labelled, randomised study (ARMOR) in T2DM patients treated with either tesaglitazar 2 mg (n=98) or pioglitazone 45 mg (n=38) for six months. Patients randomised to pioglitazone were not included in the PK-PD assessment. Table 2 shows baseline characteristics for the tesaglitazar patients.

Table 2. Tesaglitazar patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>SCr&lt;sup&gt;a&lt;/sup&gt; (μmol/L)</th>
<th>CL&lt;sub&gt;iorthalamate&lt;/sub&gt; (mL/min/1.73m&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>MDRD&lt;sup&gt;b&lt;/sup&gt; (mL/min/1.73m&lt;sup&gt;2&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>56</td>
<td>94</td>
<td>67</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Range</td>
<td>45-78</td>
<td>56-179</td>
<td>35-106</td>
<td>54-174</td>
<td>54-166</td>
</tr>
</tbody>
</table>

Gender: 50 males and 44 females; Race: 77 Caucasians, 15 Blacks and 2 Asians
<sup>a</sup> Serum creatinine
<sup>b</sup> Calculated GFR by the MDRD formula

3.1.3 Patient studies with tesaglitazar (Paper III and IV)

Data from three large clinical phase II and III studies with tesaglitazar were included in Paper III and IV. The GLAD and SIR studies were randomised, double-blind, placebo controlled, three months, dose ranging studies in patients with T2DM (GLAD), or in subjects with insulin resistance (SIR). The GALLANT6 study was a randomised, double blind, six months, phase III study in patients with T2DM treated with either tesaglitazar or pioglitazone. Patient characteristics are presented in Table 3.

Table 3. Tesaglitazar patient characteristics (median and ranges)

<table>
<thead>
<tr>
<th>Study population</th>
<th>GLAD</th>
<th>SIR</th>
<th>GALLANT6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Phase</td>
<td>II</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>Study population</td>
<td>T2DM</td>
<td>IRS</td>
<td>T2DM</td>
</tr>
<tr>
<td>Tesaglitazar doses (mg)</td>
<td>0.1, 0.5, 1, 2, 3 and placebo</td>
<td>0.1, 0.25, 0.5, and placebo</td>
<td>0.5, 1</td>
</tr>
<tr>
<td>Treatment duration</td>
<td>12 weeks</td>
<td>12 weeks</td>
<td>24 weeks</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>412</td>
<td>377</td>
<td>671</td>
</tr>
<tr>
<td>Naïve to treatment</td>
<td>130</td>
<td>377</td>
<td>81</td>
</tr>
<tr>
<td>Gender (♂/♀)</td>
<td>242/170</td>
<td>289/88</td>
<td>353/318</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58 (32-80)</td>
<td>50 (29-77)</td>
<td>58 (22-85)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>88 (46-140)</td>
<td>94 (56-140)</td>
<td>87 (43-186)</td>
</tr>
<tr>
<td>Renal function (mL/min)</td>
<td>68 (29-163)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91 (29-185)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91 (35-186)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated by Cockroft-Gault using lean body weight as measure of body weight. <sup>b</sup> Calculated by the MDRD formula
In paper IV, patients were grouped according to their disease state (disease group: DGR), ranging from non-diabetic subjects with insulin resistance (DGR1), drug-naïve T2DM patients (DGR2-3, GLAD and GALLANT6 study, respectively) and T2DM patients previously treated with anti-diabetic agents (DGR 4-5, GLAD and GALLANT6 study, respectively).

### 3.2 Measurements and variables (biomarkers)

Pharmacokinetic and pharmacodynamic data were collected in all five studies, and the number of PK and PD samples per study is presented in Table 4. Actual dosing and plasma sampling times were used in each analysis, protocol times were used for the urine data in Paper I.

<table>
<thead>
<tr>
<th></th>
<th>RENAL</th>
<th>ARMOR</th>
<th>GLAD</th>
<th>SIR</th>
<th>GALLANT6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>Paper I</td>
<td>II</td>
<td>III-IV</td>
<td>IV</td>
<td>IV</td>
</tr>
<tr>
<td>Subjects</td>
<td>41</td>
<td>94</td>
<td>412</td>
<td>377</td>
<td>671</td>
</tr>
<tr>
<td>PK</td>
<td>707+323+326&lt;sup&gt;a&lt;/sup&gt;</td>
<td>547</td>
<td>1283</td>
<td>1187</td>
<td>1899</td>
</tr>
<tr>
<td>FPG</td>
<td>4035</td>
<td>3529</td>
<td>6342</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c</td>
<td>1548</td>
<td>6291</td>
<td>5817</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb</td>
<td>3115</td>
<td>2430</td>
<td>1238</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL</td>
<td>2612</td>
<td>5615</td>
<td>1238</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL&lt;sub&gt;iothalamate&lt;/sub&gt;</td>
<td>253</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDRD</td>
<td>704</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Corresponds to number of tesaglitazar and metabolite samples in plasma, and number of samples in urine (tesaglitazar and metabolite)

### 3.2.1 Blood sampling schedules

#### 3.2.1.1 Pharmacokinetics

In the RENAL study (Paper I), plasma samples for determination of tesaglitazar and its metabolite were drawn at day 1 and 42±3 after start of treatment: at pre-dose, 1, 2, 4, 12 and 24 hours post-dose. Trough samples were collected at day 14 and 28. Four additional samples were obtained at day 3±1, 6±1, 9±1 and 21±2 after last dose intake. Urine samples for determination of tesaglitazar and its metabolite were collected in the intervals: 0-6, 6-12, 12-24 hours at day 1, and during 0-24 hours at day 42±3.

In the ARMOR study (Paper II), tesaglitazar plasma samples were collected throughout the study period. Trough samples were collected at week 4, 8, 12, 16, 20 and 24, in all patients randomised to tesaglitazar. An additional PK sample was collected at the follow-up visit 4 weeks after last dose
intake. In a subset of patients, three additional samples (0.5-1, 1-2 and 3-4 hours post dose intake) were collected at week 4 and 20.

In the GLAD and SIR studies (Paper III-IV), sparse plasma samples were collected throughout the treatment period, at week 2, 8 and 12 weeks and 3 weeks after the last dose. In GALLANT6, sparse plasma samples were collected at week 4, 12 and 24, and at the follow-up visit 3 weeks after last dose intake. Plasma concentration samples below lower limit of quantification (LLOQ) were omitted from the PK analysis.

3.2.1.2 Pharmacodynamics
In the ARMOR study (Paper II), GFR was measured, using iothalamate clearance (CL_{iothalamate}), at baseline, week 12 and 24. Patients with reduced CL_{iothalamate} or increased S-creatinine, according to pre-specified criteria, had additional renal assessments at 4, 8 and 12 weeks after last dose intake. GFR calculated by the MDRD formula (Modified Diet in Renal Disease^1) was used as an additional measure of renal function and was obtained at baseline, at monthly visits and at 4 and 8 weeks after ending treatment.

In the GLAD, SIR and GALLANT6 studies, FPG, FI, Hb and HbA1c (not measured in SIR) were measured during run-in, bi-weekly or monthly during treatment and at the follow-up visit 3 weeks after last dose intake.

3.2.2 Bioanalysis
Concentrations of tesaglitazar and its acyl glucuronide in plasma were determined using a reversed-phase liquid chromatography and mass spectrometry^63. A similar method was used for determination of these analytes in urine. LLOQ were 0.003 and 0.01 μmol/L for tesaglitazar and its metabolite, respectively. The plasma protein binding of tesaglitazar was determined by ultrafiltration (Paper I). The centrifuge (Hettich Zentrifugen ROTA-FIXA/KS) used, was tempered to 37°C and run for 1 h at 2600 rpm. The plasma samples were filtered at 1200 g (2600 rpm) using Centrifree-filter at 37°C for 20 min and the samples were analysed as described above.

Central laboratories were used for analysis of all pharmacodynamic samples (FPG, FI, Hb and HbA1c), according to standard laboratory procedures.
3.3 Data analysis

3.3.1 Non-linear mixed effects modelling

Population PK and PK-PD modelling with NONMEM\textsuperscript{64} was used throughout this thesis to assess the pharmacokinetics and exposure-response properties of tesaglitazar. This approach applies non-linear mixed effects models to repeated measures from a group of individuals\textsuperscript{65} and enables estimation of fixed effect parameters, which represent the typical (population, $\theta$) values of the parameters and random effect parameters at two or more levels. Data from all individuals are used, and the fixed and random parameters are simultaneously estimated, making it possible to include sparse and heterogeneous data in the assessment\textsuperscript{66}.

For example, a parameter for an individual in a model (e.g. Baseline$_i$) can be described by:

$$\text{Baseline}_i = \theta_{\text{Baseline}} + \eta_i$$

where $\eta_i$ represents the difference between the $i^{th}$ individual’s value of baseline (Baseline$_i$) and the population (typical) value of baseline ($\theta_{\text{Baseline}}$). The $\eta_i$ values are assumed to normally distributed in the population, with a mean of zero, and a variance of $\omega^2$. Hence, $\omega^2$ is the estimated interindividual variability (IIV) of $\theta_{\text{Baseline}}$. In biology, parameters are often assumed to be lognormally distributed and therefore IIV on a parameter is often implemented as:

$$\text{Baseline}_i = \theta_{\text{Baseline}} \times e^{\eta_i}$$

One important aspect of population PK-PD modelling is understanding and quantifying sources of IIV, i.e. evaluating if and how covariates can explain differences in PK and PK-PD responses between individuals. A covariate is typically a patient descriptor such as age, gender, renal function, concomitant medication etc. Inclusion of a covariate can be exemplified by:

$$\text{Baseline}_i = \theta_{\text{Baseline}} \times [1 + \theta_{\text{age}} \times (\text{AGE-50})] \times e^{\eta_i}$$

where $\theta_{\text{Baseline}}$ represents the typical baseline for 50 year old individuals, and $\theta_{\text{age}}$ is the relative change in baseline by changing age.
Due to other sources of variability than differences between individuals (e.g. assay error, errors in dosing and sampling times, and model misspecification) the observation \((\text{Obs}_{i,j})\) will differ from the individual prediction \((\text{Pred}_{i,j})\) of that observation. This discrepancy, often denoted as residual error, can be described by:

\[
\text{Obs}_{i,j} = \text{Pred}_{i,j} + \epsilon_{i,j}
\]

where \(\epsilon_{i,j}\) is assumed to be normally distributed with a mean of zero and an estimated variance of \(\sigma^2\). In this example, the residual error is additive, but other residual models can also be used such as a proportional or a combination of both. In general, proportional residual error models are used.

In NONMEM\(^{64}\), parameters are estimated through a parametric maximum likelihood approach, whereby a joint function (the objective function) of all model parameters and the data (the observation) is evaluated. The maximum likelihood parameter estimates are the parameter estimates producing the greatest probability that the given data occur. NONMEM estimates parameters by minimising the extended least square objective function, which is approximately proportional to minus two times the logarithm of the likelihood (-2\log likelihood) of the data\(^{64}\).

### 3.3.2 Model assessment

#### 3.3.2.1 Model comparison

Graphical diagnostics, using the Xpose program (version 3.1)\(^{67}\) and comparison of competing models using the objective function values (OFV) in the likelihood ratio test, guided model development. The likelihood ratio test is used to assess statistical significance between nested models. For two nested models, the difference in OFV (minus twice the log likelihood) is approximately chi-squared \((\chi^2)\) distributed. When the FOCE-INTER method is used, a difference in OFV>3.84 (one degree of freedom) is significant at the 5% level, corresponding value for \(p<0.01\) is 6.63 ref.\(^{68}\). For non-nested competing models, the model producing the lowest OFV is favourable to the model with the higher OFV (provided the same number of parameters).

Covariates were evaluated by forward and backward inclusion/deletion in a stepwise fashion, where covariates were forward included in the model at the \(p<0.05\) level, and backwards deleted at \(p<0.01\), using the likelihood ratio test.

#### 3.3.2.2 Predictive performance

Visual and numerical predictive checks (VPC/NPC)\(^{69}\) were performed to assess the final population PK and PK-PD models (Paper I, III and IV). From each model, 500 or 1000 data sets were simulated using final model parameter estimates and original data sets. The data were evaluated by
graphical or numeric comparisons between the model predicted median and 95%-prediction interval, and the observed median and all individual observations over time.

3.3.3 Software

All population PK and PK-PD analyses were performed using the software NONMEM (version V or VI, level 1)\(^64\) with the first-order conditional estimation (FOCE) method, with or without interaction (INTER). NONMEM was run on Linux (Redhat version 9) and compiled with Gnu Compiler Collection (version 3.2.2). The post-processor Xpose 3.1\(^67\) was used for model diagnostic purposes and for exploration of possible covariate relationships. Xpose runs in a Splus environment (Insightful Corporation, version 2000 for Windows). Splus was also used for data manipulation, exploratory analysis and graphics.

3.3.4 Pharmacokinetic models

In paper II-IV one compartment models with first order absorption and elimination were used to describe the pharmacokinetic properties of tesaglitazar. Impact of covariates were investigated on apparent clearance (CL/F) and apparent volume of distribution (V/F). Due to the sparse sampling schemes, no covariate effects were evaluated on the absorption rate constant (k\(_a\)). The results from the PK analyses were utilized in the different PK-PD analyses, where individual predictions of average tesaglitazar plasma concentrations (C\(_{\text{average}}\)) were used as a measure of tesaglitazar exposure.

3.3.4.1 Interconversion of tesaglitazar (Paper I)

In paper I, a mechanistic population PK model was developed for tesaglitazar and its metabolite (an acyl glucuronide) following repeated oral administration of tesaglitazar in subjects with varying renal function. The aim was to derive a PK model that could explain the finding of approximately 2-3 times higher plasma exposure of tesaglitazar in subjects with impaired renal function compared to healthy controls, given the observation that tesaglitazar is predominantly metabolised and only 20% of tesaglitazar is found unchanged in the urine.

Two hypotheses for the cause of the increased tesaglitazar exposure in subjects with impaired renal function were tested. Hypothesis A: the metabolism of tesaglitazar was assumed to be correlated with the renal function. This was tested by incorporating a correlation between \(\text{CL}_{\text{iohexol}}\) and the metabolic clearance of tesaglitazar. Hypothesis B: the increased tesaglitazar exposure was due to interconversion between the acyl glucuronide and tesaglitazar. An additional compartment was incorporated to mimic biliary excretion of the acyl glucuronide into the gut, and thereafter interconversion
and reabsorption of tesaglitazar into the central compartment of the parent drug. These processes were modelled as rate constants, $k_{bm}$ and $k_{int}$, respectively.

The two hypotheses were tested one at a time and were compared by differences in OFV and goodness of fit. Figure 5 gives an overview of the model structure and the model parameters, including the two hypotheses.

![Figure 5. Structural model overview including the two hypotheses](image)

3.3.5 PK-PD models

### 3.3.5.1 Model for the PK and renal function interrelationship (Paper II)

In the ARMOR study, tesaglitazar was found to reduce GFR, and since renal function affects the exposure of tesaglitazar (as described in Paper I) it was necessary to describe both these processes simultaneously (Paper II). Figure 6 illustrates the interrelationships between tesaglitazar and renal function.
A one-compartment PK model with first order absorption was used to describe the PK of tesaglitazar. Renal function was included a priori as a covariate influencing the apparent clearance (CL/F).

An indirect response model was developed to account for the effect delay between tesaglitazar plasma concentrations and renal function\(^1\). Both CL\(_{\text{iotha-lamate}}\) and MDRD were used as measures of GFR with separate residual errors for the two different biomarkers. The tesaglitazar effect was introduced on the zero order input function of GFR (k\(_{\text{in}}\)). The following parameters were estimated; GFR baseline (GFR\(_{\text{baseline}}\): parameterised as k\(_{\text{in}}\)/k\(_{\text{out}}\)), first order rate constant (k\(_{\text{out}}\)) which determines the delay in GFR change to a change in tesaglitazar exposure (Cp), maximal reduction in GFR (E\(_{\text{max}}\)) and the tesaglitazar plasma concentration achieving half maximal reduction in GFR (EC\(_{50}\)) according to the following equation.

\[
\frac{d\text{GFR}}{dt} = k_{\text{in}} \cdot \left[ 1 - \frac{E_{\text{max}} \cdot \text{Cp}}{(EC_{50} + \text{Cp})} \right] - k_{\text{out}} \cdot \text{GFR}
\]

Covariates evaluated were; gender, age, body weight, race and concomitant medications (angiotensin converting enzyme (ACE) inhibitors, angiotensin-II receptor antagonists, NSAID’s and thiazide diuretics). Covariates were assessed for effect on CL/F, V/F, GFR\(_{\text{baseline}}\) and EC\(_{50}\). Impact of concomitant medications were only evaluated for possible PD interaction on EC\(_{50}\).
3.3.5.2 Mechanistic FPG-Hb-HbA1c model (Paper III-IV)

A population PK-PD model characterising the relationships between tesaglitazar exposure, FPG, HbA1c and Hb was developed using data from the GLAD study (Paper III). Emphasis was to describe the release and aging of red blood cells (RBC) including the FPG dependent glycosylation process of RBCs to HbA1c. The model was developed sequentially, firstly the PK of tesaglitazar, secondly the relationship between tesaglitazar exposure and FPG, thirdly the mechanistic model for the interrelationship between FPG and HbA1c and lastly, four different hypotheses for the mechanism of the tesaglitazar effect on Hb were tested. After the separate models were established, a simultaneous analysis of the final FPG-Hb-HbA1c models was performed.

The individual Bayesian PK parameters from the population PK analysis were used to predict each patients average tesaglitazar plasma concentration during the treatment period. An indirect response model was developed for the effect of tesaglitazar exposure on FPG over time. A transit compartment model was used to characterise the relationship between FPG and HbA1c over time (Figure 7).

Figure 7. Schematic representation of the mechanism-based model for the FPG-HbA1c relationship

The four, in series coupled, transit compartments describe RBC aging, starting with a zero order release of RBC into circulation (K_{in RBC}). The first order
rate constant ($K_{tr}$) defines the RBC transition from one age stage to the next until the cell dies. At any given age stage, the RBC can become glycosylated to HbA1c as a function of FPG ($K_{glucose} \cdot FPG^\lambda$). $K_{in\ RBC}$, RBC lifespan, $K_{glucose}$ and $\lambda$ were estimated in the model, including between patient variability on $K_{in\ RBC}$, RBC lifespan and $\lambda$.

Tesaglitazar affects Hb in a dose- and time dependent manner. The model was further extended with the purpose to test and evaluate the most likely, out of four possible, mechanisms for the tesaglitazar induced Hb effect, and also assess the potential impact on HbA1c given the different mechanisms. The tested hypotheses were:

- inhibition of RBC production
- shortening of RBC lifespan
- non-selective RBC elimination
- haemodilution or redistribution of RBC

The different models were tested one at a time in the final FPG-Hb-HbA1c model and were compared by differences in OFV and plausibility of parameter estimates.

The potential impact of covariates were evaluated and covariates were: age, gender, body weight, renal function (assessed by CrCL) and prior anti-diabetic treatment (drug naïve or previously treated).

The FPG-Hb-HbA1c model was re-evaluated using the GALLANT6 study, data which had not been used to develop the model (Paper IV). The sub-models were assessed sequentially with emphasis on how well the model could predict the new study data of longer duration and in a new patient population. Since the inclusion criteria differed between GLAD and GALLANT6, the simulations accounted for these differences. In GALLANT6, drug-naïve patients were enrolled if their HbA1c were within 7-10%, and pre-treated patients if HbA1c<10%. In the GLAD study and in drug-naïve patients, FPG had to be >7 mmol/L during the run-in period (three visits). For pre-treated patient, the third visit during the run-in period had to be >7 mmol/L. Further, both drug-naïve and pre-treated patients were excluded if FPG was ≥13.3 mmol/L at any of the three run-in visits. All model parameters were assumed to be as estimated from the GLAD study, except FPG baseline and each model component (FPG and Hb-HbA1c, respectively) was evaluated externally by visual predictive checks (VPC).

Lastly, the FPG-Hb-HbA1c model was re-estimated by combining the GLAD and GALLANT6 data.
3.3.5.3 Mechanistic FPG-FI model (Paper IV)

The mechanistic model, proposed by Topp et al.\cite{60}, that describes the basal relationships between FPG, fasting insulin (FI) and β-cell mass (BCM) in normal subjects was further developed to include impact of disease stage on insulin sensitivity (IS) and BCM, as well as influence of tesaglitazar treatment (Paper IV). The data used in the assessment originated from three clinical studies with tesaglitazar (SIR, GLAD and GALLANT6), which included non-diabetic, insulin resistance subjects and patients with T2DM. In all, five disease stages were assessed (defined in Section 3.1.3).

The net changes in FPG, FI and BCM in healthy subjects were described by three differential equations\cite{60} and a description of the model parameters is included in Table 5.

\[
\frac{dFPG}{dt} = R_0 - \left( E_{G0} + IS \cdot FI \right) \cdot FPG
\]

\[
\frac{dFI}{dt} = \frac{BCM \cdot \sigma \cdot FPG^2}{\left( \alpha^2 + FPG^2 \right)} - k \cdot FI
\]

\[
\frac{dBCM}{dt} = \left( -d_0 + R_1 \cdot FPG - R_2 \cdot FPG^2 \right) \cdot BCM
\]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_0$</td>
<td>rate of FPG production at zero glucose</td>
</tr>
<tr>
<td>$E_{G0}$</td>
<td>total glucose effectiveness at zero insulin</td>
</tr>
<tr>
<td>$IS$</td>
<td>insulin sensitivity</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>maximum insulin secretion rate per mass unit of β-cell</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>FPG level producing half the maximum insulin secretion</td>
</tr>
<tr>
<td>$k$</td>
<td>first-order elimination rate of FI</td>
</tr>
<tr>
<td>$d_0$</td>
<td>BCM death rate at zero glucose</td>
</tr>
<tr>
<td>$R_1$ and $R_2$</td>
<td>rate constants describing glucose dependent β-cell adaptation</td>
</tr>
</tbody>
</table>

Note that changes in BCM is not dependent on FI, and that reduced insulin sensitivity alone does not cause hyperglycaemia since β-cell adaptation acts as a negative feedback bringing FPG back towards normal (set-point).
The impact of disease stage was implemented as an offset in \( \beta \)-cell adaptation causing a higher FPG set-point in T2DM patients. Thus the equation for change in BCM was expanded to:

\[
\frac{dBCM}{dt} = \left( -d_0 + R_1 \cdot FPG' - R_2 \cdot FPG'' \right) \cdot BCM
\]

where \( FPG' \) was defined as \( FPG' = FPG - OFFSET \). In T2DM patients FPG is higher than \( FPG' \) since the \( OFFSET \) is causing a hyperglycaemic FPG set-point. \( \beta \)-cell adaptation at different FPG levels is illustrated for a healthy subject and T2DM patient in Figure 8.

\[\text{Figure 8. Beta-cell adaptation rate versus FPG. The black solid curve represents the change in a healthy individual and the grey in an T2DM patient. The dotted vertical lines mark the physiological fixed points in each of the two individuals. This is a point of attraction and beta-cell adaptation acts with a negative feedback to bring the FPG back to this set-point. At FPG much higher than the physiological fixed point severe glucose toxicity causes a positive feedback (below the horizontal line).}\]
Insulin sensitivity (IS) was originally set to a fixed value\textsuperscript{60}. A non-linear and positive relationship between insulin elimination and IS was added\textsuperscript{31, 70, 71} according to:

\[ k = k_H \cdot \left( \frac{IS}{IS_H} \right)^{REL_{k-S}} \]

where \(k\) and \(IS\) are the insulin elimination rate and insulin sensitivity, respectively. \(REL_{k-S}\) is the power slope for change in \(k\) by change in \(IS\). \(IS_H\) and \(k_H\) are the values in healthy subjects\textsuperscript{60}.

Tesaglitazar treatment effects were incorporated on β-cell adaptation (OFFSET) and on insulin sensitivity (IS). The effect on OFFSET was direct and implemented with a Emax model as:

\[ FPG' = FPG - OFFSET \cdot \left( 1 - \frac{C_{average}^\gamma}{\left( C_{average}^\gamma + EC_{50B}^\gamma \right)} \right) \]

where \(C_{average}\) is the individual predicted average tesaglitazar concentration in plasma, \(EC_{50B}\) is the concentration achieving reduction in OFFSET by 50% and \(\gamma\) is the sigmoidicity factor.

The tesaglitazar effect on IS was implemented as an indirect effect as:

\[ \frac{dIS_t}{dt} = k_{in} \cdot \left( 1 + \frac{E_{max,S} \cdot C_{average}}{EC_{50S} + C_{average}} \right) - k_{out} \cdot IS_t \]

which makes insulin sensitivity (IS) a variable that change with treatment over time and which replaces IS in the equation for change in FPG as presented above. \(k_{in}\) is a zero order rate constant for the production of IS, and \(k_{out}\) is a first order rate constant determining the time course of the indirect response in IS. Insulin sensitivity at baseline is defined as \(IS_0 = k_{in}/k_{out}\). \(E_{max,S}\) and \(EC_{50,S}\) are maximal improvement in insulin sensitivity and average tesaglitazar concentration achieving half maximal improvement in IS.

The effect of discontinuing prior anti-diabetic treatment at enrolment (i.e. a wash-out effect) was included on OFFSET and IS. The full model including the interrelationships between tesaglitazar exposure, FPG, FI, BCM and IS is illustrated in Figure 9.

FPG and FI were assumed to be at steady state with respect to each other, given the current BCM and IS. Further, at the first visit it was assumed that BCM and insulin sensitivity were at steady state in all patients.

The model was simultaneously fit to the FPG and FI data. The model parameter values were fixed according to Topp et al\textsuperscript{60}. except for: insulin sensitivity at baseline (IS), OFFSET, \(k_{out}\), \(Rel_{k-S}\), \(wash-out_B\) and \(wash-out_S\), \(E_{max,S}\).
Between patient variability was included on IS, OFFSET, wash-out$_B$, wash-out$_S$, EC$_{50}$, and on the residual error terms for FPG and FI, and were assumed to be log-normally distributed, except for wash-out$_B$.

Figure 9. Schematic illustration of the mechanistic FPG-FI model, incorporating insulin sensitivity (IS) and β-cell mass (BCM). Changes from the steady state are illustrated as indirect effects for all four variables, indicated by solid or broken arrows. However, responses in FPG and FI are relatively fast and therefore assumed at steady state relative to one another, at any given level of IS and BCM. Drug treatment exerts an indirect effect on IS and BCM which explains the delay in the response of FI and FPG. The indirect effects of drug treatment and the effect of IS on the elimination of FI are additions to the model from that originally suggested$^{60}$. This has been indicated by red line colour.
4 Results

4.1 Pharmacokinetic results

4.1.1 Interconversion of tesaglitazar (Paper I)

The analysis showed that the most likely cause of the increased tesaglitazar plasma exposure in subjects with impaired renal function is that the decreased renal elimination of the acyl glucuronide causes an increase in plasma exposure of the metabolite which leads to increased levels of metabolite undergoing interconversion and subsequently an accumulation of tesaglitazar (hypothesis B). This model was statistical favourable ($\Delta$OFV = 217, p<0.0001) and produced good fit to the data (Figure 10) compared to the model where the tesaglitazar metabolism was assumed to be dependent on renal function (hypothesis A).

![Graphs showing time course of tesaglitazar and its metabolite concentrations](Figure 10)

Figure 10. Visual predictive check of tesaglitazar (A) and its metabolite (B) in plasma in subjects with impaired renal function (left) and controls (right). The bold solid and dotted lines represent observed and model predicted median. The grey areas within the dotted lines are the model 95% prediction interval. The circles are the observations.
Metabolism was the major elimination route for tesaglitazar, with only a minor part (~1%) being renally cleared. For the acyl glucuronide, three elimination pathways were incorporated into the model; renal, non-renal and biliary excretion. The metabolite that was biliary excreted was assumed to be hydrolysed back to tesaglitazar and subsequently re-absorbed into the circulation. The lower renal elimination of the acyl glucuronide will lead to increased numbers of interconversion cycles in subjects with impaired renal function compared to subjects with normal renal function. Concomitant probenecid use (n=4) was found to inhibit the renal elimination of both tesaglitazar and its metabolite. These results were later confirmed in a traditional drug-drug interaction study (unpublished data), indicating the strong power of population PK modelling to identify covariate relationships. The separation of the elimination and interconversion processes was possible due to the simultaneous assessment of tesaglitazar and the acyl glucuronide in plasma and urine in subjects with varying renal functions, since the ratio between tesaglitazar and the metabolite vary with varying renal function.

4.1.2 Pharmacokinetics of tesaglitazar (Paper II-IV)

In the larger clinical studies (ARMOR, SIR, GLAD and GALLANT6) only tesaglitazar in plasma was determined. Therefore, less complex PK models were used to describe the pharmacokinetic properties of tesaglitazar. The interrelationship between tesaglitazar exposure and renal function is presented in Section 4.2.1. One-compartment PK models with first order absorption and elimination were used with consistent results across the studies (Table 6). At steady state, apparent clearance (CL/F) was approximately 0.13 L/h and was found to be influenced primarily by renal function. CL/F decreased by approximately 0.75-0.9% per decreasing unit in renal function (at renal function values below the median values in each study population). The relationship between renal function and individual predictions of tesaglitazar plasma exposure (AUC) is found in Figure 11. Overall between patient variability in CL/F was approximately 35-40% and decreased to about 25-30% after accounting for differences in renal function. In the two dose-ranging studies (SIR and GLAD) small dose, and time-dependent effects on CL/F were found. These were modelled using a power function (dose) and a step function (time). No effect of dose or time were identified in the ARMOR and GALLANT6 studies. Tesaglitazar had a small apparent volume of distribution (11-13 L) and a relatively long half-life (50-70 h). The sampling scheme in the studies were not optimised to estimate the absorption rate constant (Ka), which therefore had to be fixed in ARMOR and GALLANT6 (2.3 and 2.4 h⁻¹, respectively). Ka was estimated in the SIR+GLAD analysis to 2.33 h⁻¹.
Table 6. Parameter estimates and associated relative standard errors (RSE) from the population PK model assessments of tesaglitazar

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>ARMORa Paper II (n=94)</th>
<th>GLAD+Sir Paper III (n=582)</th>
<th>GALLANT6 Paper IV (n=672)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural model parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F (L/h) (θ₁)</td>
<td>0.130 (4.4)</td>
<td>0.147 (2.4)</td>
<td>0.136 (2.7)</td>
</tr>
<tr>
<td>Impact of covariates on CL/F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal function (θ₂, θ₃)</td>
<td>0.75 (14)b</td>
<td>0.90 (6.7)c</td>
<td>0.91 (5.4)d</td>
</tr>
<tr>
<td>Gender (%)/unit change</td>
<td>-13 (35)</td>
<td>n.s.</td>
<td>-17 (13)</td>
</tr>
<tr>
<td>Time (% difference in ♀)</td>
<td>n.s.</td>
<td>-10 (13)e</td>
<td>n.s.</td>
</tr>
<tr>
<td>Dose (θ₅) (%)</td>
<td>n.e.</td>
<td>-7.5 (16)e</td>
<td></td>
</tr>
<tr>
<td>V/F (L)</td>
<td>13 (5.3)</td>
<td>11 (2.5)</td>
<td>12 (5.0)</td>
</tr>
<tr>
<td>Impact of race on V/F (% difference in Blacks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V/F (L)</td>
<td>n.e.</td>
<td>n.e.</td>
<td>-35 (39)</td>
</tr>
<tr>
<td>Ka (h⁻¹)</td>
<td>2.4 (fixed)</td>
<td>2.33 (18)</td>
<td>2.3 (fixed)</td>
</tr>
<tr>
<td>Inter-patient variability (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F</td>
<td>25 (23)</td>
<td>28 (8.5)</td>
<td>30 (8.5)</td>
</tr>
<tr>
<td>V/F</td>
<td>33 (33)</td>
<td>35 (22)</td>
<td>44 (47)</td>
</tr>
<tr>
<td>Ka</td>
<td>n.s.</td>
<td>150 (53)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Residual variability (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F</td>
<td>26 (8.0)</td>
<td>23 (4.4)</td>
<td>23 (4.2)</td>
</tr>
</tbody>
</table>

Data are presented as parameter estimates (relative standard error). CL/F, apparent clearance; V/F, apparent volume of distribution; F, bioavailability; Ka, absorption rate constant; n.s., not significant; n.e., not estimated.

a Parameter estimates from the simultaneous PK-PD assessment between tesaglitazar and renal function.
b CL/F = θ₁ (1+θ₂γ(GFR-100)), where GFR is either Clᵢohexol or calculated GFR by the MDRD formula.
c CL/F = θ₁ (1+θ₂γ(CrCL-76)) for CrCL≤76 mL/min and CL/F= θ₁ (1+θ₃γ(CrCL-76)) when CrCL>76 mL/min at week 2 receiving 1 mg of tesaglitazar.
d CL/F = θ₁ (1+θ₂γ(MDRD-90)) for MDRD≤90 mL/min, CL/F= θ₁ (1+θ₃γ(CrCL-90)) when MDRD>90 mL/min.
e at week 8: CL/F = θ₁ (1+θ₄γ), at week 12: CL/F = θ₁ (1+θ₄γ) (1+θ₃γ) .
f CL/F= θ₁ Dosef⁹⁶

g proportional error model
4.2 Pharmacodynamic results

4.2.1 Interrelationship between tesaglitazar exposure and renal function (Paper II)

Since renal function affects tesaglitazar exposure, and tesaglitazar affects renal function, a simultaneous analysis of this interrelationship was performed. The one-compartment tesaglitazar PK model is presented in Section 4.1.2. The integrated indirect response model adequately described both the magnitude and time course of the reduction in renal function (GFR). The mean GFR at baseline was estimated to be 100 mL/min/1.73m², and decreased to a pseudo steady state after approximately 12 weeks of treatment (76 mL/min/1.73m²) with only minor decrease in GFR thereafter. All patients had deterioring renal function and no demographic covariate could explain differences in response. However, patients concomitantly treated with an ACE-inhibitor were significantly more sensitive to tesaglitazar exposure compared to those not treated with ACE inhibitors (EC₅₀ 0.75 vs. 1.75 μmol/L). When discontinuing treatment, tesaglitazar concentrations fell with a mean half-life of about 67 h, and the majority of the plasma samples were below LLOQ (0.003 μmol/L) at the first follow-up visit (four weeks after last dose intake). GFR returned slowly towards baseline, and the data and model suggest a return to baseline 8-10 weeks after last dose.

Figure 11. The relationship between individual estimates of tesaglitazar exposure, normalised to 1 mg (AUC₁mg), and renal function (calculated GFR by the MDRD formula), n=1554.
Due to the interrelationship between tesaglitazar exposure and renal function, steady state will occur at a later time point and also at a different level compared to if PK and GFR were independent. In this situation, where CL/F depends on both GFR and EC$_{50}$, the $C_{average}$ at steady state can be calculated as a function of dose (2 mg), dosing interval (24h), non-renal clearance, renal clearance, EC$_{50}$ and GFR$_{baseline}$. At high baseline GFR and in patients not treated with an ACE inhibitor, $C_{average}$ at steady state is expected to be approximately 15-20% higher compared to if there was no interrelationship.
between PK and GFR. The increase in $C_{\text{average}}$ is higher (25-30%) in patients treated with ACE inhibitors due to their lower EC$_{50}$. In patients with low renal function, the increase in $C_{\text{average}}$ is less due the larger proportion of total clearance being non-renal.

The inclusion of calculated GFR (by the MDRD formula) improved the ability to characterise the time course of the change in GFR, since the primary variable for GFR (CL$_{\text{iothalamate}}$) was only collected at baseline, 3 and at 6 months of treatment.

**Table 7.** Indirect response model parameter estimates and associated relative standard errors (RSE) from the simultaneous PK-PD model assessment of tesaglitazar and its interrelationship with renal function.

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Estimate (RSE, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural model parameters</td>
<td></td>
</tr>
<tr>
<td>$GFR_{\text{baseline}}$ (mL/min/1.73m$^2$)</td>
<td>100 (2.0)</td>
</tr>
<tr>
<td>$K_{\text{out}}$ (day$^{-1}$)</td>
<td>0.055 (13)</td>
</tr>
<tr>
<td>$E_{\text{max}}$ (%)</td>
<td>40 (6.4)</td>
</tr>
<tr>
<td>EC$_{50}$ (μmol/L)</td>
<td>1.7 (17)</td>
</tr>
<tr>
<td>Impact of ACE inhibitor use on EC$_{50}$ (%)</td>
<td>-56 (18)</td>
</tr>
<tr>
<td>Inter patient variability (%)</td>
<td></td>
</tr>
<tr>
<td>$GFR_{\text{baseline}}$</td>
<td>16 (22)</td>
</tr>
<tr>
<td>EC$_{50}$</td>
<td>69 (45)</td>
</tr>
<tr>
<td>Residual error magnitude in GFR (%)$^b$</td>
<td></td>
</tr>
<tr>
<td>CL$_{\text{iothalamate}}$</td>
<td>20 (6.7)</td>
</tr>
<tr>
<td>MDRD</td>
<td>11 (5.6)</td>
</tr>
</tbody>
</table>

$K_{\text{out}}$, first order rate constant for change in GFR; $E_{\text{max}}$, maximal reduction in GFR; EC$_{50}$, tesaglitazar concentration achieving half maximal reduction in GFR; CL$_{\text{iothalamate}}$, measure of renal function by plasma iothalamate clearance; MDRD, calculated GFR by the MDRD formula.

$^a$ linear model
$^b$ proportional error model

4.2.2 FPG-Hb-HbA1c model (Paper III-IV)

The FPG-Hb-HbA1c model was developed on data from the GLAD study (Paper III), and later re-assessed on the GALLANT6 study (Paper IV). A summary of PD baseline characteristics including biomarkers used in Paper IV is presented in **Table 8**.
Table 8. Baseline characteristics at enrolment for the PD biomarkers stratified by dose and disease group, median (range).

<table>
<thead>
<tr>
<th>Study</th>
<th>FPG (mmol/L)</th>
<th>HbA1c (%)</th>
<th>Hb (g/L)</th>
<th>FI (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIR</td>
<td>(4.0-9.8)</td>
<td>n.m.</td>
<td>(116-177)</td>
<td>(19-812)</td>
</tr>
<tr>
<td>GLAD&lt;sub&gt;naive&lt;/sub&gt; &lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.6</td>
<td>6.8</td>
<td>146</td>
<td>62</td>
</tr>
<tr>
<td>GLAD&lt;sub&gt;prior&lt;/sub&gt; &lt;sup&gt;b&lt;/sup&gt;</td>
<td>(6.5-13.3)</td>
<td>(5.5-13.1)</td>
<td>(112-176)</td>
<td>(10-347)</td>
</tr>
<tr>
<td>GALLANT6&lt;sub&gt;naive&lt;/sub&gt; &lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.2</td>
<td>7.6</td>
<td>146</td>
<td>78</td>
</tr>
<tr>
<td>GALLANT6&lt;sub&gt;prior&lt;/sub&gt; &lt;sup&gt;b&lt;/sup&gt;</td>
<td>(6.6-13.3)</td>
<td>(7-9.8)</td>
<td>(129-177)</td>
<td>(20-250)</td>
</tr>
<tr>
<td></td>
<td>(2.2-15.4)</td>
<td>(4.1-10)</td>
<td>(91-197)</td>
<td>(8-483)</td>
</tr>
</tbody>
</table>

n.m. not measured
<sup>a</sup> patients naïve to anti-diabetic treatment
<sup>b</sup> patients previously treated with anti-diabetic drugs

4.2.2.1 Model development based on the GLAD study (Paper III)

An indirect response model, with a stimulatory drug effect (E<sub>max</sub> model) on K<sub>out</sub> FPG, best described the FPG data. Patients discontinuing prior anti-diabetic treatment at enrolment had an increase in FPG over time, and patients on placebo had on average 16% higher FPG at end of treatment. The onset of the treatment effect was slow, and a new FPG steady state was reached after approximately 12 weeks of treatment. Females were more sensitive to tesaglitazar exposure and had 50% lower EC<sub>50</sub> FPG compared to males.

The mechanism-based PK-PD model with a linked chain of four, in series coupled, transit compartments could well describe the FPG-HbA1c interaction. Females had a slightly lower (10%) release rate of RBC (K<sub>in</sub> RBC) compared to males and the mean RBC lifespan was estimated to 135 days. The RBC lifespan was determined by the change in glycosylation rate following changes in FPG (and HbA1c) over time. The rate at which FPG glycosylates Hb to HbA1c (K<sub>glucose</sub>) was estimated to 0.00018 day<sup>-1</sup>/FPG<sup>λ</sup>. This corresponds to a glycosylation rate of approximately 0.1% per day at a FPG of 10 mmol/L. The non-linear relationship between FPG and HbA1c was described by a power function with a slope (λ) of 0.74. This relation is likely to be non-linear due to fasting glucose not being directly proportional to 24 hr-average glucose<sup>72, 73</sup>. The proportion of RBC being glycosylated increased with increasing age of the cell<sup>74</sup>.
The haemodilution model produced the lowest OFV and also reasonable estimate for the RBC lifespan. This mechanism is partly supported by external data\textsuperscript{75, 76}, however the exact mechanism is still not yet understood. The indirect tesaglitazar effect was incorporated as a change in the volume of distribution of RBC. Since the model for haemodilution affects all circulating RBC equally, the decrease in Hb would not translate to any additional effect on HbA1c. If the decrease in Hb would have been caused by tesaglitazar-dependent decrease in Hb production, a transient increase in HbA1c followed by a return to its original baseline would be expected, e.g. no long-term effect on HbA1c. If the Hb effect was caused by shortening of RBC lifespan or non-selective Hb elimination, a long-term decrease in HbA1c would be expected, more pronounced for the former mechanism. Final parameter estimates for the simultaneous FPG-Hb-Hb1c model is found in Table 9, and Figure 13 presents the results of the VPC’s for FPG, HbA1c and Hb receiving 2 mg of tesaglitazar.

\textbf{Figure 13.} Visual predictive checks for FPG, HbA1c and Hb receiving 2 mg of tesaglitazar. Upper panels (FPG and HbA1c) include pre-treated patients, and the two lower panels show drug naïve patients. The VCP for Hb was not stratified for prior treatment. The thin solid lines are the 95% prediction intervals and the bold line is the predicted median response. The circles are the observed data, and the dotted line is a non-parametric smooth of the observed data. Day zero is the day of randomisation.
Table 9. Parameter estimates (and relative standard errors) of the FPG-Hb-HbA1c model based on the GLAD, and combined GLAD+GALLANT6 study data

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Estimate GLAD</th>
<th>Estimate GLAD+GALLANT6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FPG model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPG\textsubscript{baseline} at enrolment (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior anti-diabetic treatment</td>
<td>8.2 (1.0)</td>
<td>7.4 (0.8)</td>
</tr>
<tr>
<td>Drug-naïve</td>
<td>8.7 (1.2)</td>
<td>8.9 (1.1)</td>
</tr>
<tr>
<td>Impact of prior anti-diabetic treatment on Kin\textsubscript{FPG} (%), FPG\textsubscript{wash-out}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.4 (8.4)</td>
<td>16.9 (5.8)</td>
</tr>
<tr>
<td>E\textsubscript{max} FPG (%)</td>
<td>70 (7.5)</td>
<td>85 (9.8)</td>
</tr>
<tr>
<td>EC\textsubscript{50} FPG males (μmol/L)</td>
<td>1.5 (18)</td>
<td>1.94 (20)</td>
</tr>
<tr>
<td>EC\textsubscript{50} FPG females (μmol/L)</td>
<td>0.88 (25)</td>
<td>1.26 (14)</td>
</tr>
<tr>
<td>K\textsubscript{out} FPG (day\textsuperscript{-1})</td>
<td>0.037 (6.5)</td>
<td>0.032 (4.7)</td>
</tr>
<tr>
<td>IIV\textsuperscript{a} FPG\textsubscript{baseline}</td>
<td>14 (8.6)</td>
<td>18 (5.5)</td>
</tr>
<tr>
<td>IIV FPG\textsubscript{wash-out}</td>
<td>80 (15)</td>
<td>86 (11)</td>
</tr>
<tr>
<td>IIV EC\textsubscript{50} FPG</td>
<td>110 (17)</td>
<td>88 (31)</td>
</tr>
<tr>
<td>IIV Residual error</td>
<td>36 (12)</td>
<td>38 (8.2)</td>
</tr>
<tr>
<td>Correlation (FPG\textsubscript{baseline} - EC\textsubscript{50} FPG)</td>
<td>n.s.</td>
<td>-0.45 (16)</td>
</tr>
<tr>
<td>Residual variability (%)</td>
<td>9.6 (2.4)</td>
<td>9.1 (1.8)</td>
</tr>
<tr>
<td><strong>FPG-HbA1c model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC lifespan (day)</td>
<td>135 (7.0)</td>
<td>91.2 (2.8)</td>
</tr>
<tr>
<td>K\textsubscript{in} RBC males (g/L/day)</td>
<td>1.11 (7.0)</td>
<td>1.66 (2.8)</td>
</tr>
<tr>
<td>K\textsubscript{in} RBC females (g/L/day)</td>
<td>1.02 (7.0)</td>
<td>1.51 (2.8)</td>
</tr>
<tr>
<td>K\textsubscript{glucose} (1/day/FPG\textsuperscript{4} mmol/L)</td>
<td>0.00018 (13)</td>
<td>0.00041 (4.2)</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>0.743 (5.2)</td>
<td>0.552 (2.2)</td>
</tr>
<tr>
<td>IIV K\textsubscript{in} RBC</td>
<td>7.1 (9.4)</td>
<td>7.2 (5.6)</td>
</tr>
<tr>
<td>IIV (\lambda)</td>
<td>5.9 (15)</td>
<td>8.6 (7.0)</td>
</tr>
<tr>
<td>IIV Residual error</td>
<td>17 (32)</td>
<td>41 (7.5)</td>
</tr>
<tr>
<td>Residual error HbA1c (%)</td>
<td>3.0 (1.9)</td>
<td>4.1 (1.7)</td>
</tr>
<tr>
<td><strong>Haemodilution model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K\textsubscript{out} dilution (day\textsuperscript{-1})</td>
<td>0.030 (6.5)</td>
<td>0.026 (5.2)</td>
</tr>
<tr>
<td>E\textsubscript{max} dilution (%)</td>
<td>68 (31)</td>
<td>48 (24)</td>
</tr>
<tr>
<td>EC\textsubscript{50} dilution (μmol/L)</td>
<td>8.2 (40)</td>
<td>5.4 (30)</td>
</tr>
<tr>
<td>IIV EC\textsubscript{50} dilution</td>
<td>56 (21)</td>
<td>54 (28)</td>
</tr>
<tr>
<td>IIV Residual error</td>
<td>26 (24)</td>
<td>22 (25)</td>
</tr>
<tr>
<td>Residual error Hb (%)</td>
<td>5.0 (3.6)</td>
<td>3.1 (1.2)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Inter individual variability, expressed as coefficient of variation (%)

---

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4.2.2.2 Re-assessment of the FPG-Hb-HbA1c model using the GALLANT6 study (Paper IV)

The FPG-Hb-HbA1c model was evaluated externally on the GALLANT6 study, data on which the model has not been developed. The assessment was performed as described in Section 3.3.3.2. The inclusion criteria for GALLANT6 were taken into account when making the model predictions. The VPC showed that the FPG and Hb sub-models could predict the GALLANT6 data well. Patients previously treated with two anti-diabetic agents had higher FPG values than predicted, not unexpected since this sub-population was not included in the data used for the model development. The predictions of HbA1c in the drug-naïve patients were accurate. HbA1c in the pre-treated patients was under-predicted at all times, but both the time course and magnitude of change in HbA1c were reasonable.

The FPG-Hb-HbA1c model components were re-estimated in a sequential manner using the GLAD and GALLANT6 study data (Table 9, right column). Small changes were observed in the FPG model parameter estimates, especially notably were differences in baseline characteristics and identified negative correlations between FPGbaseline and EC50 FPG, as well as between EC50 FPG and FPGwash-out (-0.45 and -0.44 respectively). Parameters in the mechanistic FPG-Hb-HbA1c model also changed somewhat: K in RBC increased (1.5 g/L/day), RBC lifespan decreased (91 days) and the glycosylation rate increased (0.00041 day⁻¹/FPG⁻¹) and was less dependent on FPG (λ, 0.55). A comparison between the typical model prediction of the steady state relationship between FPG and HbA1c, including external data of the relationship between FPG and HbA1c at steady state is illustrated in Figure 14.

![Figure 14](image-url)

**Figure 14.** Qualification of the estimated relationship between FPG and HbA1c (bold, red lines), including external data (thin lines) of glucose and HbA1c correlations. The histograms give the FPG distributions at baseline. The left panel shows the results from the GLAD analysis, and the right from GALLANT6 (bold blue dashed line is the updated FPG-HbA1c relationship from the re-estimated model).
Small changes in the parameters for the haemodilution model were observed, $k_{\text{out dilution}}$ was somewhat lower indicating a slower change in Hb than estimated from the GLAD study.

4.2.3 FPG-FI model (Paper IV)

The mechanistic model for FPG and FI, including components for β-cell mass and insulin sensitivity could adequately describe the FPG and FI data over time in the heterogeneous patient population (Figure 15). The impact of disease stage on BCM was modelled through the OFFSET parameter and patients with T2DM typically had physiological set points changed to 8-9 mmol/L (compared to 5.6 mmol/L in healthy subjects) whereas non-diabetic insulin resistant subjects had no deterioration in BCM. Insulin resistance was present in all disease stages and IS was 40-60% of that in healthy subjects. T2DM patients discontinuing prior anti-diabetic treatment at enrolment showed deterioration in FPG and FI (wash-out effects), and these effects were implemented on BCM and IS.

The treatment response in FI was fast due to the rapid improvement in IS, and new steady state in FI was reached within weeks. The change in FPG was initially fast, thereafter slower, and steady state was reached within months of treatment. This is likely a result of fast initial improvement in IS and a slower upward adaptation in BCM. In non-diabetic, insulin resistant subjects BCM changed in the opposite direction due the large improvement in IS that initially reduced FPG more than the treatment reduced the OFFSET. The inclusion of a positive relationship between insulin elimination and IS improved the model fit of the FI data. A negative correlation was found between OFFSET and IS, indicating that patients with lower insulin sensitivity tend to also have lower BCM.

In the investigated dose range the exposure-response relation was nearly linear for IS (EC$_{50S}$ greater than the observed exposure range). Regarding the exposure-response on the FPG set-point, EC$_{50B}$ was within the exposure range. The two EC$_{50}$ parameters were highly correlated on the individual level, indicating a shared mechanistic pathway, possibly through free fatty acid (FFA) which is reduced by tesaglitazar.

In Figure 16, median model predictions for FPG, FI, IS and BCM after a treatment with tesaglitazar 1 mg is illustrated.
Figure 15. Visual predictive checks for FPG and FI based on the mechanistic FPG-FI model. Upper panels show drug-naive T2DM patients treated with 0.5, 1, 2 or 3 mg of tesaglitazar. Lower panels show non-diabetic insulin resistant subjects from the SIR study randomised to 0.5 or 1 mg of tesaglitazar. The black and blue, solid lines represent observed and model-predicted median, respectively. The blue area within the dotted lines represents the model 95%-prediction interval (PI). The black circles represent observations within the PI and observations outside the PI are represented by a non-black plotting symbol that is unique for each individual within the panel.
Figure 16. The change in BCM, IS (denoted S in the figure), FI and FPG relative to values in healthy subjects simulated using the mechanistic PK-PD model, for all five disease groups (DGR). The median response to 1 mg daily dosing of tesaglitazar is displayed.
5 Discussion

5.1 Interconversion of tesaglitazar (Paper I)

Tesaglitazar is predominantly metabolised (to an acyl glucuronide) and 20% of the tesaglitazar dose is found unchanged in the urine. Thus, it is not apparent that renal function would affect the exposure of tesaglitazar in plasma. However, in the RENAL study, it was found that tesaglitazar exposure was 2-3 higher in subjects with low renal function. A mechanism-based population PK model was derived with the aim to explain this finding. The result demonstrates that the most likely explanation is that, the decreased renal elimination of the acyl glucuronide causes an increase of metabolite in plasma in subjects with impaired renal function, which leads to increased amount of metabolite undergoing interconversion and subsequently accumulation of tesaglitazar in plasma.

A number of independent data support this mechanism, including that the interconversion occurs via biliary secretion, that the hydrolysis of the acyl conjugate takes place in the gut with subsequent re-absorption as tesaglitazar. The acyl glucuronide of tesaglitazar is stable in plasma (unpublished data). Many anionic drugs and their conjugated metabolites are metabolised in the liver with subsequent urine and biliary excretion. The acyl glucuronide of tesaglitazar is a substrate for the two biliary efflux transporters MRP-2 and BCRP (unpublished data). In the tesaglitazar ADME study, mean radioactivity in faeces was ~8% after intravenous administration. Glucuronides, following biliary secretion can become substrates for the microfloral β-glucuronidases and hydrolysed back to the parent compound. Lastly, the absorption of tesaglitazar is fast and complete.

Interconversion, also called “futile cycling” has been reported for other drugs forming acyl glucuronides. No data has yet been presented of this type of interconversion in man, since it is stipulated that it would require intravenous dosing of the acyl glucuronide to solve the clearance equations. Thus, studies only report apparent clearances of the aglycone, ignoring the reversibility of the metabolism.

The present analysis shows that it is possible to characterise and quantify interconversion of a drug forming an acyl glucuronide. This was made possible due to the simultaneous assessment of both plasma and urine data of tesaglitazar and its metabolite in subjects with varying degrees of renal function. This enables separation of the elimination and interconversion proc-
esses, since the ratio between tesaglitazar and the metabolite varies with varying renal functions.

5.2 Interrelationship between tesaglitazar exposure and renal function (Paper II)

Since there is an interdependency between renal function and the exposure of tesaglitazar it is necessary to describe and quantify these processes in a simultaneous analysis. Given this interrelationship between glomerular filtration rate (GFR) and tesaglitazar exposure both these variables will be affected by each other leading to lower (GFR) and greater ($C_{\text{average}}$) steady state levels compared to if these effects were independent. For the same reason, steady state will be reached at a later time point.

A population PK-PD model was developed for tesaglitazar and GFR in T2DM patients receiving daily doses of tesaglitazar (2 mg), which adequately described tesaglitazar exposure and GFR over time. GFR decreased in all patients, and those treated with an ACE inhibitor were found to be more sensitive to tesaglitazar. When discontinuing treatment, mean GFR returned slowly towards baseline and the data and model suggest that the effect on GFR is reversible. Using an additional measure of renal function (MDRD) was pivotal in determining the time course of the changes in renal function, especially since post treatment measures of CLiothalamate was only available in few patients.

The mechanism of which tesaglitazar affects GFR is not understood. Tesaglitazar, being a potent PPAR $\alpha/\gamma$ agonist, could inhibit renal vasodilatory prostaglandins through a PPAR $\alpha$ mediated mechanism. A reduction of these prostaglandins leads to a constriction of the afferent arterioles and a subsequent reduction in GFR$^{87,88}$. In addition, PPAR $\gamma$ agonists have been shown to affect the renin angiotensin system$^{89,90}$, which potentially could mediate a vasodilatory effect on the efferent arterioles. Theoretically, a combination of these effects could explain the relatively large effects on renal function seen after treatment with high doses of tesaglitazar.

5.3 FPG-Hb-HbA1c model (Paper III-IV)

A mechanism-based PK-PD model for the relationship between FPG and HbA1c was developed to describe and quantify the physiological processes related to RBC glycosylation and turnover, and to evaluate the impact of tesaglitazar exposure on this system (Paper III).

The glycosylation of RBC forming HbA1c, is a chemical, non-enzymatic reaction between glucose and RBC, and the concentrations of HbA1c should
be near-proportional to the time-average concentrations of glucose within the erythrocytes\textsuperscript{73}. It has also been reported that the proportion of RBC being glycosylated increases according to the age of the cell\textsuperscript{74}.

An empiric indirect response models described the relationship between FPG and tesaglitazar exposure over time. The mechanism-based FPG-HbA\textsubscript{1c} model aimed to mimic the release and aging of RBC and the FPG dependent glycosylation process, using a number of, in series coupled, transit compartments. This allows the lifespan of RBC to vary between cells within an individual, compared to other types of life-span models\textsuperscript{21, 91} where the lifespan is assumed to be identical for all cells within one subject. The mean RBC lifespan was estimated to 135 days, which is in agreement with the literature\textsuperscript{92}. In the present analysis, the relationship between FPG and HbA\textsubscript{1c} was found to be non-linear, which was significantly better than a direct proportional relationship as suggested in a population PD model by deWinter et al\textsuperscript{59}. The likely explanation to the non-linear relationship is that fasting glucose and time-average glucose are not directly proportional to each other\textsuperscript{72, 73}.

The mechanistic PD model is not dependent on presence of (or changes in) Hb data, since the estimation of the RBC lifespan and the glycosylation process is determined by the changes in FPG and HbA\textsubscript{1c} over time. However, tesaglitazar affects Hb, and the model was further developed to investigate the mechanisms behind this. Hemodilution was the mechanism that was best supported by the data. PPAR agonists have shown to increase plasma volume as well as affect renal sodium regulation\textsuperscript{75, 76} which support the result. However, the cause of the Hb effect is not completely understood and other mechanisms have been suggested\textsuperscript{93}.

The population PK-PD model was re-evaluated using data from a 6 months, phase III study with tesaglitazar. It accurately predicted the response of FPG, Hb and HbA\textsubscript{1c} in drug-naïve patients. In patients previously treated with anti-diabetic drugs, HbA\textsubscript{1c} was under-predicted at all times. Plausible explanations could be a model structure not suitable for extrapolation to lower FPG ranges and unknown differences in patient populations between the studies.

5.4 FPG-FI model (Paper IV)

Tesaglitazar activates both PPAR \(\alpha\) and \(\gamma\) receptors, which improves peripheral insulin sensitivity, increases peripheral glucose uptake and decreases hepatic glucose output, similar to PPAR \(\gamma\) agonists\textsuperscript{94}. Activation of PPAR \(\alpha\) stimulates the uptake and catabolism of free fatty acids (FFA), promotes lipolysis, and enhances high density lipoprotein (HDL) synthesis, resulting in improved lipid metabolism\textsuperscript{95, 96}.
A PD model proposed by Topp et al\textsuperscript{60}, which describes the interrelationship between glucose, insulin and beta-cell mass (BCM) in healthy individuals was implemented and further developed on data from three clinical studies with tesaglitazar. This is the first time (to my knowledge) that the model has been fitted to data. The FPG-FI model could adequately describe all observations, even though the data originated from a heterogeneous population, ranging from non-diabetic insulin resistant subjects to T2DM patients. Patients were assumed to differ from healthy subjects by an offset in the beta-cell adaptation (i.e. lower BCM) and lower insulin sensitivity coupled with lower insulin elimination. The reason for implementing the effect of disease on BCM as an offset in beta-cell adaptation is due to actual BCM not being measured. Untreated T2DM patients were predicted by the model to have approximately 40\% relative BCM compared to healthy individuals, which is in agreement with the data from the literature\textsuperscript{97-99}. The model identified a strong correlation between insulin sensitivity and insulin elimination, which has been reported earlier with PPAR agonists\textsuperscript{70, 71, 100}. Both of these processes are affected by the level of FFA, which is reduced by tesaglitazar treatment\textsuperscript{45}.

The effect of tesaglitazar treatment on FPG, FI, BCM and IS predicted by the model is in line with the assumed mechanism of action. Insulin sensitivity improved rapidly and greatly in both insulin resistant subjects and in patients with T2DM, which is expected given the PPAR \(\alpha/\gamma\) activation. As a consequence to the improved insulin sensitivity, the FI was reduced during the first few weeks of treatment. BCM reached a new steady state after about six months of the treatment. In insulin resistant subjects there was a fast improvement in S and FI, but little effect on FPG and BCM. DeWinter at al included impact of disease progression on insulin sensitivity and beta-cell function and evaluated the effects of drug treatment on these processes\textsuperscript{59}. Disease progression was not included in the present analysis since the studies were only of 3 or 6 months duration. If including data from longer studies, the FPG-FI model could be improved to also include disease progression of BCM and IS.
6 Conclusions

In this thesis different mechanism-based PK, PK-PD and disease models describing important safety and efficacy biomarkers used in anti-diabetic clinical drug development have been presented. These models were based on clinical data generated during development of the PPAR α/γ agonist tesaglitazar, however, they are generic and could serve as template models that can contribute to valuable understanding of drug action and (T2DM) disease, and thereby support decision making during development of future anti-diabetic drugs.

- The PK model for tesaglitazar provides a modelling framework to evaluate interconversion of the metabolite (an acyl glucuronide) based on the simultaneous analysis of plasma and urine data from a group of subjects with varying renal function. The model and data give insight into the likely mechanism of the increased tesaglitazar exposure in renally impaired subjects, and separate elimination and interconversion processes without dosing of the metabolite.

- A simultaneous PK-PD assessment was warranted as tesaglitazar exposure and renal function affect each other. The model and data provide valuable information on the effect of tesaglitazar on renal function including, magnitude of the reduction, sub-groups at risk and the full time course.

- The FPG-Hb-HbA1c model describes, in a mechanistically reasonable manner, the interplay between important diabetic biomarkers. It provides plausible description of the release and aging of RBC as well as the FPG dependent glycosylation of RBC to HbA1c.

- The FPG-Hb-HbA1c model gives a modelling framework to evaluate potential mechanisms of the tesaglitazar effect on Hb and further, provides understanding on how the different mechanisms would affect HbA1c.
• The FPG-Hb-HbA1c model can accurately predict the outcome of a new 6 months study when accounting for the different patient population. The results from the joint analysis can be used to more accurately predict long-term HbA1c response based on short-term FPG data.

• The PK-PD model including components for β-cell mass and insulin sensitivity precisely describes FPG and FI data in a heterogeneous population of non-diabetic insulin resistant subjects and patients with T2DM. It provides mechanistic understanding on the complex glucose homeostasis, and a realistic characterisation of the impact of disease stage and drug treatment on the system.
7 Populärvetenskaplig sammanfattning

Att utveckla läkemedel tar lång tid, kostar mycket pengar och de allra flesta substanser som studeras blir aldrig färdiga läkemedel. Innan ett nytt läkemedel kan börja användas måste man visa att det är säkert och effektivt i den patientgrupp man avser att behandla. Många studier behövs göras, först i celler och djur, sen på människor, i den så kallade kliniska fasen.

Matematiska modeller används för att beskriva substansens öde i kroppen, samt hur substansen i sin tur påverkar kroppen. Dessa modeller ger värdefull kunskap om hur det tänkta läkemedlet fungerar. Kunskap som kan används när man tar beslut om man skall fortsätta utveckla substansen, och för att visa att läkemedlet är effektivt och säkert att användas på patienter i samhället.


Denna avhandling beskriver fyra modeller som togs fram under utvecklingen av en substans (tesaglitazar) för behandling av T2DM. Den första modellen gav värdefull förståelse kring tesaglitazars öde i kroppen och kunde förklara varför individer med dålig njurfunktion hade högre halter av tesaglitazar i kroppen jämfört med de med god njurfunktion. Den andra modellen beskriver tesaglitazars negativa påverkan på njurfunktionen. Den tredje modellen ger ökad förståelsen kring hur glukos binder till röda blodkroppar och bildar HbA1c. Den fjärde och sista modellen beskriver kroppens komplicerade reglering av glukos, hur T2DM negativt påverkar detta system samt hur tesaglitazar förbättrar den störda glukosregleringen.

Dessa modeller har varit viktiga för att öka förståelsen kring T2DM, samt tesaglitazar positiva och negativa effekter. Modellerna kan användas i utvecklingen av nya läkemedel för behandling av T2DM och ökar möjligheterna att tidigt visa om substansen har de önskade effekterna.
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