Population pharmacokinetic/pharmacodynamic modelling of the hypothalamic-pituitary-gonadal axis

Christoffer Wenzel Tornøe

Kgs. Lyngby 2005
IMM-PHD-2005-154

IMM

Technical University of Denmark
Informatics and Mathematical Modelling
Building 321, DK-2800 Kongens Lyngby, Denmark
Phone +45 45253351, Fax +45 45882673
reception@imm.dtu.dk
www.imm.dtu.dk

IMM-PHD: ISSN 0909-3192

Preface

This Ph.D. thesis was prepared at Informatics and Mathematical Modelling (IMM) at Technical University of Denmark (DTU) in partial fulfillment of the requirements for acquiring the Ph.D. degree in Engineering.

The project was financially supported by CIT (Center for IT) and Ferring Pharmaceuticals A/S as part of the Computer Supported Drug Development project. The work was carried out in the period from October 2002 to July 2005 in collaboration with Ferring Pharmaceutical A/S, Informatics and Mathematical Modelling at DTU, and Department of Pharmaceutical Biosciences, Division of Pharmacokinetics and Drug Therapy at Uppsala University.

The topic of the Ph.D. thesis is population pharmacokinetic/pharmacodynamic (PK/PD) modelling of the hypothalamic-pituitary-gonadal (HPG) axis. The thesis consists of a summary report and five scientific research papers written during the Ph.D. study and published/submitted to international journals.

The work was supervised by Henrik Madsen and Henrik Aalborg Nielsen (IMM, DTU), E. Niclas Jonsson (Uppsala University), and Henrik Agersø (Ferring Pharmaceuticals A/S).

Kgs. Lyngby, July 2005

Christoffer Wenzel Tornøe

Abstract

The present thesis deals with different aspects of population pharmacokinetic/pharmacodynamic (PK/PD) modelling of the male hypothalamic-pituitary-gonadal (HPG) axis. The thesis consists of a summary report and five scientific research papers.

An overview of the main topics covered in the thesis is provided in the summary report including PK/PD modelling in drug development, the pathological, physiological, and pharmacological aspects of the male HPG axis, and a detailed description of the methodology behind non-linear mixed-effects modelling based on stochastic differential equations (SDEs).

The main objective of the work underlying this thesis was to develop mechanism-based population PK/PD models of the HPG axis. The HPG axis is a multivariate closed-loop control system consisting of regulatory hormonal feedback mechanisms. The number and complexity of the physiological mechanisms involved in such models makes them difficult to develop and are often too complex to be conveniently described by empirical models. Hence, the use of SDEs in population PK/PD modelling was used as a tool to systematically develop a mechanism-based model of the HPG axis following treatment with gonadotropin-releasing hormone (GnRH) agonist triptorelin and GnRH antagonist degarelix in a combined model.

The use of SDEs in non-linear mixed-effects modelling was investigated by implementing the Extended Kalman Filter in the NONMEM software. Non-linear mixed-effects models based on SDEs extend the first-stage model of the hierarchical structure by decomposing the intra-individual variability into two types of noise, i.e. a system noise term representing unknown or incorrectly specified dynamics and a measurement noise term accounting for uncorrelated errors

such as assay error. This setup makes identification of structural model misspecification feasible by quantifying the model uncertainty, which subsequently provides the basis for systematic population PK/PD model development.

To support the model building process, the SDE approach was applied to clinical PK/PD data and used as a tool for tracking unexplained variations in parameters, identifying complicated non-linear dynamic dependencies, and deconvolving the functional feedback relationships of the HPG axis. The developed mechanism-based model of the HPG axis consisted of four compartments where the secretion of readily releasable LH from a pool compartment was stimulated and inhibited by the plasma triptorelin and degarelix concentrations, respectively. Circulating LH stimulated the testosterone secretion while the delayed testosterone feedback on the non-basal LH synthesis and release was modelled through a receptor compartment where testosterone stimulates the production of receptors. The derived mechanism-based model of the HPG axis was able to account for the observed LH and testosterone concentration-time profiles following treatment with both GnRH agonist triptorelin and GnRH antagonist degarelix thereby indicating that the model is sufficient at mimicking the underlying physiology of the endocrine system.

KEY WORDS: Population PK/PD modelling; non-linear mixed-effects modelling; NONMEM; stochastic differential equations; Extended Kalman Filter; systematic population PK/PD model building; HPG axis; GnRH agonist triptorelin; GnRH antagonist degarelix.

Resumé

Denne afhandling omhandler forskellige aspekter af population farmakokinetisk/ farmakodynamisk (PK/PD) modellering af den mandlinge hypothalamus-hypofyse-gonade (HPG) akse. Afhandlingen består af en sammenfatning samt fem videnskabelige publikationer.

En oversigt over denne afhandlings hovedemner er givet i sammentfatning indeholdende emner såsom populations PK/PD modellering i lægemiddeludvikling, patologiske, fysiologiske, og farmakologiske aspekter ved den mandlige HPG akse samt en detaljeret beskrivelse af metoden bag ikke-lineær mixed-effekters modellering baseret på stokastiske differentialligninger (SDE'er).

Hovedformålet med denne afhandling var at udvikle en mekanistisk-baseret population PK/PD model af HPG aksen. HPG aksen er et multivariat lukket-sløjfe kontrolsystem, som består af regulatoriske hormonale feedback mekanismer. Antallet og kompleksiteten af de fysiologiske mekanismer, som indgår i sådanne modeller, gør dem svære at udvikle og er ofte for komplekse til at blive beskrevet af empiriske modeller. Brugen af SDE'er i populations PK/PD modelling blev derfor brugt som et redskab til systematisk at udvikle en mekanistisk-baseret model af HPG aksen efter behandling med gonadotropin-releasing hormon (GnRH) agonist triptorelin og GnRH antagonist degarelix i en kombineret model.

Anvendelsen af SDE'er i ikke-lineær mixed-effekts modellering blev undersøgt ved implementering af det udvidede Kalman Filter i software programmet NONMEM. Ikke-lineære mixed-effekts modeller baseret på SDE'er udvider residualfejlsmodellen i den hierakiske struktur ved at dekomponere den intra-individuelle variabilitet til et system støj led, som repræsenterer ukendt eller inkorrekt specificeret dynamik og et målestøjsled, som tager højde for ukorreleret støj såsom

målestøj. Dette setup muliggør identifikation af strukturelle modelmisspecifikationer ved at kvantificere modellens usikkerhed som efterfølgende giver en basis for systematisk population PK/PD model udvikling.

SDE metoden blev anvendt på kliniske PK/PD data til at supportere modelbygningsprocessen og brugt som et redskab til sporing af uforklarede variationer i parametre, identifikation af komplicerede ikke-lineære dynamiske afhængigheder og afkodning af funktionelle feedback forbindelser i HPG aksen. Den udviklede mekanistisk-baseret model af HPG aksen bestod af fire kompartments, hvor sekretionen af hurtig tilgængelig LH fra et pool kompartment stimuleredes og inhiberedes af hhv. plasma triptorelin og degarelix koncentrationer. Cirkulerende LH stimulerer testosteron sekretionen, mens det forsinkede testosteron feedback på den ikke-basale LH syntese og frigivelse blev modelleret gennem et receptor kompartment, hvor testosteron stimulerer receptor produktionen. Den opstillede mekanistisk-baseret model af HPG aksen kunne gøre rede for de observede LH og testosteron koncentration-tids profiler efter behandling med GnRH agonist triptorelin og GnRH antagonist degarelix, hvilket indikerer at modellen er tilstrækkelig til at efterligne den underliggende fysiologi for det endokrine system.

STIKORD: Population PK/PD modellering; ikke-lineær mixed-effekts modellering; NONMEM; stokastiske differentialligninger; systematisk population PK/PD modelbygning; HPG aksen; GnRH antagonist degarelix; GnRH agonist triptorelin.

List of publications

The thesis is based on the following five scientific research papers, which will be referred to by their Roman numerals in the text.

I. C. W. Tornøe, H. Agersø, H. A. Nielsen, H. Madsen, and E. N. Jonsson. Population pharmacokinetic modeling of a subcutaneous depot for GnRH antagonist degarelix. *Pharm. Res.*, **21**(4):574–584 (2004).

II. C. W. Tornøe, H. Agersø, H. Madsen, E. N. Jonsson, and H. A. Nielsen. Non-linear mixed-effects pharmacokinetic/pharmacodynamic modelling in NLME using differential equations. *Comput. Methods Programs Biomed.*, **76**(1):31–40 (2004).

III. C. W. Tornøe, H. Agersø, H. A. Nielsen, H. Madsen, and E. N. Jonsson. Pharmacokinetic/Pharmacodynamic Modelling of GnRH Antagonist Degarelix: A Comparison of the Non-linear Mixed-Effects Programs NONMEM and NLME. *J. Pharmacokinet. Pharmacodyn.*, **31**(6):441–461 (2004).

IV. C. W. Tornøe, R. V. Overgaard, H. Agersø, H. A. Nielsen, H. Madsen, and E. N. Jonsson. Stochastic Differential Equations in NONMEM: Implementation, Application, and Comparision with Ordinary Differential Equations. *Pharm. Res.*, **22**(8):1247–1258 (2005).

V. C. W. Tornøe, H. Agersø, T. Senderovitz, H. A. Nielsen, H. Madsen, M. O. Karlsson, and E. N. Jonsson. Mechanism-based population PK/PD modeling of the hypothalamic-pituitary-gonadal axis following treatment with GnRH agonist triptorelin and GnRH antagonist degarelix. *Pharm. Res.*, Submitted (2005).

The following publications prepared during the Ph.D. study were not directly related to the present research and will therefore not be addressed in this thesis.

- C. W. Tornøe, J. L. Jacobsen, H. Madsen. Grey-box pharmacokinetic/ pharmacodynamic modelling of a euglycaemic clamp study. *J. Math. Bio.*, **48**(6):591-604 (2004).
- C. W. Tornøe, J. L. Jacobsen, O. Pedersen, T. Hansen, H. Madsen. Grey-box Modelling of Pharmacokinetic/Pharmacodynamic Systems. *J. Pharmacokinet. Pharmacodyn.*, **31**(5):401-417 (2004).
- R. V. Overgaard, E. N. Jonsson, C. W. Tornøe, and H. Madsen. Non-Linear Mixed-Effects Models with Stochastic Differential Equations. Implementation of an Estimation Algorithm. *J. Pharmacokinet. Pharmacodyn.*, **32**(1), In press (2005).

Abbreviations & symbols

Abbreviations

ACTH	Adrenocorticotropic hormone
ADME	Absorption, distribution, metabolism, and excretion
AR	Autoregressive
AUC	Area under the curve
CRH	Corticotropin-releasing hormone
CTS	Clinical trial simulations
CV	Coefficient of variation
EKF	Extended Kalman filter
EVID	Event identifier
FDA	Food and drug administration
FO	First-order
FOCE	First-order conditional estimation
FSH	Follicle-stimulating hormone
GAM	General additive modelling
GH	Growth hormone
GHRH	Growth hormone-releasing hormone
GLP	Good laboratory practice
GnRH	Gonadotropin-releasing hormone
GOF	Goodness-of-fit
HPG	Hypothalamic-pituitary-gonadal
HS	Healthy subjects

IIV	Inter-individual variability
IM	Intramuscular
IOV	Inter-occasion variability
IPP	Individual PK parameters
IV	Intravenous
LC	Liquid chromatography
LH	Luteinizing hormone
LLOQ	Lower limit of quantification
LRT	Likelihood ratio test
MEIA	Microparticle enzyme immunoassay
ML	Maximum likelihood
MS	Mass spectrometry
M&S	Modelling and simulation
NCA	Non-compartmental analysis
NDA	New drug application
ODE	Ordinary differential equation
OFV	Objective function value
PC	Prostate cancer
PCP	Prostate cancer patients
PD	Pharmacodynamics
PDE	Partial differential equation
PK	Pharmacokinetics
PK/PD	Pharmacokinetics/pharmacodynamics
PNLS	Penalized non-linear least squares
PoC	Proof of concept
Q-Q	Quantile-quantile
RSD	Relative standard deviation
RSE	Relative standard error
SC	Subcutaneous
SCM	Stepwise covariate modelling
SDE	Stochastic differential equation
STS	Standard two-stage
Te	Testosterone
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone

Symbols

Δ	Hessian
ϵ	Residual error
η	Inter-individual random-effects
∇	Gradient
Ω	Inter-individual covariance
ϕ	Individual parameter
Σ	Measurement error covariance
σ_w	Diffusion term
θ	Population mean parameter
C_e	Effect-compartment concentration
C_{max}	Maximal drug concentration
C_p	Plasma concentration
d	Input
e	Measurement error
E_{max}	Maximal effect
EC_{50}	Concentration producing half the maximal effect (potency)
F	Absolute bioavailability
Fr	Fraction
k	First-order rate constant
K	Kalman gain
K_{in}	Zero-order constant for production of response
k_{e0}	Rate constant from effect-compartment to out
k_{out}	First-order constant for loss of response
l	Individual log-likelihood function
L	Population likelihood function
P	State covariance
R	Output prediction covariance
t	Time
$t_{1/2}$	Half-life
t_{max}	Time to maximal drug concentration
w	Standard Wiener process
x	State variable
y	Measurement
Z	Covariates

Contents

Preface **i**

Abstract **iii**

Resumé **v**

List of publications **vii**

Abbreviations & symbols **ix**

1 Introduction **1**

1.1 Background 1

1.2 Aims of the thesis 2

1.3 Outline 2

2 PK/PD modelling in drug development **5**

2.1 Clinical drug development 5

2.2 PK/PD modelling 6

3 HPG axis **13**

3.1 Pathological aspects 13

3.2 Physiological aspects 14

3.3 Endocrine system 16

3.4 Pharmacological aspects 17

4 Methodology **19**

4.1 Terminology and notation 19

4.2 Non-linear mixed-effects modelling 20

4.3 Extended Kalman Filter 27

4.4 Systematic population PK/PD model building framework 30

5 Materials & Methods **33**

5.1 Compounds 33

5.2 Analytical methods 34

5.3 Overview of studies 34

5.4 Data analysis 36

6 Results & Discussion **41**

6.1 Population PK modelling of SC and IM depots 41

6.2 SDEs in population PK/PD modelling 47

6.3 Mechanism-based PK/PD modelling of the HPG axis 56

7 Conclusions **61**

Acknowledgements **65**

References **67**

Papers

I Population Pharmacokinetic Modeling of a Subcutaneous Depot for GnRH Antagonist Degarelix 77

II Non-linear mixed-effects pharmacokinetic/pharmacodynamic modelling in NLME using differential equations 79

III Pharmacokinetic/Pharmacodynamic Modelling of GnRH Antagonist Degarelix: A Comparison of the Non-linear Mixed-Effects Programs NONMEM and NLME 81

IV Stochastic Differential Equations in NONMEM: Implementation, Application, and Comparision with Ordinary Differential Equations 83

V Mechanism-based population PK/PD modeling of the hypothalamic-pituitary-gonadal axis following treatment with GnRH agonist triptorelin and GnRH antagonist degarelix 85

Chapter 1

Introduction

1.1 Background

Prostate cancer (PC) is a leading cause of illness and death among men, and is responsible for almost 3% of deaths in men older than 55 years [12, 53]. The management of PC has mainly focused on the gonadotropin-releasing hormone (GnRH) receptor where Decapeptyl (triptorelin) was developed as the first generation of GnRH agonists by Ferring Pharmaceuticals A/S. Ferring is currently developing a new generation of GnRH analogues where the first in this new class of drugs is the GnRH antagonist degarelix.

Population pharmacokinetic/pharmacodynamic (PK/PD) modelling is becoming an important decision support tool for faster and more efficient drug development. In order to apply population PK/PD modelling to the development of degarelix, it is necessary to understand the physiology of the hypothalamic-pituitary-gonadal (HPG) axis on which GnRH analogues act. The number and complexity of the physiological mechanisms involved in modelling physiological systems such as the HPG axis are often too complex to be conveniently described by empirical PK/PD models. Thus, there is an increasing need to develop new sophisticated computational methods and models to ensure the optimal utilization of the PK/PD data from projects such as the degarelix development project.

Stochastic differential equations (SDEs) provide an attractive modelling approach for systematic population PK/PD model development by allowing information about unmodelled dynamics of the system to be extracted from data. The key advantage of using SDEs compared to ordinary differential equations (ODEs) is that they allow for decomposition of the residual error into a system noise term representing unknown or incorrectly specified dynamics and a measurement noise term accounting for uncorrelated errors such as assay error. SDEs have been proven beneficial in other scientific areas to optimize the model building process for non-population data by pinpointing model deficiencies and iteratively improve the model using a systematic model building framework [44, 45].

1.2 Aims of the thesis

The overall aims of the work presented in this thesis were to develop population PK/PD models for drugs acting on the HPG axis and to investigate the use of advanced computational methods in non-linear mixed-effects modelling.

The specific aims of the thesis were:

- To build population PK models for the absorption of GnRH antagonist degarelix and GnRH agonist triptorelin from subcutaneous (SC) and intramuscular (IM) depots.
- To implement SDEs in non-linear mixed-effects modelling software and explore possible applications of SDEs in population PK/PD modelling.
- To develop a mechanism-based population PK/PD model of the HPG axis, which would be able to describe and predict the PK/PD response following treatment with GnRH agonist triptorelin and GnRH antagonist degarelix.

1.3 Outline

The remainder of this thesis is organized in the following way.

In **Chapter 2**, the phases of clinical drug development are explained along with basic PK/PD concepts and different PK/PD modelling approaches.

The background pathological, physiological, and pharmacological aspects of the HPG axis are introduced in **Chapter 3**.

The methodology developed in this thesis are described in **Chapter 4**. A brief introduction to SDEs is provided in Section 4.2.1 while a detailed description of the theory behind the use of SDEs in non-linear mixed-effects modelling is included in Sections 4.2–4.3. A systematic population PK/PD model building framework based on SDEs is presented in Section 4.4.

Chapter 5 includes a description of the materials and methods used throughout the Ph.D. project. Information about the two compounds GnRH agonist triptorelin and GnRH antagonist degarelix, the analytical methods, and the studies used in the population PK/PD modelling of the HPG axis are specified in Sections 5.1–5.3. A description of the applied data analysis is provided in Section 5.4.

An overview of the most important results obtained in the Ph.D. project is given in **Chapter 6**. The results from Papers I–V are linked together and discussed (with a broader perspective than in the papers) in Sections 6.1–6.3.

The overall conclusions according to the thesis objectives are presented in **Chapter 7** along with future perspectives.

Chapter 2

PK/PD modelling in drug development

PK/PD modelling is a multi-disciplinary field requiring expertise from many scientific areas. In the following chapter, a brief introduction to the phases of clinical drug development is provided along with different aspects of PK/PD modelling.

2.1 Clinical drug development

The objective of clinical drug development is to provide relevant information on safety and efficacy of the drug to enable physicians to treat patients optimally [2]. Drug development is a costly and time consuming process. Current costs of bringing a new drug to market is estimated to be as high as 0.8 to 1.7 billion US dollars [80]. On average, it takes 6–12 years to bring a new drug through all the regulatory hurdles before being marketed [22]. Each new drug application (NDA) submission to the regulatory authorities consists of an average 68 clinical trials with a total of 4,300 subjects [8].

Clinical drug development is an information-gathering process, which can be thought of as two successive learning versus confirming cycles. The first cycle (i.e. Phase I and Phase IIa) addresses the question of whether benefit over

existing therapies can be expected. It involves *learning* (Phase I) what is the largest short-term dose that can be administered to healthy male subjects without causing harm, and then *confirming* (Phase IIa) whether that dose induces some measurable short-term benefit in patients for whom the drug is intended for, i.e. proof of concept (PoC). An affirmative answer at this first stage provides the justification for a more elaborate second cycle. This next cycle (i.e. Phase IIb and Phase III) attempts to *learn* (Phase IIb) what is an optimal dose regimen to achieve an acceptable benefit/risk and ends with the formal clinical (Phase III) trials of that regimen versus a comparator. If the trial(s) reject the null hypothesis of no incremental benefit of the new drug over the comparator (or occasionally no less benefit), the drug is approvable. For some drugs, the regulatory authorities require additional post-marketing studies (Phase IV) to evaluate long-term effects or to obtain new indications [69].

Occasionally, the outcome of a clinical trial is unsatisfactory and the development might have to be terminated or the trial has to be repeated with potential huge extra costs in the late development phases. It is therefore of great importance to use all available information to increase the probability that a given trial gives the required information to make a decision as early as possible whether to terminate or progress the development program. The regulatory agencies have recently stressed the need to improve the critical path of drug development and initiatives have been taken to have end-of-phase 2A (EOP2A) meetings with industry focusing on PK/PD modelling and simulation (M&S) [80].

2.2 PK/PD modelling

The present introduction to PK/PD modelling does not cover all the different aspects of PK/PD modelling with equal emphasis. The classical PK/PD modelling is only briefly mentioned whereas more space is devoted to mechanism-based and population PK/PD modelling, which currently is the main area of focus in the research community.

Pharmacokinetics (PK) is the study of the time course of and factors affecting the drug movement in the body and includes the processes of drug absorption, distribution, and elimination, i.e.

- **Absorption** is the process by which unchanged drug proceeds from site of administration to the systemic circulation (site of measurement within the body).
- **Distribution** is the process of reversible transfer of a drug to and from

the site of measurement, eg. blood and muscle.

- **Elimination** is an irreversible process. The elimination pathway is in general determined by the drug's physio-chemical properties where lipophilic compounds are subject to metabolism (in liver and/or blood) while hydrophilic compounds are subject to excretion (in kidneys and/or bile).

Pharmacodynamics (PD) is the description of the time course and factors controlling the drug effect on the body, i.e. the study of drug-target (receptor) interactions and signal transduction processes.

The aim of PK/PD modelling is to link the PK and PD to establish and evaluate the dose-exposure-effect relationship (where effect refers to both efficacy and toxicity) following drug administration [66].

The three main objectives for modelling PK/PD data are to describe, understand, and predict [22], i.e.

- PK/PD modelling is a concise way to describe and summarize data from clinical studies. Current understanding of the modelled system can be conceptualized and competing hypotheses of mechanisms of drug action can be rejected/accepted.
- Understanding the PK/PD of a drug can be used to explain the observed variability by identifying and quantifying factors influencing the PK/PD.
- Linking the dose-concentration-effect relationships in a PK/PD model facilitates the prediction of the concentration-time profiles resulting from new dosing regimens, pharmacological variability within the system (disease, drug-drug interactions) and between systems (*in vitro-in vivo* correlations, allometric scaling, inter-individual variability).

The integration of PK/PD principles into clinical drug development contribute to a more rational and efficient drug development process by supporting lead candidate differentiation, interpretation of efficacy and safety, and identification of clinical target concentrations/doses [22, 69]. Clinical trial simulations (CTS) is an emerging technique to guide the design of future clinical studies through assessment of optimal power, sample size, and dose selection.

2.2.1 PK/PD models

Pharmacometrics is the science of developing and applying statistical methods and mathematical models in a biological context for characterizing, under-

standing, and predicting the PK/PD of a drug [3]. It is a multi-disciplinary field requiring expertise from pharmacology, physiology, pathology, mathematics, statistics, and computer science to build PK/PD models, which are able to describe the absorption, distribution, metabolism, and excretion (ADME) of a drug, mechanism of drug action, underlying physiology, as well as disease progression.

The basic components of PK/PD models are explained below and visualized in Figure 2.1.

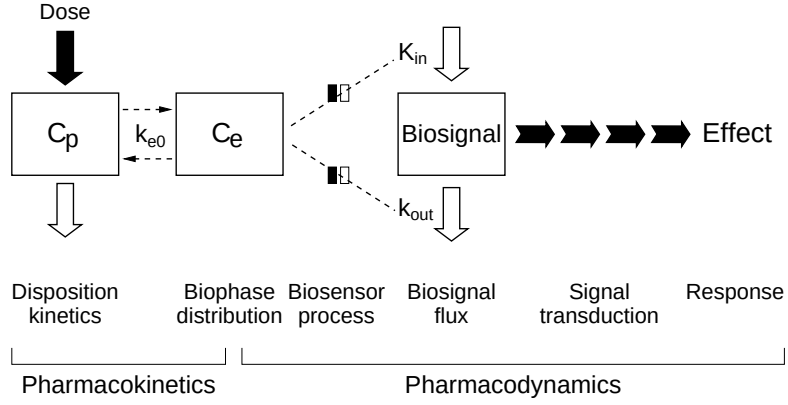

Figure 2.1: Basic components of PK/PD models [51].

PK models are typically straightforward to develop and can be derived from basic principles such as conservation of mass, Fick's laws of diffusion, and Michaelis-Menten kinetics. The time course of plasma drug concentration is often assumed in equilibrium with the biophase concentration from where the drug exerts it's action [68].

PD models are much more complicated compared to PK models due to the vast number of pharmacological and physiological mechanisms controlling the drug response. The biosensor process involves drug-receptor interactions, which lead a cascade of intracellular events. These events are typically very difficult to model since they require detailed information about drug affinity (i.e. drug-receptor complex formation and dissociation) as well as receptor density, conformation, occupancy, and activation (i.e. intrinsic efficacy) [16, 51].

Many drugs act via indirect mechanisms and the biosensor process may either stimulate or inhibit the production or loss of an endogenous mediator (biosignal flux) [34]. These mediators do not necessarily represent the final observed response, which may be further downstream from the receptor activation. The

signal transduction processes, which are all the physiological processes between activation of the target and the generation of response, account for post-receptor time delays due to functional adaptations, desensitization, and tolerance development following acute or long-term drug exposure, disease progression, and drug-drug interactions [51].

PK/PD models should ideally rely upon the current state of knowledge about the pharmacology of the drug and the physiology of the system. When the rate-limiting step on the path to system response is due to pre-receptor non-equilibrium (e.g. biophase distribution), a simple effect-compartment model [68] is commonly used to account for the observed delay between the plasma drug concentration and the effect whereas rate-limiting steps further downstream result in indirect response models [35, 81]. Rarely, the time constants of the PK/PD model are such that neither step is strictly rate-limiting due to e.g. time-varying systems, which then requires the PK/PD model to be able to describe both mechanisms. However, it is often necessary to simplify the models due to parameter identifiability issues and the limited information available in sparsely sampled clinical PK/PD data.

PK/PD models can generally be classified as being empirical, mechanistic, or physiological [11, 88], i.e.

Empirical models are based purely on a mathematical description of e.g. plasma concentration-time data of a drug. The mathematical functions or differential equations are employed without regard to any mechanistic aspects of the modelled system. This method is quick but can rarely be used to extrapolate to other dosing regimens.

Mechanism-based models aim at mimicking the data generation mechanism of the underlying physiological system thereby enabling the description and prediction of multiple drugs acting on the same system. The estimation of all model parameters is generally only possible by using data and information from several studies.

Physiological models imply certain mechanisms or entities that have physiological, biochemical, or physical significance. Contrary to mechanism-based models, physiological models use flow rates (fluxes) through particular organs or tissues along with experimentally determined ratios, e.g. the ratio between the blood and tissue concentration. The advantage of the physiological approach is that biosimulations of events such as fever or heart failure can be performed. The disadvantage is that the mathematics become very complex and it is impossible to estimate all model parameters.

A combination of empirical, mechanism-based, and physiological models is commonly used in PK/PD modelling where certain parts of the system may be modelled empirically, while other parts are modelled based on *a priori* physiological knowledge about the system. This approach is often preferred due to issues associated with identification and estimation of model parameters.

Mechanism-based models aim at separating drug-related parameters from system-related parameters. The drug-related parameters relate to the physical and chemical drug properties (e.g. affinity and efficacy), while the system-specific part of the model describes the underlying physiology and is consistent across drugs [16, 51]. The benefits of formulating a mechanism-based PK/PD model, compared to an empirical formulation, are

- The development of the model may lead to a greater understanding of the system,
- The model neatly summarizes the current state of knowledge about the system,
- The system-specific part of the model can be used for all drugs acting on that system,
- The drug-specific part of the model can be used to test hypothesis on mechanism of drug action,
- The model can be used to identify gaps in the current knowledge that may be addressed in future studies, and
- If the model is correctly specified, it should have better simulation and prediction properties than corresponding empirical models, which lack the ability to characterize systems and dosing regimens other than those they have been developed for.

2.2.2 Population PK/PD modelling

The definition of population PK/PD is the study of the sources of variability among individuals (both healthy volunteers and patients) receiving clinical relevant doses of drug [1]. The magnitude of unexplained (random) variability is of great importance in clinical drug development for the design of dosing regimens since the efficacy and safety of a drug might decrease as unexplained variability increases [71, 79]. The population approach was first applied in clinical drug development because only sparse PK data could easily be obtained in the target patient population. PK/PD modelling was therefore the only option to an adequate and efficient interpretation of such data [3].

One of the main objectives of population PK/PD modelling is to characterize and explain the different sources of variability [22, 79]. The population model framework consists of the following three sub-models (see Figure 2.2).

1. The *structural sub-model* describes the overall trend in the data using fixed-effects parameters, e.g. clearance and volume.
2. In the *statistical sub-model*, the variability is accounted for using different levels of random-effects, i.e.
   (a) Inter-individual variability (IIV) in response, which describes biological population variability that cannot be explained solely in terms of the measurable independent variables.
   (b) Intra-individual (residual) variability, which arise due to assay error and structural model approximations.
   (c) Inter-occasion variability (IOV) accounting for variation between study occasions.
3. The *covariate sub-model* expresses the relationship between covariates and model parameters using fixed-effects parameters.

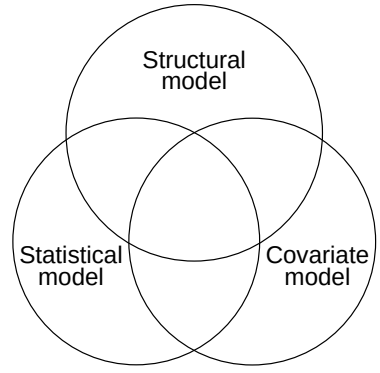

Figure 2.2: Schematic illustration of the population PK/PD model [30].

Population PK/PD modelling is commonly performed by one of the following two methods, i.e.

Standard two-stage (STS) method analyzes the individual data separately and the sample mean and variances are calculated using the individual estimated parameters. The STS method is often used to analyze PK data from studies involving intensive sampling performed on a limited number of individuals. The method is simple and intuitive but the IIV is generally overestimated [71].

Non-linear mixed-effects modelling uses a hierarchical model structure, which allows for simultaneous estimation of the inter- and intra-individual variability (random effects) as well as the influence of measured concomitant effects or covariates on the fixed-effects parameters. This method enables the analysis of PK/PD data from both sparse-sampled and unbalanced study designs.

The non-linear mixed-effects modelling approach is typically the preferred method in population PK/PD modelling because it provides reliable predictions of the variability and because it is the only practical method for analyzing data from multiple studies in a single data analysis. The estimation of parameters in non-linear mixed-effects models is the topic of Section 4.2.

Chapter 3

Hypothalamic-pituitary-gonadal axis

The HPG axis is the hormone system that controls the release of sex hormones. In order to be able to model the HPG axis after treatment with GnRH analogues one needs to understand the connection between the disease, physiological system, and the mechanism of drug action (see Figure 3.1). A brief description of the pathological, physiological, and pharmacological aspects of the male HPG axis is therefore presented in the following.

3.1 Pathological aspects

Prostate cancer (PC) is a leading cause of cancer mortality and morbidity among men in the industrialized world second only to lung cancer and is responsible for almost 3% of deaths in men older than 55 years [12, 53]. A widely recognized feature of PC is its high sensitivity to androgen deprivation. Androgen deprivation may be achieved by bilateral orchiectomy, by administration of oestrogens, or by administration of GnRH analogues. The aim of the different treatments is to suppress and maintain serum testosterone to castrate levels, i.e. testosterone levels below 0.5 ng/mL.

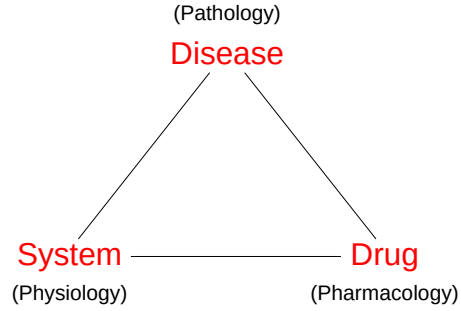

Figure 3.1: The connection between disease (pathology), system (physiology), and mechanism of drug action (pharmacology).

3.2 Physiological aspects

3.2.1 Hypothalamus

The hypothalamus is a small region located within the brain and is part of the central nervous system. Hypothalamic hormones play a pivotal role in the regulation of body functions such as eating, sexual functions and behaviors, blood pressure and heart rate, maintenance of body temperature, and emotional states just to name a few. The hypothalamus hormones are produced in nerve cells (i.e. neurons) and the release is modulated by other neurons. The hypothalamus thereby serves as the major link between the nervous and endocrine systems [25].

The hypothalamic hormones are released into blood vessels that connect the hypothalamus and the pituitary gland (i.e. the hypothalamic-hypophyseal portal system). The hypothalamic hormones are commonly referred to as releasing or inhibiting hormones because they promote or inhibit the release of pituitary hormones. The major hypothalamic hormones are [25, 85]

- Corticotropin-releasing hormone (CRH) is part of the hormone system regulating carbohydrate, protein, and fat metabolism as well as sodium and water balance in the body.
- Gonadotropin-releasing hormone (GnRH) controls sexual and reproductive functions.
- Thyrotropin-releasing hormone (TRH) is part of the hormone system controlling the metabolic processes of all cells.

- Growth hormone-releasing hormone (GHRH) is an essential component of the system promoting the organism's growth.
- Somatostatin affects bone and muscle growth with the opposite effect of GHRH.

3.2.2 Pituitary

The pituitary gland is located in the brain directly below the hypothalamus consisting of two parts, i.e. the anterior and posterior pituitary.

The anterior pituitary produces several hormones that either stimulate target glands to produce hormones or directly affect organs. The anterior pituitary hormones include [25, 85]

- Adrenocorticotropic hormone (ACTH) stimulates the release of hormones from the adrenal cortex.
- Luteinizing hormone (LH) stimulates testosterone production in the testes.
- Follicle-stimulating hormone (FSH) stimulates sperm production.
- Thyroid-stimulating hormone (TSH) stimulates the release of thyroid hormones.
- Growth hormone (GH) promotes the body's growth and development.

The posterior pituitary does not itself produce hormones. Instead, it stores the hormones vasopressin and oxytocin, which are produced by neurons in the hypothalamus. Both hormones are collected at the ends of the neurons, which are located in the hypothalamus and extend to the posterior pituitary [25].

3.2.3 Gonads

The gonads (i.e. the testes) serve two major functions: Spermatozoa and steroid sex hormone (androgen) synthesis, which are necessary for the development and function of the male reproductive organs and secondary sex characteristics. The principal androgenic steroid is testosterone, which primarily is secreted from the testes but also from the adrenal glands. Its main function is to stimulate the development and growth of the male genital tract [25].

3.3 Endocrine system

Endocrinology is the study of endocrine glands and hormones of the body and their related disorders. Endocrine glands are organs or clusters of cells producing hormones, which are secreted directly into the bloodstream without passing through tubes or ducts and carried via the blood to their target cells. The endocrine system is a complex control system of glands producing hormones, which help to control bodily metabolic activity and obtain homeostasis by hormonal feedback loops including the pituitary, thyroid, parathyroid, and adrenal gland as well as the pancreas, ovaries, and testes [85].

The feedback mechanisms of the endocrine control system with respect to the male HPG axis is illustrated in Figure 3.2. The HPG hormone system is activated by GnRH, which is secreted regularly in short bursts from neurons in the hypothalamus. GnRH acts as an endogenous agonist that selectively stimulates the gonadotrophic cells in the anterior pituitary gland to synthesize and release the gonadotropins LH and FSH [25]. The responsiveness of the gonadotrophs to GnRH varies under different conditions, but seems to be correlated with the number of GnRH receptors, and is only activated by pulsatile GnRH stimulation [40, 43].

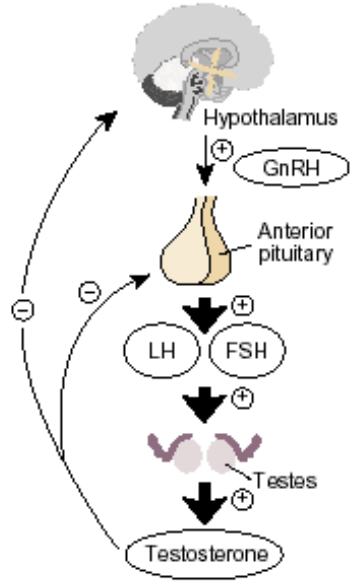

Figure 3.2: Schematic illustration of the feedback mechanisms that control the endocrine system of the male HPG axis [25].

LH interacts with receptors on the plasma membrane of testicular Leydig cells, which induces *de novo* synthesis of androgens, primarily testosterone [23, 56]. FSH and testosterone are key regulators of the testicular Sertoli cells, which support and nourish the sperm cells during their maturation. The Sertoli cells secrete inhibin which prevents pituitary FSH release. Activin, which is produced in the Leydig cells, stimulate FSH secretion and thus has the opposite effect of inhibin [25].

Rising levels of circulating testosterone appear to have a negative short- and long-loop feedback action on the pituitary and hypothalamus, respectively, thereby reducing the LH release from the pituitary as well as the hypothalamic GnRH secretion. The feedback mechanisms are still not fully understood, but testosterone seems to be able to suppress the release of gonadotropins by decreasing pituitary sensitivity to GnRH. Other findings indicate that testosterone feedback takes place at the hypothalamic level by testosterone modulating the frequency of the hypothalamic pulse generator [9].

3.4 Pharmacological aspects

The potency of GnRH and its analogous as stimulators or inhibitors of pituitary gonadotropin secretion have made them highly useful in the therapy of sex hormone-dependent tumors. The mechanism of action of GnRH agonist and GnRH antagonist[1] are explained in the following.

3.4.1 GnRH agonists

Prostate cancer patients have for many years been treated with GnRH agonists [72]. GnRH agonists are similar in structure and function to natural GnRH, but are about 60 times more potent than the natural hormone [12]. GnRH agonists act by competitive binding to and stimulation of pituitary GnRH receptors resulting in initial stimulation of gonadotropin and testosterone secretion. Continuous stimulation of the pituitary by chronic administration of long-acting GnRH agonists will result in down-regulation of pituitary GnRH receptors followed by receptor desensitization with subsequent inhibition of LH production [40]. This, in turn causes a suppression of gonadal steroids (primarily testosterone), on which continued growth of hormone-sensitive PC cells depends [23, 56, 73].

[1]The definition of an agonist is a ligand that binds to a receptor and alters the receptor state resulting in a biological response, while an antagonist is a ligand that reduces the action of another ligand.

3.4.2 GnRH antagonists

In contrast to treatment with GnRH agonists, GnRH antagonists block the pituitary GnRH receptors causing immediate suppression of gonadotropin secretion which result in medical castration, i.e. testosterone levels below 0.5 ng/mL. The initial flare-up effect (LH and testosterone surge) of GnRH agonists is thereby avoided with GnRH antagonists [26, 70, 86]. This is particularly important in clinical situations where a fast and profound suppression of testosterone is desired, such as symptomatic PC (e.g. pain or spinal cord compression) [61].

Previously, the short duration of action of GnRH antagonists formulations compared to the agonists formulations (where one-month, three-month, and even longer depot formulations are available [21, 48]) has limited the use of the GnRH antagonists in the treatment of PC along with histamine-mediated side-effects [70]. In order to prolong the duration of action of GnRH antagonists, several studies have been performed with sustained release formulations (microcapsules or microgranules) [42, 60, 62, 63]. Recently, the first GnRH antagonist with prolonged effect, abarelix, was approved by the U.S. Food and Drug Administration (FDA) for treatment of a subpopulation of PC patients with a one-month depot formulation [78].

Chapter 4

Methodology

The terminology and notation used in this chapter is initially presented followed by a detailed description of the developed methodology for implementing SDEs in non-linear mixed-effects modelling.

4.1 Terminology and notation

To ease the notation, bold symbols refer to vector or matrix representation. Capital Greek letters symbolizes population PK/PD parameters.

The symbol $p(X)$ denotes the density of X, while $p(X|Y)$ symbolizes the conditional density of X given Y. Conditioning on not explicitly stated variables is represented by "$\cdot$", i.e. $p(X|\cdot)$.

Subscript notation $i(j|j-1)$ refers to the j^{th} prediction based on all $j-1$ previous measurements for individual i. $\boldsymbol{\mathcal{Y}}_{ij}$ represents all observations of the i^{th} individual up to time t_{ij}.

The notation $\boldsymbol{l}_i\Big|_{\hat{\boldsymbol{\eta}}_i}$ refers to the individual log-likelihood function $\boldsymbol{l}_i$ evaluated at the empirical Bayes estimates $\hat{\boldsymbol{\eta}}_i$.

4.2 Non-linear mixed-effects modelling

Population PK/PD data analysis is typically performed using non-linear mixed-effects modelling. Non-linear mixed-effects models can be thought of as a hierarchical model structure where the variability in response is split into inter- and intra-individual variability as illustrated in Figure 4.1.

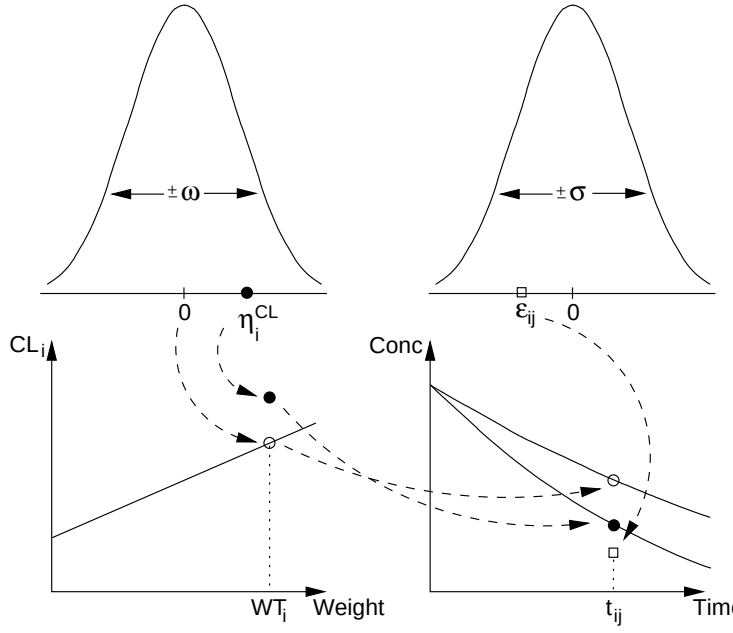

Figure 4.1: Schematic illustration of the hierarchical model structure in non-linear mixed-effects models. Inter-individual variability (**Left**) and intra-individual (residual) variability (**Right**). Population (○) and individual (●) parameter/prediction and observed concentration (□) [67].

4.2.1 Stochastic differential equations

Stochastic state-space or *grey-box* models consist of SDEs describing the dynamics of the system in continuous time system equations (4.1) and a set of

discrete time measurement equations (4.2) [45, 46, 76, 77], i.e.

$$d\boldsymbol{x}_t = \boldsymbol{g}(\boldsymbol{x}_t, \boldsymbol{d}_t, \boldsymbol{\phi})\, dt + \boldsymbol{\sigma}_w\, d\boldsymbol{w}_t\,, \qquad \boldsymbol{w}_t - \boldsymbol{w}_s \in N(\boldsymbol{0}, |t-s|\boldsymbol{I}) \tag{4.1}$$

$$\boldsymbol{y}_j = \boldsymbol{f}(\boldsymbol{x}_j, \boldsymbol{\phi}) + \boldsymbol{e}_j\,, \qquad \boldsymbol{e}_j \in N(\boldsymbol{0}, \boldsymbol{\Sigma}) \tag{4.2}$$

where $\boldsymbol{x}$ is the state vector, $\boldsymbol{d}$ is the input vector, $\boldsymbol{\phi}$ is the parameter vector, t is the time variable, $\boldsymbol{\sigma}_w\, d\boldsymbol{w}$ is the system noise, and $\boldsymbol{I}$ is the identity matrix. The measurement vector is denoted by $\boldsymbol{y}$ while $\boldsymbol{e}$ is the measurement error with mean zero and covariance $\boldsymbol{\Sigma}$. The non-linear functions $\boldsymbol{g}(\cdot)$ and $\boldsymbol{f}(\cdot)$ describe the relationship between the dynamics of the states and the states and the observations, respectively.

The structural model function $\boldsymbol{g}(\cdot)$ is commonly referred to as the drift term while the matrix $\boldsymbol{\sigma}_w$ is a scaling diffusion term [28]. The standard Wiener process $\boldsymbol{w}$ (also referred to as Brownian motion) is a non-stationary stochastic process with mutually independent (orthogonal) increments $(\boldsymbol{w}_t - \boldsymbol{w}_s)$ which are Gaussian distributed with mean zero and covariance $|t-s|\boldsymbol{I}$. If the diffusion term $\boldsymbol{\sigma}_w$ is zero, the system of SDEs in Eq. (4.1) reduces to a set of ODEs. The usual physiological interpretation of the parameters is thereby preserved in the SDE model formulation.

The difference between a state-space model based on SDEs and ODEs is illustrated by simulation of an exponential decay in Figure 4.2. The measurements of the two models look very similar while the time evolution of the underlying state is noticeably different. This is because the system noise influences the time evolution of the underlying state making it a stochastic variable in the SDE model. In the ODE model, the residual noise does not affect the time evolution of the deterministic state.

4.2.2 Non-linear mixed-effects modelling based on SDEs

Non-linear mixed-effects models based on SDEs extend the first-stage model of the hierarchical structure by decomposing the intra-individual variability into system noise representing unknown or incorrectly specified dynamics and a measurement noise term accounting for uncorrelated errors such as assay error [55].

The first-stage model describes the intra-individual (residual) variability. It consists of a structural model described by a system of SDEs in Eq. (4.3) and a set of measurement equations in Eq. (4.4), which describes the difference

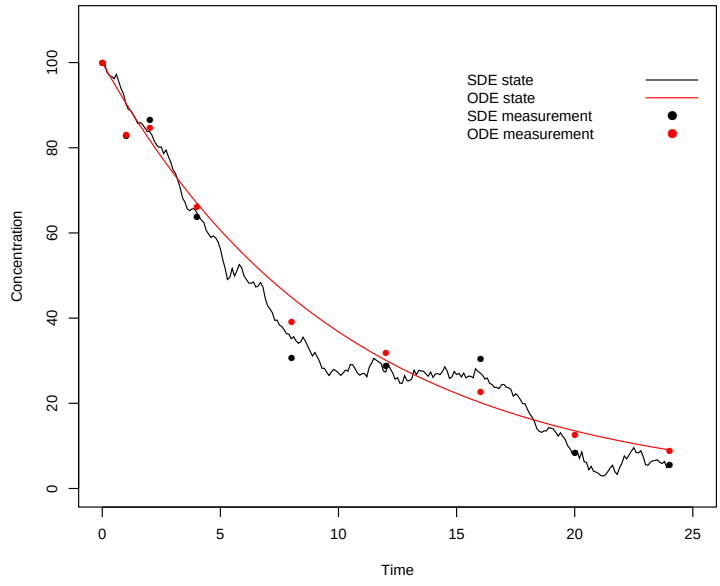

Figure 4.2: Simulation of a state-space model based on SDEs (black) and ODEs (red). States (–) and measurements (•).

between the structural model predictions and the observations, i.e.

$$d\boldsymbol{x}_{it} = \boldsymbol{g}(\boldsymbol{x}_{it}, \boldsymbol{d}_{it}, \boldsymbol{\phi}_i)\, dt + \boldsymbol{\sigma}_w\, d\boldsymbol{w}_{it}\,, \quad \boldsymbol{w}_{it} - \boldsymbol{w}_{is} \in N(\boldsymbol{0}, |t-s|\boldsymbol{I}) \tag{4.3}$$

$$\boldsymbol{y}_{ij} = \boldsymbol{f}(\boldsymbol{x}_{ij}, \boldsymbol{\phi}_i) + \boldsymbol{e}_{ij}\,, \quad \boldsymbol{e}_{ij} \in N(\boldsymbol{0}, \boldsymbol{\Sigma}) \tag{4.4}$$

where the subscript notation ij refers to individual i at time t_{ij}. The first-stage model in Eqs. (4.3)–(4.4) is identical to Eqs. (4.1)–(4.2) except for the dependency on the individual (i.e. denoted by subscript i).

The first-stage density is specified by [55]

$$p_1(\boldsymbol{\mathcal{Y}}_{in_i}|\boldsymbol{\phi}_i, \boldsymbol{\Sigma}, \boldsymbol{\sigma}_w, \boldsymbol{d}_i) = \left(\prod_{j=2}^{n_i} p(\boldsymbol{y}_{ij}|\boldsymbol{\mathcal{Y}}_{i(j-1)}, \cdot)\right) p(\boldsymbol{y}_{i1}|\cdot) \tag{4.5}$$

where $\boldsymbol{\mathcal{Y}}_{ij} = [\boldsymbol{y}_{i1}, \ldots, \boldsymbol{y}_{ij}]$ represents all observations of the i^{th} individual up to time t_{ij}, n_i are the total number of observations for individual i, while the conditioning on $\boldsymbol{\phi}_i$, $\boldsymbol{\Sigma}$, $\boldsymbol{\sigma}_w$, and $\boldsymbol{d}_i$ is represented by "$\cdot$".

Assuming that the conditional densities on the right hand side in Eq. (4.5) are well approximated by Gaussian densities, the first-stage distribution density is completely characterized by a sequence of means and covariances as specified by the right hand side of (4.5). The conditional densities describe the distribution of the following measurement conditioned on all the previous measurements, so that the mean and covariance of the conditional distribution is identical to the prediction and covariance of the following measurement, i.e. the one-step prediction $\hat{\boldsymbol{y}}_{i(j|j-1)}$ and its associated covariance $\boldsymbol{R}_{i(j|j-1)}$ [55]. Assuming a Gaussian density, it is completely described by

$$\hat{\boldsymbol{y}}_{i(j|j-1)} = E[\boldsymbol{y}_{ij}|\boldsymbol{\mathcal{Y}}_{i(j-1)}, \cdot] \tag{4.6}$$

$$\boldsymbol{R}_{i(j|j-1)} = V[\boldsymbol{y}_{ij}|\boldsymbol{\mathcal{Y}}_{i(j-1)}, \cdot] \tag{4.7}$$

where $\hat{\boldsymbol{y}}_{i(j|j-1)}$ and $\boldsymbol{R}_{i(j|j-1)}$ are the conditional mean and covariance, respectively, of $\boldsymbol{y}_{ij}$ conditioned on all previous measurements up to time $t_{i(j-1)}$ for individual i denoted by $\boldsymbol{\mathcal{Y}}_{i(j-1)} = [\boldsymbol{y}_{i1}, \ldots, \boldsymbol{y}_{i(j-1)}]$.

The one-step prediction errors are calculated by

$$\boldsymbol{\epsilon}_{ij} = \boldsymbol{y}_{ij} - \hat{\boldsymbol{y}}_{i(j|j-1)}\,, \qquad \boldsymbol{\epsilon}_{ij} \in N(\boldsymbol{0}, \boldsymbol{R}_{i(j|j-1)}) \tag{4.8}$$

The one-step predictions $\hat{\boldsymbol{y}}_{i(j|j-1)}$ and the associated covariance $\boldsymbol{R}_{i(j|j-1)}$ can be calculated recursively using the Extended Kalman Filter (EKF) (see Section 4.3 for further details).

The second-stage model describing the inter-individual variability (IIV) is included in the same way as for ODEs. The individual parameters $\boldsymbol{\phi}_i$ are modelled as

$$\boldsymbol{\phi}_i = \boldsymbol{h}(\boldsymbol{\theta}, \boldsymbol{Z}_i)\exp(\boldsymbol{\eta}_i)\,, \qquad \boldsymbol{\eta}_i \in N(\boldsymbol{0}, \boldsymbol{\Omega})\,, \qquad \boldsymbol{\phi}_i > 0 \tag{4.9}$$

where $\boldsymbol{h}(\cdot)$ denotes the structural type parameter model, which is a function of the fixed-effects parameters $\boldsymbol{\theta}$, covariates $\boldsymbol{Z}_i$, and random-effects parameters $\boldsymbol{\eta}_i$ influencing $\boldsymbol{\phi}_i$. This parameterization is chosen to constrain the individual parameters to be non-negative, which is appropriate for the models considered in this thesis. The random-effects $\boldsymbol{\eta}_i$ are realistically assumed independent and multivariate Gaussian distributed with zero mean and IIV covariance matrix $\boldsymbol{\Omega}$. The three levels of random-effects $\boldsymbol{w}_{it}$, $\boldsymbol{e}_{ij}$, and $\boldsymbol{\eta}_i$ are assumed mutually independent for all i, t, and j.

The marginal density of $\boldsymbol{\mathcal{Y}}_{in_i}$ is obtained from the conditional density of $\boldsymbol{\mathcal{Y}}_{in_i}$ given the random effects $\boldsymbol{\eta}_i$ and the marginal distribution of $\boldsymbol{\eta}_i$, i.e.

$$p(\boldsymbol{\mathcal{Y}}_{in_i}|\boldsymbol{\theta}, \boldsymbol{\Sigma}, \boldsymbol{\sigma}_w, \boldsymbol{\Omega}) = \int p_1(\boldsymbol{\mathcal{Y}}_{in_i}|\boldsymbol{\eta}_i, \boldsymbol{\theta}, \boldsymbol{\Sigma}, \boldsymbol{\sigma}_w, \boldsymbol{d}_i)\ p_2(\boldsymbol{\eta}_i|\boldsymbol{\Omega})\, d\boldsymbol{\eta}_i \tag{4.10}$$

where the conditional density of $\boldsymbol{\mathcal{Y}}_{in_i}$ given the random-effects $\boldsymbol{\eta}_i$ is denoted by $p_1(\boldsymbol{\mathcal{Y}}_{in_i}|\cdot)$ while $p_2(\boldsymbol{\eta}_i|\boldsymbol{\Omega})$ is the marginal distribution of $\boldsymbol{\eta}_i$.

The population likelihood function based on the marginal density in Eq. (4.10) can be written as the following product of integrals

$$\begin{aligned} L(\boldsymbol{\theta}, \boldsymbol{\Sigma}, \boldsymbol{\sigma}_w, \boldsymbol{\Omega}) &\propto \prod_{i=1}^{N} \int p_1(\boldsymbol{\mathcal{Y}}_{in_i}|\boldsymbol{\eta}_i, \boldsymbol{\theta}, \boldsymbol{\Sigma}, \boldsymbol{\sigma}_w, \boldsymbol{d}_i) p_2(\boldsymbol{\eta}_i|\boldsymbol{\Omega}) d\boldsymbol{\eta}_i \\ &= \prod_{i=1}^{N} \int \exp(\boldsymbol{l}_i)\, d\boldsymbol{\eta}_i \end{aligned} \tag{4.11}$$

where N is the number of individual and $\boldsymbol{l}_i$ is the *a posteriori* individual log-likelihood function (log of right hand side of Eq. (4.5)).

The integral in Eq. (4.11) does in general not have a closed-form expression when the first-stage model is non-linear in $\boldsymbol{\eta}$ and can therefore not be evaluated analytically [58]. Approximations therefore have to be made in order to be able to estimate the model parameters.

4.2.3 Approximations of the population likelihood function

The three main likelihood approximations available in NONMEM [7] are the Laplacian approximation, the first-order conditional estimation (FOCE) method, and the first-order (FO) method listed in order of decreasing accuracy.

The Laplacian method of evaluating the exact marginal likelihood consists of using a second-order Taylor expansion of $\boldsymbol{l}_i$ around the value of $\boldsymbol{\eta}_i$ which minimizes $\boldsymbol{l}_i$, i.e. the mode of the posterior distribution for $\boldsymbol{\eta}_i$ given $\boldsymbol{\theta}$, $\boldsymbol{\Sigma}$, $\boldsymbol{\sigma}_w$, and $\boldsymbol{\Omega}$ denoted by $\hat{\boldsymbol{\eta}}_i$ [7, 13, 82, 90].

Consider a second-order Taylor expansion of $\boldsymbol{l}_i$ around $\hat{\boldsymbol{\eta}}_i$, i.e.

$$\boldsymbol{l}_i \approx \boldsymbol{l}_i\big|_{\hat{\boldsymbol{\eta}}_i} + \frac{\partial \boldsymbol{l}_i}{\partial \boldsymbol{\eta}_i}\Big|_{\hat{\boldsymbol{\eta}}_i}^{T} (\boldsymbol{\eta}_i - \hat{\boldsymbol{\eta}}_i) + \frac{1}{2} (\boldsymbol{\eta}_i - \hat{\boldsymbol{\eta}}_i)^T \frac{\partial^2 \boldsymbol{l}_i}{\partial \boldsymbol{\eta}_i \partial \boldsymbol{\eta}_i^T}\Big|_{\hat{\boldsymbol{\eta}}_i} (\boldsymbol{\eta}_i - \hat{\boldsymbol{\eta}}_i) \tag{4.12}$$

where $\hat{\boldsymbol{\eta}}_i$ commonly is referred to as the empirical Bayes estimates. The integral

in Eq. (4.11) can be approximated using Eq. (4.12) by

$$\begin{aligned}\int \exp(\boldsymbol{l}_i)\, d\boldsymbol{\eta}_i &\approx \int \exp\left[\boldsymbol{l}_i + \nabla \boldsymbol{l}_i^T (\boldsymbol{\eta}_i - \hat{\boldsymbol{\eta}}_i) + \frac{1}{2} (\boldsymbol{\eta}_i - \hat{\boldsymbol{\eta}}_i)^T \Delta \boldsymbol{l}_i (\boldsymbol{\eta}_i - \hat{\boldsymbol{\eta}}_i)\right] d\boldsymbol{\eta}_i \\ &= \int \exp\left[\boldsymbol{l}_i + \nabla \boldsymbol{l}_i^T \boldsymbol{\eta}_i + \frac{1}{2} \boldsymbol{\eta}_i^T \Delta \boldsymbol{l}_i \boldsymbol{\eta}_i\right] d\boldsymbol{\eta}_i \\ &= \int \exp\left[\boldsymbol{l}_i + \frac{1}{2} \boldsymbol{\eta}_i \Delta \boldsymbol{l}_i^{-1} \boldsymbol{\eta}_i - \frac{1}{2} \nabla \boldsymbol{l}_i^T \Delta \boldsymbol{l}_i^{-1} \nabla \boldsymbol{l}_i\right] d\boldsymbol{\eta}_i \\ &= (2\pi)^{k/2} \left|\Delta \boldsymbol{l}_i\right|^{-1/2} \exp\left[\boldsymbol{l}_i - \frac{1}{2} \nabla \boldsymbol{l}_i^T \Delta \boldsymbol{l}_i^{-1} \nabla \boldsymbol{l}_i\right]\end{aligned} \tag{4.13}$$

where $\boldsymbol{\eta}_i$ is a k-dimensional random-effects vector, $\nabla \boldsymbol{l}_i = \frac{\partial \boldsymbol{l}_i}{\partial \boldsymbol{\eta}_i}\Big|_{\hat{\boldsymbol{\eta}}_i}$ is the gradient vector, and $\Delta \boldsymbol{l}_i = \frac{\partial^2 \boldsymbol{l}_i}{\partial \boldsymbol{\eta}_i \partial \boldsymbol{\eta}_i^T}$ is the Hessian matrix.

The gradient vector $\nabla \boldsymbol{l}_i$ of the *a posteriori* individual log-likelihood with respect to the random effects will vanish when the expansion is made around the true maximum since

$$\frac{\partial \boldsymbol{l}_i}{\partial \boldsymbol{\eta}_i}\Big|_{\hat{\boldsymbol{\eta}}_i} = 0 \tag{4.14}$$

The Laplacian approximation of the population likelihood function in Eq. (4.11) thereby becomes

$$L(\boldsymbol{\theta}, \boldsymbol{\Sigma}, \boldsymbol{\sigma}_w, \boldsymbol{\Omega}) \propto \prod_{i=1}^{N} \int \exp(\boldsymbol{l}_i)\, d\boldsymbol{\eta}_i \propto \prod_{i=1}^{N} |\Delta \boldsymbol{l}_i|^{-1/2} \exp(\boldsymbol{l}_i)\Big|_{\hat{\boldsymbol{\eta}}_i} \tag{4.15}$$

Assuming a Gaussian conditional density for the first-stage distribution density, the individual *a posteriori* log-likelihood function $\boldsymbol{l}_i$ and its Hessian $\Delta \boldsymbol{l}_i$ are calculated by

$$\boldsymbol{l}_i = -\frac{1}{2} \sum_{j=1}^{n_i} \left(\boldsymbol{\epsilon}_{ij}^T \boldsymbol{R}_{i(j|j-1)}^{-1} \boldsymbol{\epsilon}_{ij} + \log |2\pi \boldsymbol{R}_{i(j|j-1)}|\right) - \frac{1}{2} \boldsymbol{\eta}_i^T \boldsymbol{\Omega}^{-1} \boldsymbol{\eta}_i - \frac{1}{2} \log |2\pi \boldsymbol{\Omega}| \tag{4.16}$$

and

$$\Delta \boldsymbol{l}_i = \sum_{j=1}^{n_i} \left[\frac{\partial^2 \boldsymbol{\epsilon}_{ij}}{\partial \boldsymbol{\eta} \partial \boldsymbol{\eta}^T}\Big|_{\hat{\boldsymbol{\eta}}_i} \boldsymbol{\epsilon}_{ij} \boldsymbol{R}_{i(j|j-1)}^{-1} - \frac{\partial \boldsymbol{\epsilon}_{ij}}{\partial \boldsymbol{\eta}}\Big|_{\hat{\boldsymbol{\eta}}_i}^T \boldsymbol{R}_{i(j|j-1)}^{-1} \frac{\partial \boldsymbol{\epsilon}_{ij}}{\partial \boldsymbol{\eta}}\Big|_{\hat{\boldsymbol{\eta}}_i}\right] - \boldsymbol{\Omega}^{-1} \tag{4.17}$$

The key element in the evaluation of the population likelihood function in Eq. (4.15) is thus the Hessian matrix $\Delta \boldsymbol{l}_i$. The numerical evaluation of second-order partial derivatives to form the Hessian is usually quite sensitive leading to uncertainty in the objective function and to optimization problems.

The contribution of the second-order partial derivatives in the Hessian matrix is usually negligible compared to that of the square of the first-order partial derivatives since the second-order Taylor expansion in Eq. (4.12) is made around the value of $\boldsymbol{\eta}_i$ which minimizes $\boldsymbol{l}_i$. The terms involving second-order partial derivatives in Eq. (4.17) are therefore disregarded in the FOCE method [7] and the Hessian is approximated by

$$\Delta \boldsymbol{l}_i \approx -\sum_{j=1}^{n_i} \nabla \boldsymbol{\epsilon}_{ij}^T \boldsymbol{R}_{i(j|j-1)}^{-1} \nabla \boldsymbol{\epsilon}_{ij} - \boldsymbol{\Omega}^{-1} \tag{4.18}$$

where $\nabla \boldsymbol{\epsilon}_{ij} = \frac{\partial \boldsymbol{\epsilon}_{ij}}{\partial \boldsymbol{\eta}}\big|_{\hat{\boldsymbol{\eta}}_i}$ is the gradient vector of the one-step prediction error $\boldsymbol{\epsilon}_{ij}$ with respect to the random effects $\boldsymbol{\eta}$, respectively.

The objective function of the FOCE method thereby becomes

$$-2 \log L(\boldsymbol{\theta}, \boldsymbol{\Sigma}, \boldsymbol{\sigma}_w, \boldsymbol{\Omega}) \propto \sum_{i=1}^{N} \Big[\log \big| \Delta \boldsymbol{l}_i \big| - 2 \boldsymbol{l}_i \Big] \tag{4.19}$$

The simplest (and least accurate) approximation of the population likelihood function is the FO method where the first-order Taylor expansion of the likelihood function is made around the population parameter values, i.e. $\hat{\boldsymbol{\eta}} = 0$.

The iterative FOCE method is repeated until convergence of the numerical minimization routine using the following algorithm.

- The maximum likelihood (ML) estimates of the population parameters are those values of $\boldsymbol{\theta}$, $\boldsymbol{\Sigma}$, $\boldsymbol{\sigma}_w$, and $\boldsymbol{\Omega}$ that minimizes the objective function in Eq. (4.19) (i.e. maximizes the likelihood in Eq. (4.15)) denoted by $\hat{\boldsymbol{\theta}}$, $\hat{\boldsymbol{\Sigma}}$, $\hat{\boldsymbol{\sigma}}_w$, and $\hat{\boldsymbol{\Omega}}$, i.e.

$$\big(\hat{\boldsymbol{\theta}}, \hat{\boldsymbol{\Sigma}}, \hat{\boldsymbol{\sigma}}_w, \hat{\boldsymbol{\Omega}}\big) = \arg \min_{\boldsymbol{\theta}, \boldsymbol{\Sigma}, \boldsymbol{\sigma}_w, \boldsymbol{\Omega}} \Big\{ -2 \log L \left(\boldsymbol{\theta}, \boldsymbol{\Sigma}, \boldsymbol{\sigma}_w, \boldsymbol{\Omega}\right) \Big\} \tag{4.20}$$

- For each value of $\hat{\boldsymbol{\theta}}$, $\hat{\boldsymbol{\Sigma}}$, and $\hat{\boldsymbol{\Omega}}$ produced by the minimization routine, the inter-individual random-effects parameters $\boldsymbol{\eta}$ are estimated by their posterior modes by maximizing Eq. (4.16).

A measure of the uncertainty of the final parameter estimates can be calculated using Fisher's information matrix using the inverse Hessian matrix for the log-likelihood function.

Further information about the FOCE method as implemented in NONMEM can be found in NONMEM Users Guide - part VII [7].

4.3 Extended Kalman Filter

The EKF [36, 37] provides an efficient recursive algorithm to calculate the conditional mean and covariance for the assumed Gaussian conditional densities needed to evaluate the FOCE objective function in Eq. (4.19) [28, 52].

The EKF equations can be grouped into two parts, i.e. prediction and update equations. The prediction equations predict the state and output variables one-step ahead (i.e. until the next measurement) while the update equations update the state predictions with the newly obtained measurement.

The one-step *state prediction equations* of the EKF, which are the optimal (minimum variance) prediction of the mean and covariance, can be calculated by solving the state and state covariance prediction equations from measurement time t_{j-1} until t_j, i.e.

$$\frac{d\hat{\boldsymbol{x}}_{i(t|j-1)}}{dt} = \boldsymbol{g}(\hat{\boldsymbol{x}}_{i(t|j-1)}, \boldsymbol{d}_i, \boldsymbol{\phi}_i)\,, \qquad t \in [t_{j-1}, t_j] \quad (4.21)$$

$$\frac{d\boldsymbol{P}_{i(t|j-1)}}{dt} = \boldsymbol{A}_{it}\boldsymbol{P}_{i(t|j-1)} + \boldsymbol{P}_{i(t|j-1)}\boldsymbol{A}_{it}^T + \boldsymbol{\sigma}_w\boldsymbol{\sigma}_w^T\,, \; t \in [t_{j-1}, t_j] \quad (4.22)$$

with initial conditions as specified by the EKF update equations (see Eqs. (4.29)–(4.30)). Initially, the starting conditions are

$$\hat{\boldsymbol{x}}_{i(1|0)} = \boldsymbol{x}_{i0} \quad (4.23)$$

$$\boldsymbol{P}_{i(1|0)} = \boldsymbol{P}_{i0} = \int_{t_1}^{t_2} e^{\boldsymbol{A}_{it}\,s}\boldsymbol{\sigma}_w\boldsymbol{\sigma}_w^T(e^{\boldsymbol{A}_{it}\,s})^T\,ds \quad (4.24)$$

where t_1 and t_2 are the sampling times of the two first measurements while $\boldsymbol{x}_{i0}$ are the initial states, which can be pre-specified or estimated along with the other model parameters. The integral in Eq. (4.24) specifying the initial state covariance $\boldsymbol{P}_{i0}$ is taken as the integral of the Wiener process and the dynamics of the system over the time difference between the first two measurements. This

initialization scheme has proven to be successful in other software implementations [46].

The EKF is an exact solution to the state filtering problem for linear systems, while it is a first-order approximative filter for non-linear systems. Hence, the $\boldsymbol{A}_{it}$ matrix is calculated for non-linear systems by linearizing the state equations in Eq. (4.21) using a local first-order Taylor expansion of $\boldsymbol{g}(\cdot)$ about the current state at each time instant t, i.e.

$$\boldsymbol{A}_{it} = \frac{\partial \boldsymbol{g}}{\partial \boldsymbol{x}}\Big|_{\boldsymbol{x}=\hat{\boldsymbol{x}}_{i(t|j-1)}} \tag{4.25}$$

Next, the EKF one-step *output prediction equations* are calculated by

$$\hat{\boldsymbol{y}}_{i(j|j-1)} = \boldsymbol{f}(\boldsymbol{\phi}_i, \hat{\boldsymbol{x}}_{i(j|j-1)}) \tag{4.26}$$

$$\boldsymbol{R}_{i(j|j-1)} = \boldsymbol{C}_{ij}\boldsymbol{P}_{i(j|j-1)}\boldsymbol{C}_{ij}^T + \boldsymbol{\Sigma} \tag{4.27}$$

where $\boldsymbol{C}_{ij}$ is obtained using a local first-order Taylor expansion of the measurement equation in Eq. (4.4), i.e.

$$\boldsymbol{C}_{ij} = \frac{\partial \boldsymbol{f}}{\partial \boldsymbol{x}}\Big|_{\boldsymbol{x}=\hat{\boldsymbol{x}}_{i(j|j-1)}} \tag{4.28}$$

The one-step output prediction covariance $\boldsymbol{R}_{i(j|j-1)}$ is thus the sum of the state covariance associated with the observed states ($\boldsymbol{C}_{ij}\boldsymbol{P}_{i(j|j-1)}\boldsymbol{C}_{ij}^T$) and the covariance of the actual measurement ($\boldsymbol{\Sigma}$). In case of no system noise ($\boldsymbol{\sigma}_w = 0$), the one-step output prediction $\hat{\boldsymbol{y}}_{i(j|j-1)}$ and covariance $\boldsymbol{R}_{i(j|j-1)}$ will reduce to the ODE predictions $\hat{\boldsymbol{y}}_{ij}$ and residual covariance $\boldsymbol{\Sigma}$ typically used in the NONMEM likelihood function.

Finally, the one-step state and state covariance prediction estimates are updated by conditioning on the j^{th} measurement using the EKF *state update equations*, i.e.

$$\hat{\boldsymbol{x}}_{i(j|j)} = \hat{\boldsymbol{x}}_{i(j|j-1)} + \boldsymbol{K}_{ij}\big(\boldsymbol{y}_{ij} - \hat{\boldsymbol{y}}_{i(j|j-1)}\big) \tag{4.29}$$

$$\boldsymbol{P}_{i(j|j)} = \boldsymbol{P}_{i(j|j-1)} - \boldsymbol{K}_{ij}\boldsymbol{R}_{i(j|j-1)}\boldsymbol{K}_{ij}^T \tag{4.30}$$

where $\hat{\boldsymbol{x}}_{i(j|j)}$ is the updated state estimate, $\boldsymbol{P}_{i(j|j)}$ is the updated state covariance, and the Kalman gain $\boldsymbol{K}_{ij}$ is calculated by

$$\boldsymbol{K}_{ij} = \boldsymbol{P}_{i(j|j-1)}\boldsymbol{C}_{ij}^T\boldsymbol{R}_{i(j|j-1)}^{-1} \tag{4.31}$$

The optimal state estimate at time j denoted by $\hat{\boldsymbol{x}}_{i(j|j)}$ is equal to the best state prediction $\hat{\boldsymbol{x}}_{i(j|j-1)}$ before the measurement is taken plus a correction term consisting of an optimal weighting value times the difference between the measurement $\boldsymbol{y}_{ij}$ and the one-step prediction of its value. For measurements with a large variance $\boldsymbol{\Sigma}$, the Kalman gain becomes small and the measurement is weighted lightly due to the little confidence in the noisy measurement. In the limit as $\boldsymbol{\Sigma} \to \infty$, the Kalman gain becomes zero and the infinitely noisy measurement is completely ignored in the update. When the system noise is dominant implying uncertainty in the output of the system model, the measurement is heavily weighted. In the limit when $\boldsymbol{\sigma}_w \to \infty$ and $\boldsymbol{P} \to \infty$, the Kalman gain will approach 1 and the updated state will be equal to the measurement [52].

The EKF algorithm specified above and illustrated in Figure 4.3 is recursive by repeating the calculations of the one-step state and output prediction equations in Eqs. (4.21)–(4.28) as well as the state update equations in Eqs. (4.29)–(4.31) for each individual measurement.

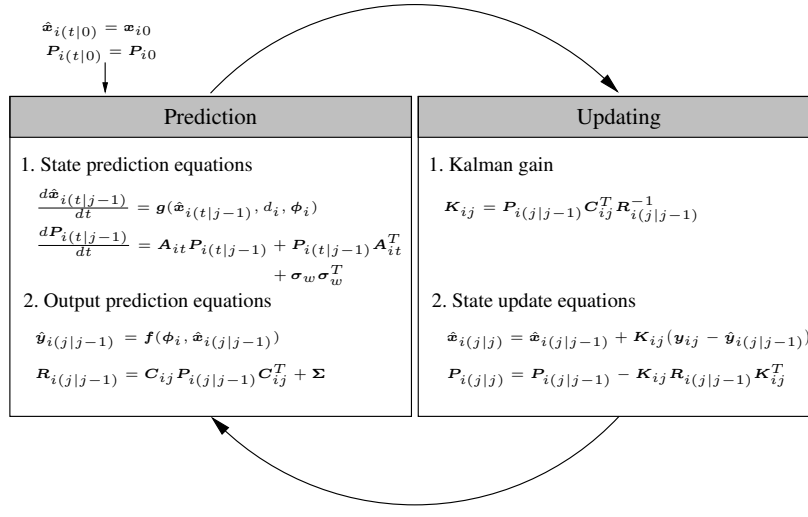

Figure 4.3: Schematic illustration of the EKF algorithm [87].

The EKF, being a linear filter, is sensitive to non-linear effects, which may result in the approximation being too crude [28]. One might consider to use subsampling[1] where the EKF equations are linearized at each subsampling instant to obtain a better approximation [47].

Several other more sophisticated statistical techniques (e.g. higher-order filters,

[1] The time interval between two measurements is divided into several subsampling instants.

particle filters, estimation functions, etc.) exist for evaluating the conditional densities in the likelihood function [54]. However, these methods increase the computational burden considerably compared to the EKF and therefore not considered in this thesis for the implementation in non-linear mixed-effects modelling.

4.4 Systematic population PK/PD model building framework

Non-linear mixed-effects modelling based on SDEs provides an appealing tool for systematic population PK/PD model development.

The main idea behind the systematic population PK/PD model building framework is to iteratively improve the model by systematically pinpointing model deficiencies. This is done by formulating a simple model structure and systematically expand it with models of increasing complexity in a manner, which is consistent with prior physiological knowledge and supported by the available data. The iterative process is continued until the model is accepted for a given purpose. The time spend on developing population PK/PD models can thereby be reduced dramatically [44, 45].

In this thesis, the proposed method is used for tracking time-varying parameters (see Paper IV), to identify dynamic dependencies, and to deconvolve functional feedback interactions of complicated physiological systems (see Paper V).

The iterative framework for systematic model development is visualized in Figure 4.4 and summarized in the algorithm below [44, 45].

Step 1 Formulate an initial ODE model derived using *a priori* knowledge about the modelled system.

Step 2 Transform the ODE model into an SDE model with a diagonal diffusion term to be able to pinpoint model deficiencies.

Step 3 Estimate the model parameters using the FOCE method together with the EKF algorithm described in Sections 4.2–4.3.

Step 4 Identify possible model misspecifications by examining the significance of the estimated diffusion terms as well as the one-step prediction error. The iterative model development is terminated if the model is accepted for the intended purpose. If the model is rejected, continue with Steps 5 to 9.

Step 5 Extend the model with state equations for the pinpointed model deficiencies.

Step 6 Estimate extended model parameters and obtain updated EKF state estimates.

Step 7 Evaluate model and repeat Steps 5 and 6 using e.g. different parameterizations until satisfactory results are obtained.

Step 8 Track unexplained variations using the updated EKF state estimates from the extended model.

Step 9 Apply non-parametric modelling methods such as general additive modelling (GAM) [24] to deconvolve functional relationship. Use the obtained information to reformulate the model and return to Step 2.

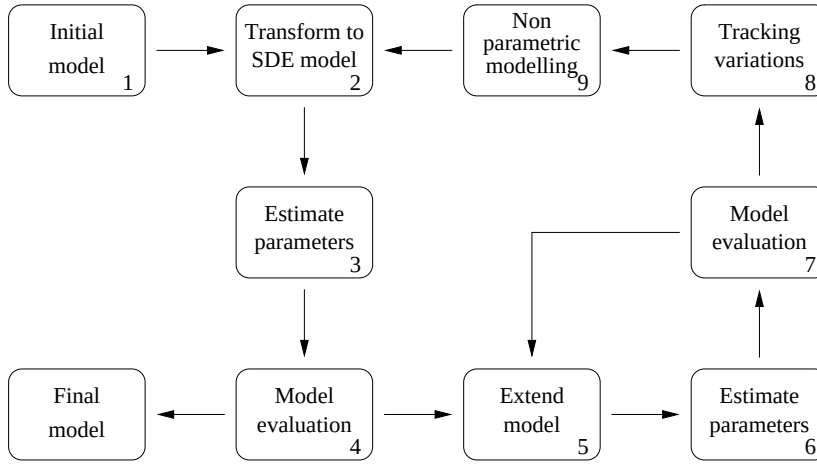

Figure 4.4: Systematic PK/PD model building framework [45].

The above mentioned approach based on SDEs is similar to the method described by Fattinger *et al.* in [19] using flexible non-parametric functions (i.e. natural cubic splines). The main difference between the two is that the SDE framework can be used to deconvolve dynamic dependencies of unknown states, inputs, and/or parameters whereas the spline method cannot. The natural cubic spline approach is, however, relative straightforward to use if the dependencies are known and only the form of the functional interactions is to be deconvolved.

Chapter 5

Materials & Methods

5.1 Compounds

5.1.1 Triptorelin

Triptorelin (pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH2) is a synthetic GnRH agonist with a longer half-life and a higher potency compared to the naturally occurring GnRH. Compared to natural GnRH, triptorelin has the amino acid L-Glycin in position 6 replaced by D-tryptophan, which increases the affinity to the GnRH receptors in the pituitary [5].

Triptorelin is the active ingredient of GnRH agonist Decapeptyl®. Decapeptyl® is available as a sustained release formulation called Decapeptyl Depot® [41]. Decapeptyl Depot® consists of two components, i.e. microparticles including the active ingredient, triptorelin-acetate embedded in a biologically degradable polymer matrix with a residual amount of microparticles hardener, Miglyol, and the suspension medium, which is an isotonic, phosphate-buffered aqueous solution containing Polysorbate 80 and an agent for increasing viscosity.

5.1.2 Degarelix

Degarelix (Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph (L-hydroorotyl)-D-4Aph (carbamoyl)-Leu-ILys-Pro-D-Ala-NH2) is a new long-acting GnRH antagonist currently being developed for prostate cancer treatment with high affinity and selectivity for GnRH receptors showing high water solubility and low histamine-releasing properties *in vitro* [10, 15, 29]. After SC administration, degarelix spontaneously forms a gel-like depot at high dose concentrations (in the mg/mL range) when it comes into contact with body fluids (see Figure 6.1 on page 42). The self-forming depot results in a sustained release of degarelix.

5.2 Analytical methods

Triptorelin and degarelix plasma concentrations were measured according to Good Laboratory Practice (GLP) by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) validated according to current guidelines for bioanalytical samples [65]. The lower limit of quantification (LLOQ) for the triptorelin and degarelix assays were 0.01 ng/mL and 0.5 ng/mL, respectively. The LH assay was based on the Microparticle Enzyme Immunoassay (MEIA) technology (Abbot Laboratories, IL). The LLOQ of the LH assay was 0.1 IU/L. Total serum testosterone concentrations were measured according to GLP by LC-MS/MS after solid-phase extraction. The LLOQ of the assay was 0.05 ng/mL.

5.3 Overview of studies

An overview of the studies used in the PK/PD data analysis described in Papers I and III–V is provided in the following and summarized in Table 5.2 on page 36.

5.3.1 Triptorelin study

The CS001 study was designed as a phase I, randomized, parallel group, single dose study. The objectives were to investigate the PK and PD of triptorelin after a single SC or IM injection of a new Decapeptyl Depot® one-month formulation in healthy male subjects. The triptorelin PK/PD data of CS001 was used for the mechanism-based modelling of the HPG axis in Paper V.

5.3.2 Degarelix studies

The CS01 study was the first in man degarelix study. The objective was to investigate the PK of degarelix in healthy male subjects in a phase I with-in group, randomized, placebo controlled, double blind dose-escalation study. The following dose levels were included in the study: placebo, 0.5, 2.0, 5.0, 10, 30 and 40 mg/subject at concentrations ranging from 5 to 30 mg/mL in the dosing solution. The degarelix PK data of CS01 was used in Paper I to model the dose-volume and dose-concentration dependent degarelix absorption from a SC depot.

The CS02 study was the first degarelix study in PC patients. It was designed as a six month, multi-center, open-labeled, 1:1:1 randomized, parallel group phase II study investigating the efficacy and safety of three dose regimens of degarelix in PC patients. The main objectives were to select a dosing regimen that would result in a) testosterone < 0.5 ng/mL in at least 70% of patients at 1 week from initial dosing and b) testosterone < 0.5 ng/ml in at least 90% of patients at 2, 4, 8, 12, 16, 20, and 24 weeks from initial dosing. Three different initiation regimens and two different maintenance treatments were tested (see Table 5.1). The initiation dose was administered either as a dose on day 0 and day 3, or as a single dose on day 0, while the maintenance dose was administered every four weeks. The degarelix PK data of CS02 was used to illustrate the application of SDEs for systematic population PK/PD model building presented in Paper IV.

Table 5.1: Dosing regimens in degarelix CS02 study.

Group	Day 0	Day 3	Maintenance
40/40/40	40 mg	40 mg	40 mg every four weeks
80/ – /20	80 mg	0 mg	20 mg every four weeks
80/80/40	80 mg	80 mg	40 mg every four weeks

The main objectives of the CS05 study was to assess safety, tolerability, and PK of degarelix in healthy male subjects. The study was designed as an open-label, dose-escalating single dose phase I study of degarelix given as an IV infusion. The following dose levels were included in the study: IV infusions of 1.5, 6, 15 and 30 μg/kg using a dosing solution concentration of 5 μg/mL. The infusion lasted 15 minutes in the two lowest dosing groups and 45 minutes in the two highest dosing groups. The CS05 study was used for the degarelix PK/PD modelling described in Papers I and III.

The objective of CS07 was to investigate the pharmacological effects of ascending single doses of degarelix administered subcutaneously to PC patients in

terms of testosterone suppression. The study design was an open-label, multicenter, parallel and sequential, ascending single dose phase II study investigating the PK, PD, and safety of degarelix in PC patients (PCP). The degarelix PK/PD CS07 data was used together with the triptorelin PK/PD CS001 data for the mechanism-based modelling of the HPG axis in Paper V.

Table 5.2: Summary of studies used in the PK/PD data analysis. The numbers in brackets are the number of samples below LLOQ.

Study	Design	Subjects	PK samples		LH samples		Te samples	
Triptorelin study								
CS001	Single SC/IM doses	58 HS	1152	(35)	1330	(0)	1300	(0)
Degarelix studies								
CS01	Single SC dose	80 HS	1484	(416)	955	(0)	1151	(9)
CS02	Repeated SC doses	129 PCP	2919	(137)	3110	(307)	3110	(336)
CS05	Single IV infusion	24 HS	516	(144)	386	(49)	386	(3)
CS07	Repeated SC doses	170 PCP	4404	(22)	4826	(207)	4827	(223)

PK, pharmacokinetic; LH, luteinizing hormone; Te, testosterone; HS, healthy subjects; PCP, prostate cancer patients.

5.4 Data analysis

5.4.1 Hardware and software

A Linux cluster consisting of 2 HP ProLiant DL360 G3 Intel Xeon with 2 x 3.06 GHz processors and 1 GB of RAM running Red Hat Linux v. 8, openMosix, and GNU Fortran compiler g77 was the main platform used for the population PK/PD analysis.

The population PK/PD modelling was primarily performed using NONMEM version V and VI beta [7] whereas NLME [59] was used for more exploratory purposes.

5.4.2 Population PK/PD analysis

All population PK/PD analysis were performed sequentially according to the individual PK parameters (IPP) method in [93]. The PK parameters were first

estimated using only the PK data. The PD parameter estimates were then obtained using the PD data and conditioning on the empirical Bayes estimates of the individual PK parameters. The reason for using a sequential instead of a simultaneous approach was mainly due to long estimation times. Furthermore, the PD were not expected to influence the PK in any of the developed PK/PD models.

Two different methods were used to handle PK and PD samples below LLOQ in the estimation of model parameters because of the different concentration-time profiles. The first time a PK measurement was below LLOQ it stayed below for the remainder of the study whereas the LH and testosterone measurements fluctuated around LLOQ throughout the study. Thus, triptorelin and degarelix plasma concentration measurements below LLOQ were omitted in the estimation (method M1 in [6]), while LH and testosterone concentration measurements below LLOQ were set to LLOQ/2 (method M5 in [6]).

The population PK/PD analysis was typically carried out in three interwoven steps with an initial exploratory data analysis followed by an iterative model development process and finally model validation [71].

5.4.2.1 Exploratory data analysis

Exploratory data analysis was used to investigate patterns and relationships in the PK/PD data by graphical and statistical analysis. Initial assumptions and hypotheses could thereby be confirmed/rejected before the complicated and time consuming PK/PD model development.

5.4.2.2 Model development

The model development was driven by the aim of building a PK/PD model that provides a satisfactory description of the PK/PD data while at the same time is feasible to work with. Thus, a minimal-model approach starting from the simplest model and expanding it until no more terms could be justified was applied to be able to estimate all model parameters, while at the same time reducing the computer run-times.

The three population PK/PD sub-models (i.e. structural, statistical, and covariate sub-models illustrated in Figure 2.2 on page 11) were developed in the following order.

1. Identify the simplest structural sub-model.

2. Identify the statistical sub-models that incorporates the random-effects.
   (a) Choose between an additive, proportional, or a combined additive and proportional residual error model based on graphical analysis of residual plots.
   (b) Apply an exponential parameter model using a diagonal IIV matrix. Test the statistical relevance of including each inter-individual random-effects and off-diagonal parameters.
3. Incorporate statistical significant and clinical relevant covariate-parameter relationships.

The three sub-models overlap as indicated in Figure 2.2 on page 11 symbolizing that a change in one of the sub-models could lead to changes in the other sub-models as investigated by Wade *et al.* in [83]. Generally, several loops of the above model building algorithm were therefore required before arriving at the final model.

The FO method was used for parameter estimation in the initial population PK/PD model building, while the FOCE method was applied during the final stages of the development process. When feasible, the structural sub-model was developed using the systematic PK/PD model building framework presented in Section 4.4 together with prior pharmacological and physiological knowledge. The covariate model building was performed with the stepwise covariate modelling (SCM) module in Perl-Speaks-NONMEM (PsN) [50] using the method described by Jonsson *et al.* in [31].

5.4.2.3 Model discrimination

Model selection was based on an understanding of the physiological system and graphical analysis using basic goodness-of-fit (GOF) plots of e.g. individual and population predictions vs. time, observed concentrations, and weighted residuals. The difference in the objective function value (OFV) produced by NONMEM was furthermore used to discriminate between hierarchical (nested) models using the likelihood ratio test (LRT). For a one parameter difference, the ΔOFV value is approximately χ^2-distributed with $\Delta\text{OFV} > 3.84$ being significant on a 5% significance level [84].

5.4.2.4 Model validation

Model validation was performed to examine the ability of the final model to describe and predict the data with which it was developed as well as data from

other studies. Depending on the objectives of the analysis, different approaches were applied including validation methods such as diagnostic checks by graphical analysis [32], bootstrapping [18], and posterior predictive check [92].

Chapter 6

Results & Discussion

This chapter provides an overview and a discussion of the most important results documented in Papers I–V. The results are presented according to the specific aims of the thesis (see Section 1.2) and divided into the following topics.

- Population PK modelling of SC and IM depots (Section 6.1)
- Implementation and application of SDEs in non-linear mixed-effects modelling (Section 6.2)
- Mechanism-based PK/PD modelling of the HPG axis (Section 6.3)

6.1 Population PK modelling of SC and IM depots

6.1.1 Degarelix PK model (Papers I, IV, and V)

Degarelix is administered as a suspension, which forms a sustained release depot after SC injections. The depot formation is the result of immediate gelling of degarelix once in contact with the SC interstitial environment (see Figure 6.1).

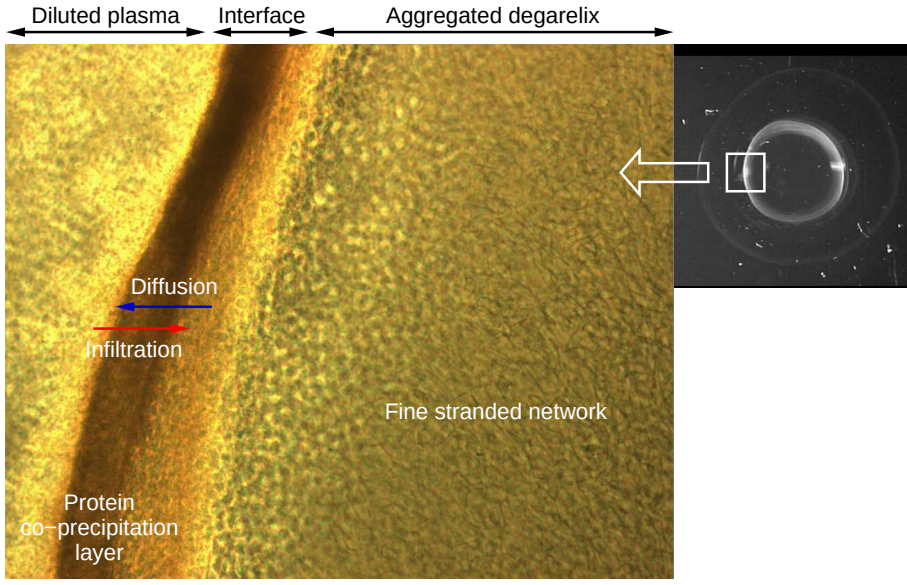

Figure 6.1: *In vitro* micro-depot of degarelix.

Degarelix is released from the depot in two distinct phases; a fast release phase right after dosing accounting for the high initial plasma concentration levels, and a slow release phase, which determines the plasma concentration levels in the maintenance phase (see Figure 6.2).

Several different factors affect the formation and the viscosity of the gel, and hence the SC release profile of degarelix. In the degarelix CS01 study, a decrease in the bioavailability was observed when the concentration in the dosing solution was increased from 5 to 30 mg/mL. Furthermore, the volume of the injected solution seemed to influence the release of degarelix from the depot; where a faster release was observed for smaller injection volumes.

A population PK model for SC administered degarelix was developed in Paper I to better understand and quantify the factors controlling the release from the SC depot. The influence of the concentration and volume of the dosing solution was investigated by assuming that the SC release could be modelled as diffusion out of a spherical SC depot. The model was derived from Fick's second law of diffusion [20] and described by a partial differential equation (PDE) (see Eq. (4) in Paper I). In order to estimate the parameters in NONMEM, it was necessary to implement an approximation of the spherical SC depot model reducing the PDE to a system of ODEs. This was done by spatial discretization of the sphere into concentric spherical shells with spatially constant flow.

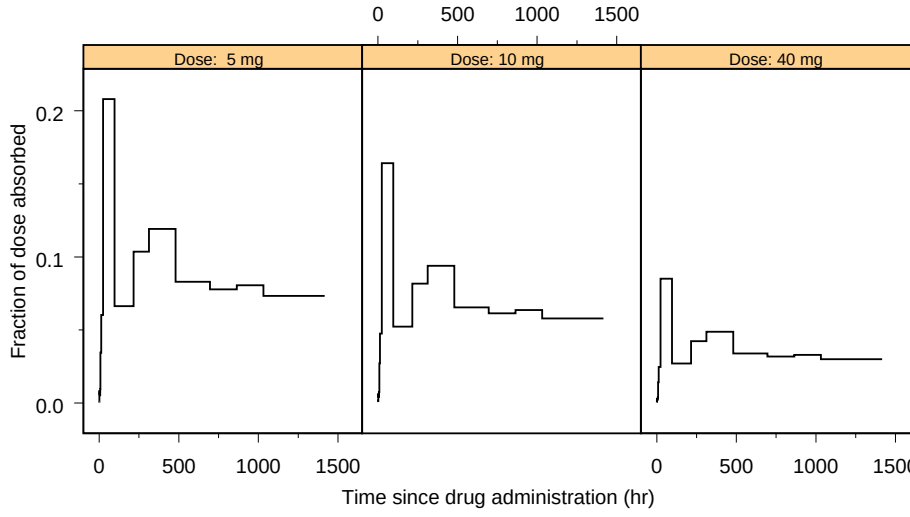

Figure 6.2: Deconvolved SC release profile for three different dose groups in the degarelix CS01 study using a flexible zero-order input model [49].

The analytical solution to the PDE was derived using theory for Sturm-Liouville problems [4] to be able to assess the error made by approximating the sphere with two concentric spherical shells. The SC depot concentration as a function of the radial distance from the core of the depot and the time since drug administration is shown in Figure 6.3 for the lowest dose group receiving a dose of 0.5 mg using a dose-volume of 0.1 mL and a dose-concentration of 5 mg/mL.

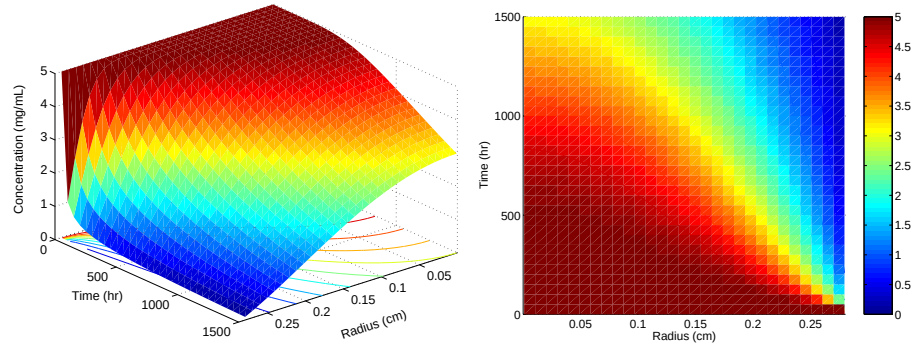

Figure 6.3: SC depot concentration as a function of radial distance from the core of the sphere (r=0 cm) to the surface (r=0.28 cm) and time since drug administration viewed from two different angles.

The spatial discretization using two spherical shells was found acceptable compared to the analytical solution when examining the SC depot concentration and exchange rate (Figures 4 and 5 in Paper I). Attempts to increase the number of shells in the SC depot model were made but it did not improve the predictions of the degarelix plasma concentrations since no concentration measurements within the SC depot were available for the estimation of model parameters.

Since bootstrapping [18] was too time consuming, the model was validated by applying the method proposed by Jonsson and Sheiner [33]. A total of 100 trials with the same design as the CS01 study were simulated. The simulated plasma degarelix concentration values at each time point were divided up into the lower 25% quantile, the middle 25%-75% quantile, and the upper 75% quantile. At each time point, the median of those three groups were calculated. This procedure was repeated for each of the 100 simulated trials and for the observed plasma degarelix concentrations. The results are shown in Figure 6.4 where the black lines are the 100 median simulated plasma degarelix concentrations while the superimposed red lines are the median observed plasma degarelix concentrations. The model seems to capture the behaviour of the observed data very well and was therefore accepted as the final model.

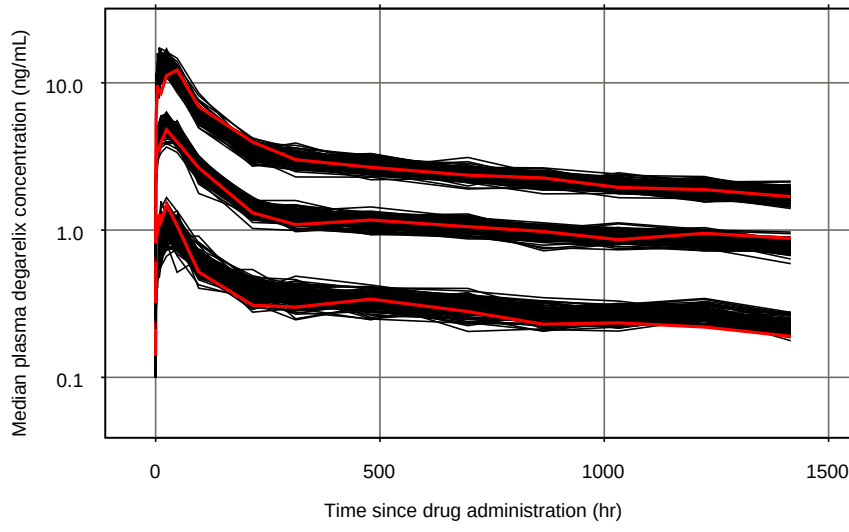

Figure 6.4: Median degarelix plasma concentrations for the lower 25%, middle 25%-75%, and upper 75% quantiles of the 100 simulated (black lines) and observed (red lines) data.

The bioavailability was estimated to decrease with increasing dose-concentration

(see Figure 7 in Paper I). The bioavailability was strangely enough estimated to be above 100% (i.e. 163%) for the group in the degarelix CS01 study receiving the lowest dose-concentration of 5 mg/mL, while the bioavailability for highest dose-concentration group of 30 mg/mL was estimated to be 36%. These estimates of the bioavailability were however in agreement with non-compartmental analysis (NCA) [75]. The explanation for estimating a bioavailability above 100% is believed to be due to uncertainty about the exact dose received, different analytic methods used in the degarelix CS01 and CS05 studies, and because of the high subject variation due to the very different study designs of the parallel SC (CS01) and IV (CS05) studies.

The dose-volume effect on the SC release was modelled by a B-spline basis [14] using a piecewise linear function to relate the unmeasured effective depot-volume to the SC injected dose-volume. The results indicated diminishing dose-volume effects at injection volumes of 1 mL and above with a maximum effective dose-volume estimate of 168% relative to the lowest dose-volume of 0.1 mL. The reason for observing diminishing dose-volume effects at larger injection volumes might be due to the formation of the SC depot, which undergoes a maturation stage where the density of the gel increases and the release rate decreases. At large dose-volumes, the maturation stage takes longer time, which results in faster diffusion out of the depot until the formation of a rigid gel. It should be noted that the degarelix CS01 study was not intended for determining a dose-volume effect on the SC release, and the design was therefore not balanced at the low dose-volumes.

The SC depot model was simplified in Paper IV since the clinical relevant SC degarelix doses would require dose-volumes larger than 1 mL. The dose-volume effect on the SC release was therefore assumed to level off at injection volumes above 1 mL, and hence only to a minor degree influence the PK of degarelix if the dose-volume would be increased beyond these levels. The new PK model was investigated using the systematic PK/PD model building framework presented in Section 4.4 by tracking variations in the absorption half-life parameter $t_{1/2,abs}$ (see Figure 1 in Paper IV). The tracked absorption half-life remained constant around 245 hr until approximately 3 days after drug adminstration where it increased to a new level for the remainder of the study. As a reasonable approximation, the SC depot was modelled with two first-order absorption components, i.e. an initial fast absorption followed by a prolonged slow release from the depot (see Figure 2 in Paper IV).

The degarelix PK model in Paper IV was further developed in Paper V using data from the degarelix CS05 and CS07 studies. A two-compartment disposition model with two first-order absorption components was used to describe the concentration-time profiles of SC administered degarelix (see Figure 1 in Paper V). Three of the PK parameters were identified from initial covariate analysis to vary with the concentration in the dosing solution. The bioavailability (F), the

slow absorption half-life ($t_{1/2,slow}$), and the fraction of the dose being absorbed via the fast absorption route (Fr) were therefore allowed to vary with the concentration of the injected dosing solution. The final degarelix PK model described the observed PK data well with good agreement between individual predicted and observed plasma degarelix concentrations.

6.1.2 Triptorelin PK model (Paper V)

A population PK model was also developed to describe the absorption of a sustained release formulation of GnRH agonist triptorelin called Decapeptyl Depot® to be used for the mechanism-based population PK/PD modelling of the HPG axis in Paper V.

Unlike degarelix, Decapeptyl Depot® is formulated to have certain desirable release characteristics. A bolus of triptorelin is released rapidly from the depot within the first 24 hr after SC and IM administration. After the initial burst, different absorption profiles are observed after SC and IM adminstration of triptorelin. SC administration of triptorelin is believed to be absorbed into the lymph before it enters the systemic circulation, while IM adminstration of triptorelin is absorbed directly into the systemic circulation from the site of injection.

The depot model for SC and IM administered triptorelin was similar in principles to the developed degarelix PK model. The initial burst was modeled as an apparent zero-order infusion where the fraction of the dose and the duration of infusion were estimated. The subsequent constant depot release was modeled by two SC compartments and one IM compartment, i.e. a SC site of injection compartment from where the drug is transferred to a transit compartment before being absorbed into the central compartment and an IM compartment with first-order absorption directly into the central compartment (see Figure 1 in Paper V).

6.2 SDEs in population PK/PD modelling

The second research project was to investigate the use of SDEs in population PK/PD modelling. It was essential to try and implement SDEs in an already available software package for parameter estimation in PK/PD models. The choice was therefore between extending the program CTSM [47] for parameter estimation in SDE models to be able to handle non-linear mixed-effects models or try and implement an algorithm for parameter estimation in SDE models in a non-linear mixed-effects software. The choice fell on the later option because it was believed that it would have a bigger impact to introduce SDEs to the PK/PD modelling community when using a software package already familiar to pharmacometricians.

The choice of programs was therefore between the NLME package [59] developed by Pinheiro and Bates and the *de facto* standard software for population PK/PD data analysis NONMEM [7] developed by Beal and Sheiner. There were pros and cons for both programs, i.e.

- The NLME package is not currently able to estimate parameters in models described by differential equations thereby limiting its use in population PK/PD modelling. It would therefore be necessary to first implement an ODE solver to be able to manage non-linear models without a closed-form solution.
- Initial results quickly revealed that it would not be possible to implement SDEs in the current version of NONMEM (i.e. version V) since it was not possible to get access to the required internal variables.

The initial choice therefore fell on the NLME package for the implementation of SDEs in non-linear mixed-effects modelling software. The attempts to implement SDEs in population PK/PD modelling is described in the following.

6.2.1 Development of the nlmeODE package (Paper II)

In order to be able to implement the EKF algorithm in NLME, it was first necessary to implement an ODE solver to be able to handle non-linear PK/PD models without closed-form solutions. For that purpose, the nlmeODE package [74] was developed by combining NLME with the odesolve package [64] in R (www.r-project.org) [27]. The odesolve package provides an interface to the Fortran ODE solver lsoda (Livermore solver for ordinary differential equations, with automatic algorithm selection) [57], which can be used to solve initial value problems for systems of first-order ODEs.

The numerical stability and the rate of convergence of the optimization algorithm in NLME were attempted increased by simultaneous solution of the sensitivity equations associated with the system of differential equations. These sensitivity equations were automatically derived by nlmeODE and passed to the gradient attribute of the NLME object. Unfortunately, this feature only increased the estimation time and made the estimation extremely unstable for larger systems of ODEs.

The nlmeODE package was written primarily for population PK/PD modelling using a similar syntax as NONMEM's NMTRAN for easy specification of complicated dosing regimens. The nlmeODE package allows for sequential and simultaneous PK/PD data analysis, and can handle the most common types of PK/PD models, multiple doses/infusions as well as estimation of bioavailability and rate/duration of infusions. The PK data of the anti-asthmatic drug theophylline was used to illustrate the syntax of nlmeODE and available tools for data analysis in R in Paper II.

Plans of porting the nlmeODE package to S-PLUS were skipped since S-PLUS does not have an ODE solver implemented. Attempts were however made to try and port the odesolve package to S-PLUS but without success due to the different lexical scoping rules of R and S-PLUS. The results from the comparison of NONMEM and NLME in Paper III were awaited before making the decision to develop nlmeODE further (see Section 6.2.2).

6.2.2 Comparison of the non-linear mixed-effects programs NONMEM and NLME (Paper III)

The purpose of Paper III was mainly to compare the non-linear mixed-effects programs NONMEM and NLME/nlmeODE using PK/PD degarelix data from CS05 and illustrate the level of model complexity NLME and nlmeODE could handle.

NONMEM and NLME are both parametric non-Bayesian likelihood approaches proposing different approximations of the population likelihood function. The FOCE method in NONMEM is compared with the ML method in the alternating algorithm proposed by Lindstrom and Bates as implemented in NLME since these seemed to be the most similar. The FOCE method is generally considered more accurate than Lindstrom and Bates alternating algorithm since the FOCE method uses an expansion around the estimated random effects only and not like the LME approximation in NLME, which makes it around both the estimated fixed and random effects. The alternating algorithm is though supposed to be less computer intensive than the FOCE method because the penalized non-linear least squares (PNLS) step can be solved for all individuals

simultaneously and since the objective function can be profiled on the fixed effects.

A PK/PD model describing the degarelix CS05 PK/PD data was developed for the NONMEM and NLME comparison. The PK of degarelix following a single IV infusion was best described by a three-compartment disposition model while a turnover model with a pool compartment adequately represented the PD response of testosterone thus bringing the total number of ODEs to five with 18 parameters to be estimated.

The obtained parameter and relative standard error (RSE) estimates were consistent between NONMEM and NLME with a few exceptions, while the model predictions were almost identical. Both methods required approximately the same number of function evaluations but the computation times were significantly longer using NLME together with nlmeODE compared to NONMEM. This was mainly due to the implementation of nlmeODE in R, which is an interpreted language while NONMEM is written in the compiled language Fortran. The results indicated that the two likelihood approximations in NONMEM and NLME yield similar parameter estimates when analyzing complicated PK/PD models without closed-form solutions.

NONMEM and NLME were further compared using a parametric bootstrap procedure using 100 bootstrap replicates to evaluate the bias and precision of the obtained parameter estimates (see Figures 6 and 7 in Paper III). A comparison of the empirical means of the ratios between the NONMEM and NLME parameter estimates revealed that 16 out of the 18 parameters were significantly different from each other on a 5% significance level (see Figure 6.5).

The overall conclusions from the parametric bootstrapping study were

- The sample relative standard deviation (RSD) estimates from the parametric bootstrap procedure were noticeably more alike between the two programs than the asymptotic RSE estimates.
- The discrepancy between the RSD and RSE estimates was noticeably higher in NLME compared to NONMEM.
- The mean asymptotic RSE estimates generally underpredicted the bootstrap sample RSD estimates.
- Out of a total of 18 parameters, 3 NONMEM parameter estimates and 10 NLME parameter estimates were significantly different from their simulated parameter values on a 5% significance level.
- 16 out of the 18 parameter estimates from NONMEM and NLME were

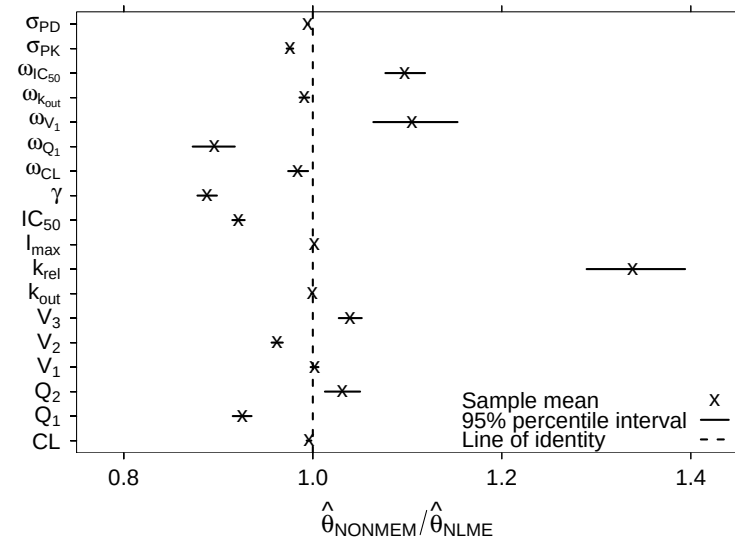

Figure 6.5: Comparison of the empirical means of the ratios between NONMEM and NLME parameter estimates from the 100 bootstrap replicates. The NONMEM and NLME parameter estimates are significantly different on a 5% significance level when the confidence interval does not include the line of unity.

considered significantly different from each other on a 5% significance level.

In general, NONMEM seemed to be superior in accuracy, stability, flexibility, and speed compared to NLME/nlmeODE but when performing graphical and statistical data analysis, NLME is preferred over NONMEM.

NONMEM VI beta was made available through Uppsala University in October 2003. Unlike NONMEM V, it was possible to get access to the state predictions in NONMEM VI beta, which should be used for the EKF implementation. The development of an nlmeSDE package was therefore put on hold because of the observed better performance of NONMEM V compared to NLME in Paper III. The implementation of SDEs in a non-linear mixed-effects software was therefore attempted in NONMEM VI beta.

6.2.3 Implementation of SDEs in NONMEM (Paper IV)

A set of Matlab (www.mathworks.com) and R functions were initially written to evaluate the FOCE population likelihood function based on SDEs. This was done to make absolutely certain that what was happening under the "hood" of NONMEM was correct thereby qualifying the implementation of the EKF in NONMEM.

The Matlab and R functions were written to handle the simplest possible case of a one-compartment PK model with an IV bolus dose. A simulation study was performed by Overgaard *et al.* in [55] to investigate the type I and type II errors associated with the introduction of system noise in non-linear mixed-effects models, i.e.

- Will significant system noise be predicted by the algorithm when none is used in the simulations (Type I error), and
- Will the algorithm fail to detect significant system noise when it is truly present in the data (Type II error).

The results showed that higher levels of system noise did not produce either additional measurement noise nor IIV, illustrating that system noise is in fact satisfactorily separable from the remaining noise parameters. Furthermore, the study demonstrated that the relationship between bias in the system noise and the level of the remaining noise parameters was small and only few type I errors occurred. A significant level of system noise could be detected when it truly was present in the simulated data (no type II errors). Finally, successful estimation was possible with sparsely sampled data having only three samples per individual [55].

Based on the results from the simulation study, it seemed feasible to extend the usual non-linear mixed-effects model with SDEs and attempts were therefore made to tweak NONMEM into using SDEs.

The recursive EKF algorithm as described in Section 4.3 was implemented in NONMEM by modifying the standard NONMEM data file and control stream.

The integral in Eq. (4.24) specifying the initial state covariance $\boldsymbol{P}_{i0}$ could not be entered directly into the NONMEM control stream. This problem was handled by adding an extra line for each individual rewinding the time variable with the time difference between the first two measurements.

Predicting and updating the states and associated covariances was slightly tricky in NONMEM because it first involved making the one-step predictions, calculat-

ing the EKF update equations, resetting all compartments, and finally update the states with the EKF updates. This was done by duplicating each observation event identifier record (EVID=0) in the data file thereby getting an EVID=0, EVID=2, and EVID=3 record where

- The one-step predictions are performed in the EVID=0 as usual.
- The EVID=2 record stores the one-step predictions from the EVID=0 record.
- The states are reset to zero and updated with the EKF updates in the subsequent EVID=3 record.

The NONMEM control stream was modified to correctly calculate the EKF equations by extensive "book-keeping" of temporary variables. The one-step predictions of the EKF are made in $DES, the EKF update equations are calculated in $PK, while the EKF output predictions are performed in $ERROR (see Appendix A in Paper IV for an example of a NONMEM SDE control stream). Further details about the implementation of SDEs in NONMEM can be found in Paper IV.

The PK data from the simulation study in [55] was used to validate the EKF implementation in NONMEM. The results were considered satisfactory since the objective function values (OFVs) from the Matlab and R functions were similar to those obtained from NONMEM. An S-PLUS script was written to automate the necessary control stream and data file modifications thereby making it relative straightforward to transform a standard NONMEM model based on ODEs into one with SDEs. However, due to the unsupported implementation in NONMEM VI beta, this is not yet something for pharmacometricians without background SDE knowledge. It is still not known when NONMEM version VI will be released and it is therefore only beta-testers who can use NONMEM with SDEs at the moment.

6.2.4 Application of SDEs in population PK/PD modelling (Paper IV and V)

Another goal of the thesis was to explore possible applications of SDEs in population PK/PD modelling. The most important investigations and findings of potential benefits of extending non-linear mixed-effects models with SDEs are summarized in the following.

One of the main features of using SDE models compared to ODE models is that the residual error is decomposed into system and measurement noise thereby

providing a more realistic description of the observed variations. Erroneous dosing, sampling history, as well as structural model misspecifications may introduce time-dependent or serial correlated residual errors. When correlated residual errors are observed due to structural model misspecifications or true physiological variations, it may be unsatisfactory that they are not included in the model. SDEs can be used to model correlated residuals and includes the statistical functionality of the commonly used continuous first-order autoregressive (AR(1)) model [38]. The SDE model structure extends the flexibility of the AR(1) model by allowing the system noise to be attributed to different model components. The system noise can be put directly on the state equation for e.g. the absorption process, which restricts the auto-correlation pattern only to be effective as long as there is absorption. This would not be possible using the continuous AR(1) model since it only acts on the measurement equation and not the dynamics of the system and will therefore act on the entire time scale.

Another source of variability is unaccounted variations in model parameters between individuals. Part of this variability can sometimes be linked to surrogate variables (e.g. demographic covariates) but most of the variability is often not predictable due to the governing processes are not fully understood or too complex to model deterministically. One way of dealing with such apparent intra-individual variability is to divide it into variation within and between study occasions using the method described in [39]. The ability to identify inter-occasion variability (IOV) though depends on the study design. The design must have information about the parameter of interest on each occasion and more than one sample per occasion must be taken on at least one occasion. Otherwise, the IOV is lumped with the inter- and intra-individual variability and it is not possible to separate out the variability in model parameters [39]. The application of SDEs for describing IOV implies time correlations and should be the choice of method when correlations are observed within occasions. However, if the IOV is unlikely to be correlated with time, the standard IOV approach described in [39] is preferred over the SDE approach.

SDEs can also be used as a diagnostic tool for model appropriateness. The significance of including system noise to a PK/PD model can be tested using the LRT since an SDE model reduces to an ODE model by fixing the diffusion term $\boldsymbol{\sigma}_w$ to zero. Significant system noise signals a potential model misspecification since errors in the structural model typically will result in significant system noise. It might however also be an indication that true physiological variations are present in the data and care should therefore be taken before drawing definitive conclusions from the system noise parameter estimates.

Clinical PK degarelix data from CS02 was used in Paper IV to illustrate the use of SDEs for tracking fluctuations in model parameters. This was done by expanding the model with a state equation for the absorption half-life that fluctuate randomly like a Wiener process. The tracking of variations in model

parameters is made possible by the way the EKF works. The one-step predictions are updated with the individual measurements at each sampling time thereby correcting for structural model deficiencies unlike an ODE approach, where the system is progressed without including additional information from the observed data (see Figure 3 in Paper IV). By plotting the updated EKF estimates of $t_{1/2,abs}$ as a function of time, it was possible to propose a reasonable approximation of the observed pattern in the absorption half-life using a two components absorption model (see Figure 1 in Paper IV). Physiological constraints on the absorption process can be imposed by using proportional system noise to prevent negative concentrations or by introducing off-diagonal elements in the diffusion term for conservation of mass balance. Several different parameterizations are often necessary to get a satisfactory result.

The introduction of SDEs to non-linear mixed-effects models might improve the parameter estimates. In particular, the inter- and intra-individual variability estimates seemed to be deflated whereas the structural parameters were less affected compared to parameter estimates from a corresponding ODE model. In Paper IV, the SDE and ODE model population parameter estimates were nearly identical since the system noise was relatively small in the final PK model. For other systems where the degree of model misspecification is more dominant (e.g. non-linear PD models of complicated physiological systems), the discrepancy between the SDE and ODE parameter estimates are expected to be greater as demonstrated for a non-linear dynamical model of a fed-batch bioreactor in [44] using a non-population approach. Preliminary results also indicated that SDEs improve the parameter standard error estimates but further simulation studies are needed to say anything definitively about it.

It is evident that an SDE model has improved simulation properties compared to an ODE model when looking at the one-step predictions (see Figure 3 in Paper IV). Whether or not SDEs can improve the predictive performance of clinical trial simulations is a bit more difficult to answer. It is not expected that PK/PD models will have better simulation properties by including SDEs when the system noise is due to model misspecifications whereas significant system noise due to true random physiological fluctuations might. The predictive performance of three summary PK variables (i.e. C_{max}, t_{max}, and AUC) was investigated for the final SDE and ODE model in Paper IV using the posterior predictive check described by Yano *et al.* in [91] (see Figure 6.6). No distinct differences between the SDE and ODE model predictions could be observed due to the relative small system noise on the slow absorption compartment.

Besides the above mentioned potential benefits of using SDEs, the main application of SDEs seems to be during the population PK/PD model development. Structural model deficiencies can be pinpointed by quantifying the model uncertainty in the system noise as illustrated in Paper IV. Furthermore, SDEs enable identification of non-linear dynamic dependencies of unknown states, in-

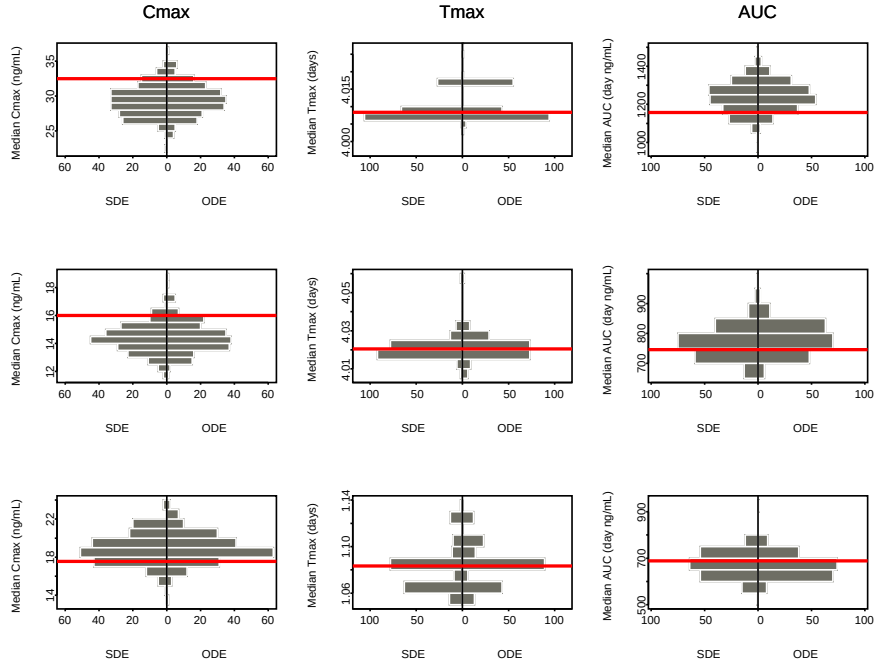

Figure 6.6: Posterior predictive check of C_{max}, t_{max}, and AUC for group 1 (**Top**), group 2 (**Center**), and group 3 (**Bottom**) in the degarelix CS02 study. SDE/ODE predicted (bars) and observed (–) median C_{max} (**Left**), t_{max} (**Middle**), and AUC (**Right**). The x-axis represents counts out of 200 replicates.

puts and/or parameters as well as deconvolution of e.g. absorption profiles and functional relationships between PK and PD.

Clinical PK/PD degarelix and triptorelin data was used in Paper V to illustrate the applications of SDEs for systematic population PK/PD model building motivated by the systematic model development scheme in [45] and presented in Section 4.4.

The feedback interactions in the mechanism-based model of the HPG axis were deconvolved using SDEs. For illustrative purposes, the one-step SDE predictions and updates of the testosterone concentration using a basic turnover model with an extra state equation for the non-basal testosterone secretion are shown in Figure 6.7 for a representative subject from the triptorelin CS001 study. The one-step SDE predictions (horizontal lines) are considerably off the observed

testosterone concentrations but are brought back on track by updating the predictions (vertical lines) with the individual measurements at each sampling time before progressing the system. The SDE framework thereby corrects for any structural model deficiencies and the updated EKF estimates can be used to identify the dynamic dependencies of the non-basal testosterone secretion and deconvolve an appropriate parametric function (see Figure 2 in Paper V).

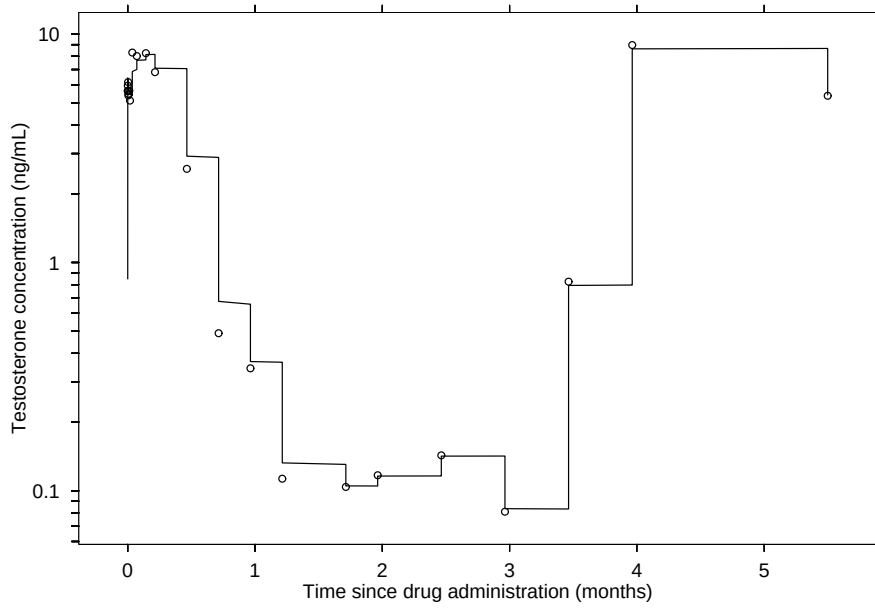

Figure 6.7: One-step predictions (–) and observed testosterone concentrations (∘) for a representative subject from the triptorelin CS001 study.

6.3 Mechanism-based PK/PD modelling of the HPG axis

The main objective of the Ph.D. thesis was to combine the results from the development of SC/IM depot PK models and the implementation of SDEs in population PK/PD modelling to build a mechanism-based model of the HPG axis.

Mechanism-based models are very time consuming to develop and computational challenging to estimate parameters in. It is necessary to correctly specify a multitude of physiological entities, their rate of formation and degradation together with potential regulatory feedback mechanisms. The number and complexity of the physiological mechanisms involved in such models makes them difficult to develop and are often too complex to be conveniently described by deterministic models. SDEs are expected to be of potential benefit for complicated PK/PD models by accounting for some of these mechanisms, while only the most important mechanisms are treated by deterministic functions. Attempts were made to formulate the final mechanism-based model of the HPG axis with SDEs but the added computational burden was of such a magnitude that the model was useless for any practical purposes. The systematic population PK/PD model building framework described in Section 4.4 was however very useful for elucidating the most important dynamic dependencies and deconvolve the functional relationships of the HPG axis.

The use of advanced computational techniques and hardware was required to optimize the model building process of the mechanism-based model the HPG axis. A Linux cluster was successfully setup for distributed execution of up to four simultaneous NONMEM runs thereby reducing the time used for model building considerably. Unfortunately, current non-linear mixed-effects software implementations do not support parallel computation and the cluster implementation can therefore not reduce the estimation time of a single run. Initiatives are however being taken to develop open-source software, which is able to distribute the time-consuming calculations on symmetric multiprocessor (SMP) computers [89].

The main problem associated with modelling a multivariate closed-loop system such as the HPG axis is that any model misspecifications in one part of the model will distort all the other parts of the model since the submodels are interdependent. Initial attempts were however made to model the two PD variables LH and testosterone separately conditioned on the observed response of the other hormone, thereby avoiding the complicated closed-loop feedbacks. This approach was not successful since the separately developed submodels were difficult to merge when abandoning the conditioning on the observed responses. It was furthermore not appropriate to switch from the FOCE method to the less accurate FO estimation method in NONMEM during the model building due to the high degree of non-linearity in the system. As a consequence, extremely long run-times (approximately 1-2 weeks per model) were experienced during the model building process. The simulation program Berkeley Madonna [17] was used as a tool for simulating the model response by specifying parameter values obtained from literature and initial NONMEM runs. This setup served as an excellent tool for developing the mechanism-based model of the HPG axis and for testing different hypothesis before attempting to estimate model parameters in NONMEM.

6.3.1 Population PK/PD model of LH and testosterone response following treatment with GnRH agonist triptorelin and GnRH antagonist degarelix (Paper V)

The main idea of building a mechanism-based model of the HPG axis, which could account for both GnRH agonist and antagonist treatment was to validate the model by having two drugs with different mechanisms of action acting on the same underlying physiological system.

The mechanism-based model of the HPG axis was developed using PK/PD data from the degarelix CS07 and triptorelin CS001 studies with more than 12,000 LH and testosterone concentration-time measurements from 228 subjects. The final PD model that best described the observed PD response of LH and testosterone concentrations following treatment with GnRH agonist triptorelin and GnRH antagonist degarelix consisted of a receptor compartment, two LH compartments (i.e. an intracellular pool compartment and a circulating LH compartment), and a compartment for circulating testosterone (see Figure 1 in Paper V). In the derived model, the secretion of readily releasable LH from the pool compartment was stimulated and inhibited by the plasma triptorelin and degarelix concentrations, respectively. Circulating LH stimulated the testosterone secretion while the delayed testosterone feedback on the non-basal LH synthesis and release was modelled through a receptor compartment where testosterone stimulates the production of receptors.

A total of 30 model parameters were successfully estimated in the final mechanism-based model of the HPG axis (see Table 3 in Paper V). Five of the parameters ($k_{e,LH}$, $k_{e,R}$, LH_{base}, and Te_{base}) were identified as study-specific parameters even though they in theory are considered to be system-specific. The distribution of the empirical Bayes estimates of the system-specific parameters with IIV ($k_{rel,LH}$, L_{max}, and L_{50}) were examined graphically by Quantile-Quantile (Q-Q) plots (see Figure 6.8).

The parameters were further investigated to verify that they were evenly distributed between the triptorelin and degarelix studies. The cumulative probability distribution of the empirical Bayes estimates from the two types of treatment were comparable and the assumption about the parameters being system-specific was accepted (see Figure 6.9).

The final model had one distinct inconsistency with respect to the mechanistic understanding of the HPG axis, i.e. the study-specific receptor stimulation of the non-basal LH synthesis and release. The physiological explanation might be found in the activation of different metabolic pathways, which results in completely different dynamic responses. Since neither endogenous hypothalamic

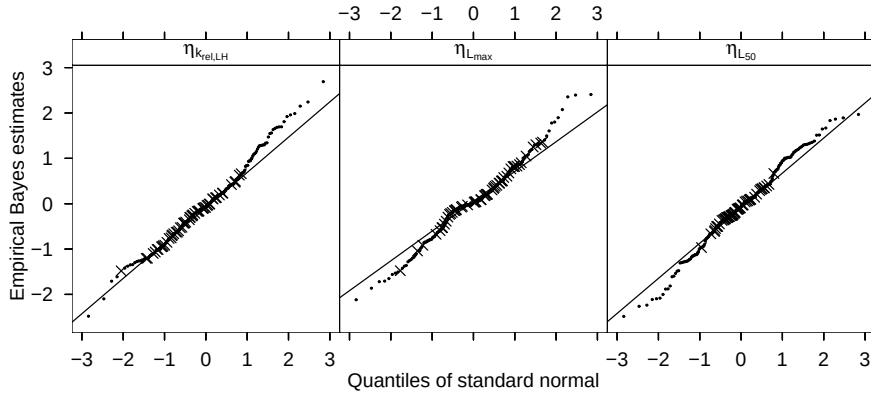

Figure 6.8: Quantile-Quantile (Q-Q) plot of the empirical Bayes estimates vs. quantiles of the standard normal distribution. All estimates would lie on the line of identity (–) if they were perfectly normal distributed. Empirical Bayes estimates from the triptorelin (×) and degarelix (·) study.

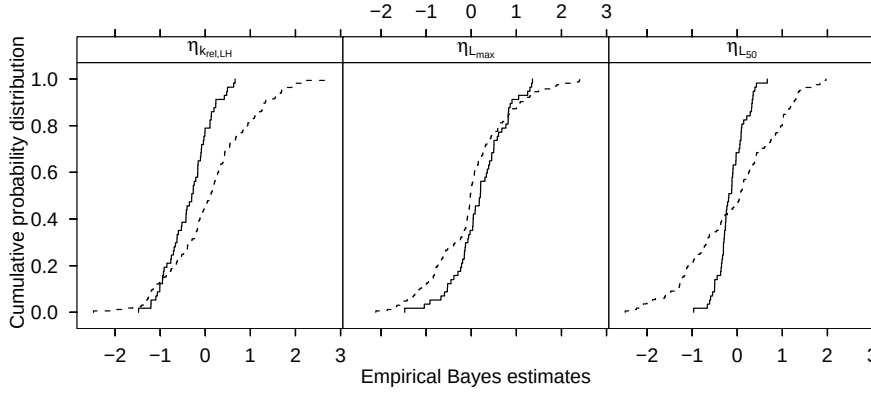

Figure 6.9: Cumulative probability distribution of the empirical Bayes estimates. Empirical Bayes estimates from the triptorelin (–) and degarelix (- - -) study.

GnRH concentrations nor changes in pituitary GnRH receptor density were measured, it was not possible to separate these two effects. Instead, an empirical

receptor compartment was used to represent the study-specific net effect of the hypothalamic GnRH and pituitary GnRH receptor response following drug treatment thereby accounting for the observed systemic down-regulation.

The different PK/PD profiles following treatment with triptorelin and degarelix was adequately captured by the mechanism-based model as illustrated in Figures 3–5 from Paper V thereby indicating that the model was sufficient at mimicking the underlying physiology of the endocrine system.

The mechanism-based model building of the HPG axis is still work in progress and the model is updated regularly with the latest available PK/PD data from the degarelix development project. Clinical trial simulations have been performed to optimize the dosing regimens for future phase III studies where a total of 1000 trials were simulated for each dosing regimen by bootstrapping the individual vector of parameter estimates with replacement thereby calculating the probabilities of obtaining 95% success rate (not shown).

Chapter 7

Conclusions

Population pharmacokinetic/pharmacodynamic (PK/PD) modelling is a powerful tool for faster and more efficient clinical drug development. The goal of modelling and simulation (M&S) is to describe, understand, and predict the clinical outcome of past and future studies. PK/PD models have evolved from being empirical descriptions of observed data to mechanism-based models, which are based on pharmacological and physiological knowledge about the modelled system. Mechanism-based PK/PD models aim at mimicking the data generation mechanism of the underlying physiological system thereby enabling the description and prediction of multiple drugs acting on the same system. Thus, new sophisticated computational methods for non-linear mixed-effects modelling are needed to be able to develop such complex models and estimate the parameters.

Different aspects of population PK/PD modelling of the hypothalamic-pituitary-gonadal (HPG) axis have been investigated in the present Ph.D. thesis and several achievements within PK modelling of subcutaneous (SC) and intramuscular (IM) depots, implementation and application of stochastic differential equations (SDEs) in non-linear mixed-effects modelling, and systematic development of a mechanism-based PK/PD model for the HPG axis have been presented.

- Population PK models were developed to describe the absorption of GnRH antagonist degarelix and GnRH agonist triptorelin from SC/IM depots.

- The initial depot model for degarelix relied on diffusion out of a spherical SC depot and was developed to quantify the influence of the concentration and volume of the dosing solution on the SC absorption profile. The depot model was later simplified using two first-order absorption components accounting for the initial fast release followed by a prolonged slow release from the depot due to diminishing dose-volume effects at clinical relevant doses of degarelix.

- The absorption of SC and IM administered triptorelin was modelled by an apparent zero-order infusion accounting for the initial burst of triptorelin and two SC compartments and one IM compartment describing the subsequent slow SC and IM release, respectively.

- Several attempts were made to implement SDEs in non-linear mixed-effects modelling software. The recursive Extended Kalman Filter (EKF) algorithm was successfully implemented in NONMEM for parameter estimation in SDE models by modifying the standard NONMEM data file and control stream.

- SDEs provide an attractive modelling approach for systematic population PK/PD model development by allowing information about unmodelled dynamics of the system to be extracted from data. This is done by decomposition of the noise affecting the system into a system noise term representing unknown or incorrectly specified dynamics and a measurement noise term accounting for uncorrelated errors such as assay error.

- The application of SDEs in systematic population PK/PD model development was investigated using clinical PK/PD data and illustrated by tracking unexplained variations in model parameters, pinpointing model deficiencies, identification of non-linear dynamic dependencies, and deconvolution of functional PK/PD relationships.

- A mechanism-based model of the HPG axis was developed, which could account for the PD response of LH and testosterone following treatment with either GnRH agonist or antagonist in a combined model. The mechanism-based model of the HPG axis was thereby validated by being able to describe the PD response for two drugs with different mechanism of action acting on the same underlying physiological system.

The primary focus of this work was on the implementation and application of SDEs during the development of population PK/PD models for the HPG axis. The application of SDEs in population PK/PD modelling was investigated using clinical PK/PD data but further simulation studies are needed to disclose all possible benefits of using SDEs. Recommendations for future work include further investigations whether the mechanism-based model of the HPG axis

can be linked to a disease progression model where testosterone stimulates the prostate cancer cell growth.

The next-generation non-linear mixed-effects modelling software are expected to be able to utilize the power of multi-processor computers simultaneously for parallel computation. This will further optimize the PK/PD modelling process and the gained speed in computation will hopefully allow for investigation of more sophisticated statistical techniques for implementing SDEs in population PK/PD modelling.

Acknowledgements

I wish to express my sincere gratitude to all who have contributed to this research. In particular, I want to thank

- My excellent team of Ph.D. advisors: Henrik Agersø, Henrik Madsen, Henrik Aalborg Nielsen, and E. Niclas Jonsson.
- Present and former Ph.D. students at IMM, especially Lasse E. Christiansen, Kim Nolsøe, Rune V. Overgaard, and Niels R. Kristensen for great discussions and table tennis matches.
- The group at Uppsala University for the introduction to fika and some great Christmas parties.
- The Experimental Medicine department at Ferring Pharmaceutical A/S for providing an excellent work environment.
- Professors Stuart L. Beal and Lewis B. Sheiner for creating something "insanely great".
- And finally, special thanks to friends and family for all their support and encouragements throughout the preparation of this thesis.

References

[1] L. Aarons. Population pharmacokinetics: theory and practice. *Br.J Clin.Pharmacol.*, 32(6):669–670, 1991.

[2] L. Aarons. Pharmacokinetic and pharmacodynamic modelling in drug development. *Stat.Methods Med.Res.*, 8(3):181–182, 1999.

[3] L. Aarons, M. O. Karlsson, F. Mentre, F. Rombout, J. L. Steimer, and A. van Peer. Role of modelling and simulation in Phase I drug development. *Eur.J.Pharm.Sci.*, 13(2):115–122, 2001.

[4] G. B. Arfken and H. J. Weber. *Mathematical Methods for Physicists: International Edition*. Academic Press, San Diego, California, 1995.

[5] J. L. Barron, R. P. Millar, and D. Searle. Metabolic clearance and plasma half-disappearance time of D-TRP6 and exogenous luteinizing hormone-releasing hormone. *J Clin.Endocrinol.Metab*, 54(6):1169–1173, 1982.

[6] S. L. Beal. Ways to fit a PK model with some data below the quantification limit. *J.Pharmacokinet.Pharmacodyn.*, 28(5):481–504, 2001.

[7] S. L. Beal and L. B. Sheiner (Eds.). *NONMEM User's Guides*. NONMEM Project Group, University of California, San Francisco, 1994.

[8] B. Brescia. Better Budgeting for Patient Recruitment. *Pharmaceutical Executive*, May, 2002.

[9] N. A. Bridges, P. C. Hindmarsh, P. J. Pringle, D. R. Matthews, and C. G. Brook. The relationship between endogenous testosterone and gonadotrophin secretion. *Clin.Endocrinol.(Oxf)*, 38(4):373–378, 1993.

[10] P. Broqua, P. J. Riviere, P. M. Conn, J. E. Rivier, M. L. Aubert, and J. L. Junien. Pharmacological profile of a new, potent, and long-acting gonadotropin- releasing hormone antagonist: degarelix. *J.Pharmacol.Exp.Ther.*, 301(1):95–102, 2002.

[11] E. R. Carson, C. Cobelli, and L. Finkelstein. *The mathematical modeling of metabolic and endocrine systems. Model formulation, identification, and validation.* John Wiley & Sons, Inc., New York, 1983.

[12] T. Cook and W. P. Sheridan. Development of GnRH antagonists for prostate cancer: new approaches to treatment. *Oncologist.*, 5(2):162–168, 2000.

[13] M. Davidian and D. M. Giltinan. *Nonlinear Models for Repeated Measurement Data.* Chapman & Hall, London, 1995.

[14] C. de Boor. *A Practical Guide to Splines.* Springer-Verlag, New York, 2001.

[15] G. de Pinieux, M. E. Legrier, F. Poirson-Bichat, Y. Courty, R. Bras-Goncalves, A. M. Dutrillaux, F. Nemati, S. Oudard, R. Lidereau, P. Broqua, J. L. Junien, B. Dutrillaux, and M. F. Poupon. Clinical and experimental progression of a new model of human prostate cancer and therapeutic approach. *Am.J.Pathol.*, 159(2):753–764, 2001.

[16] P. H. Van der Graaf and M. Danhof. Analysis of drug-receptor interactions in vivo: a new approach in pharmacokinetic-pharmacodynamic modelling. *Int.J.Clin.Pharmacol.Ther.*, 35(10):442–446, 1997.

[17] I. J. Dunn, E. Heinzle, J. Ingham, and J. E. Prenosil. *Biological Reaction Engineering: Dynamic Modelling Fundamentals with Simulation Examples.* Wiley-VCH Verlag GmbH & Co. KGaA, Weinheitn, 2003.

[18] B. Efron and R. Tibshirani. *An introduction to the bootstrap.* Chapman & Hall, London, 1993.

[19] K. E. Fattinger, D. Verotta, H. C. Porchet, A. Munafo, J. Y. Le Cotonnec, and L. B. Sheiner. Modeling a bivariate control system: LH and testosterone response to the GnRH antagonist antide. *Am.J.Physiol*, 271(4 Pt 1):E775–E787, 1996.

[20] A. Fick. Über diffusion. *Ann.Physik*, 94:59–86, 1855.

[21] C. Giberti, T. Barreca, G. Martorana, M. Truini, R. Franceschini, E. Rolandi, and L. Giuliani. Hormonal pattern and testicular histology in patients with prostatic cancer after long-term treatment with a gonadotropin-releasing hormone agonist analogue. *Eur.Urol.*, 15(1-2):125–127, 1988.

[22] R. Gieschke and J. L. Steimer. Pharmacometrics: modelling and simulation tools to improve decision making in clinical drug development. *Eur.J.Drug Metab Pharmacokinet.*, 25(1):49–58, 2000.

[23] J. E. Griffin and J. D. Wilson. *Disorders of the Testes and the Male Reproductive Tract. In Williams Textbook of Endocrinology.* W. B. Saunders Company, London, 1992.

[24] T. Hastie and R. Tibshirani. *Generalized Additive Models*. Chapman & Hall, London, 1990.

[25] S. Hiller-Sturmhöfel and A. Bartke. The Endocrine System. An Overview. *Alcohol Health & Research World*, pages 153–164, 1998.

[26] J. A. Huirne and C. B. Lambalk. Gonadotropin-releasing-hormone-receptor antagonists. *Lancet*, 358(9295):1793–1803, 2001.

[27] R. Ihaka and R. Gentleman. R: A Language for Data Analysis and Graphics. *Journal of Computational and Graphical Statistics*, 5(3):299–314, 1996.

[28] A. H. Jazwinski. *Stochastic Processes and Filtering Theory*. Academic Press, New York, 1970.

[29] G. Jiang, J. Stalewski, R. Galyean, J. Dykert, C. Schteingart, P. Broqua, A. Aebi, M. L. Aubert, G. Semple, P. Robson, K. Akinsanya, R. Haigh, P. Riviere, J. Trojnar, J. L. Junien, and J. E. Rivier. GnRH antagonists: a new generation of long acting analogues incorporating p-ureidophenylalanines at positions 5 and 6. *J.Med.Chem.*, 44(3):453–467, 2001.

[30] E. N. Jonsson. *Methodological Studies on Non-Linear Mixed Effects Model Building*. PhD thesis, Uppsala University, 1998.

[31] E. N. Jonsson and M. O. Karlsson. Automated covariate model building within NONMEM. *Pharm.Res.*, 15(9):1463–1468, 1998.

[32] E. N. Jonsson and M. O. Karlsson. Xpose–an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. *Comput.Methods Programs Biomed.*, 58(1):51–64, 1999.

[33] E. N. Jonsson and L. B. Sheiner. More efficient clinical trials through use of scientific model-based statistical tests. *Clin.Pharmacol.Ther.*, 72(6): 603–614, 2002.

[34] W. J. Jusko and H. C. Ko. Physiologic indirect response models characterize diverse types of pharmacodynamic effects. *Clin.Pharmacol.Ther.*, 56 (4):406–419, 1994.

[35] W. J. Jusko, H. C. Ko, and W. F. Ebling. Convergence of direct and indirect pharmacodynamic response models. *J Pharmacokinet.Biopharm.*, 23(1):5–8, 1995.

[36] R. E. Kalman. A New Approach to Linear Filtering and Prediction Problems. *Trans.ASME, Ser.D: J.Basic Eng.*, 82:35–45, 1960.

[37] R. E. Kalman and R. S. Bucy. New Results in Linear Filtering and Prediction Theory. *Trans.ASME, Ser.D: J.Basic Eng.*, 83:95–108, 1961.

[38] M. O. Karlsson, S. L. Beal, and L. B. Sheiner. Three new residual error models for population PK/PD analyses. *J.Pharmacokinet.Biopharm.*, 23 (6):651–672, 1995.

[39] M. O. Karlsson and L. B. Sheiner. The importance of modeling interoccasion variability in population pharmacokinetic analyses. *J.Pharmacokinet.Biopharm.*, 21(6):735–750, 1993.

[40] D. Klingmuller and H. U. Schweikert. Gonadotropin-releasing hormone: physiological and endocrinological aspects. *Recent Results Cancer Res.*, 124:1–6, 1992.

[41] K. F. Klippel, C. J. Winkler, D. Jocham, H. Rubben, B. Moser, and A. Gulati. [Effectiveness and tolerance of 1 dosage forms (subcutaneous and intramuscular) of decapeptyl depot in patients with advanced prostate carcinoma]. *Urologe A*, 38(3):270–275, 1999.

[42] E. Korkut, L. Bokser, A. M. Comaru-Schally, K. Groot, and A. V. Schally. Inhibition of growth of experimental prostate cancer with sustained delivery systems (microcapsules and microgranules) of the luteinizing hormone-releasing hormone antagonist SB-75. *Proc.Natl.Acad.Sci.U.S.A*, 88(3):844–848, 1991.

[43] M. Kovacs and A. V. Schally. Comparison of mechanisms of action of luteinizing hormone-releasing hormone (LHRH) antagonist cetrorelix and LHRH agonist triptorelin on the gene expression of pituitary LHRH receptors in rats. *Proc.Natl.Acad.Sci.U.S.A*, 98(21):12197–12202, 2001.

[44] N. R. Kristensen. *Fed-Batch Process Modelling for State Estimation and Optimal Control. A Stochastic Grey-Box Modelling Framework*. PhD thesis, Department of Chemical Engineering, Technical University of Denmark, 2002.

[45] N. R. Kristensen, H. Madsen, and S. B. Jørgensen. A method for systematic improvement of stochastic grey-box models. *Computers and Chemical Engineering*, 28(8):1431–1449, 2004.

[46] N. R. Kristensen, H. Madsen, and S. B. Jørgensen. Parameter estimation in stochastic grey-box models. *Automatica*, 40:225–237, 2004.

[47] N. R. Kristensen, H. Melgaard, , and H. Madsen. CTSM 2.3 User's Guide, 2003.

[48] J. M. Kuhn, H. Abourachid, P. Brucher, J. C. Doutres, J. Fretin, A. Jaupitre, R. Jorest, D. Lambert, J. Petit, J. Pin, J. Blumberg, and F. Dufour-Esquerre. A randomized comparison of the clinical and hormonal effects of two GnRH agonists in patients with prostate cancer. *Eur.Urol.*, 32(4):397–403, 1997.

[49] A. Lindberg-Freijs and M. O. Karlsson. Dose dependent absorption and linear disposition of cyclosporin A in rat. *Biopharm.Drug Dispos.*, 15(1): 75–86, 1994.

[50] L. Lindbom, J. Ribbing, and E. N. Jonsson. Perl-speaks-NONMEM (PsN)-a Perl module for NONMEM related programming. *Comput.Methods Programs Biomed.*, 75(2):85–94, 2004.

[51] D. E. Mager, E. Wyska, and W. J. Jusko. Diversity of mechanism-based pharmacodynamic models. *Drug Metab Dispos.*, 31(5):510–518, 2003.

[52] P. S. Maybeck. *Stochastic models, estimation, and control*. Academic Press, Inc., New York, 1979.

[53] W. G. Nelson, A. M. De Marzo, and W. B. Isaacs. Prostate cancer. *N.Engl.J.Med.*, 349(4):366–381, 2003.

[54] J. N. Nielsen, H. Madsen, and P. C. Young. Parameter Estimation in Stochastic Differential Equations: An Overview. *Annual Reviews in Control*, 24:83–94, 2000.

[55] R. V. Overgaard, E. N. Jonsson, C. W. Tornøe, and H. Madsen. Non-Linear Mixed-Effects Models with Stochastic Differential Equations. Implementation of an Estimation Algorithm. *J.Pharmacokinet.Pharmacodyn.*, 32(1): In press, 2005.

[56] K. L. Parker and B. P. Schimmer. *Pituitary Hormones and their Hypothalamic Releasing Factors. In Goodman & Gilman's The Pharmacological Basis of Therapeutics*. McGraw-Hill, London, 2001.

[57] L. R. Petzold. Automatic Selection of Methods for Solving Stiff and and Nonstiff Systems of Ordinary Differential Equations. *Siam J.Sci.Stat.Comput.*, 4:136–148, 1983.

[58] J. C. Pinheiro and D. M. Bates. Approximations to the loglikelihood function in the nonlinear mixed effects model. *Journal of Computational and Graphical Statistics*, 4(1):12–35, 1995.

[59] J. C. Pinheiro and D. M. Bates. *Mixed-Effects Models in S and S-PLUS*. Springer-Verlag, New York, 2000.

[60] T. W. Redding, A. V. Schally, S. Radulovic, S. Milovanovic, K. Szepeshazi, and J. T. Isaacs. Sustained release formulations of luteinizing hormone-releasing hormone antagonist SB-75 inhibit proliferation and enhance apoptotic cell death of human prostate carcinoma (PC-82) in male nude mice. *Cancer Res.*, 52(9):2538–2544, 1992.

[61] T. Reissmann, A. V. Schally, P. Bouchard, H. Riethmiiller, and J. Engel. The LHRH antagonist cetrorelix: a review. *Hum.Reprod.Update.*, 6(4): 322–331, 2000.

[62] G. Schwach, N. Oudry, S. Delhomme, M. Luck, H. Lindner, and R. Gurny. Biodegradable microparticles for sustained release of a new GnRH antagonist–part I: Screening commercial PLGA and formulation technologies. *Eur.J Pharm.Biopharm.*, 56(3):327–336, 2003.

[63] G. Schwach, N. Oudry, J. P. Giliberto, P. Broqua, M. Luck, H. Lindner, and R. Gurny. Biodegradable PLGA microparticles for sustained release of a new GnRH antagonist: part II. In vivo performance. *Eur.J Pharm.Biopharm.*, 57(3):441–446, 2004.

[64] R. W. Setzer. The odesolve Package. Solvers for Ordinary Differential Equations. *http://www.cran.r-project.org*, 2003.

[65] V. P. Shah, K. K. Midha, S. Dighe, I. J. McGilveray, J. P. Skelly, A. Yacobi, T. Layloff, C. T. Viswanathan, C. E. Cook, and R. D. McDowall. Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. Conference report. *Eur.J.Drug Metab Pharmacokinet.*, 16(4):249–255, 1991.

[66] L. Sheiner and J. Wakefield. Population modelling in drug development. *Stat.Methods Med.Res.*, 8(3):183–193, 1999.

[67] L. B. Sheiner and M. Rowland. Advanced Methods in Pharmacokinetics/Pharmacodynamics Course. Sils-Maria, Switzerland, 2004.

[68] L. B. Sheiner, D. R. Stanski, S. Vozeh, R. D. Miller, and J. Ham. Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine. *Clin.Pharmacol.Ther.*, 25(3):358–371, 1979.

[69] L. B. Sheiner and J. L. Steimer. Pharmacokinetic/pharmacodynamic modeling in drug development. *Annu.Rev.Pharmacol.Toxicol.*, 40:67–95, 2000.

[70] H. J. Stricker. Luteinizing hormone-releasing hormone antagonists in prostate cancer. *Urology*, 58(2 Suppl 1):24–27, 2001.

[71] H. Sun, E. O. Fadiran, C. D. Jones, L. Lesko, S. M. Huang, K. Higgins, C. Hu, S. Machado, S. Maldonado, R. Williams, M. Hossain, and E. I. Ette. Population pharmacokinetics. A regulatory perspective. *Clin.Pharmacokinet.*, 37(1):41–58, 1999.

[72] W. A. De Sy, Wilde P. De, J. M. De Meyer, J. Casselman, R. Desmet, G. Renders, and W. Schelfhout. Long term experience in the treatment of advanced prostatic cancer with decapeptyl, compared to orchiectomy. *Acta Urol.Belg.*, 56(4):581–588, 1988.

[73] A. J. Tilbrook and I. J. Clarke. Negative feedback regulation of the secretion and actions of gonadotropin-releasing hormone in males. *Biol.Reprod.*, 64(3):735–742, 2001.

[74] C. W. Tornøe, H. Agersø, E. N. Jonsson, H. Madsen, and H. A. Nielsen. Non-linear mixed-effects pharmacokinetic/pharmacodynamic modelling in NLME using differential equations. *Comput.Methods Programs Biomed.*, 76(1):31–40, 2004.

[75] C. W. Tornøe, H. Agersø, H. A. Nielsen, H. Madsen, and E. N. Jonsson. Population pharmacokinetic modeling of a subcutaneous depot for GnRH antagonist degarelix. *Pharm.Res.*, 21(4):574–584, 2004.

[76] C. W. Tornøe, J. L. Jacobsen, and H. Madsen. Grey-box pharmacokinetic/pharmacodynamic modelling of a euglycaemic clamp study. *J.Math.Biol.*, 48(6):591–604, 2004.

[77] C. W. Tornøe, J. L. Jacobsen, O. Pedersen, T. Hansen, and H. Madsen. Grey-box modelling of pharmacokinetic/pharmacodynamic systems. *J Pharmacokinet.Pharmacodyn.*, 31(5):401–417, 2004.

[78] J. Trachtenberg, M. Gittleman, C. Steidle, W. Barzell, W. Friedel, D. Pessis, N. Fotheringham, M. Campion, and M. B. Garnick. A phase 3, multicenter, open label, randomized study of abarelix versus leuprolide plus daily antiandrogen in men with prostate cancer. *J.Urol.*, 167(4):1670–1674, 2002.

[79] U.S. Food and Drug Administration. Guidance for Industry: Population Pharmacokinetics, 1999. http://www.fda.gov.

[80] U.S. Food and Drug Administration. Challenge and Opportunity on the Critical Path to New Medical Products, 2004. http://www.fda.gov.

[81] D. Verotta and L. B. Sheiner. A general conceptual model for non-steady state pharmacokinetic/pharmacodynamic data. *J Pharmacokinet.Biopharm.*, 23(1):1–4, 1995.

[82] E. F. Vonesh. A note on the use of Laplace's approximation for nonlinear mixed-effects models. *Biometrika*, 83(2):447–452, 1996.

[83] J. R. Wade, S. L. Beal, and N. C. Sambol. Interaction between structural, statistical, and covariate models in population pharmacokinetic analysis. *J.Pharmacokinet.Biopharm.*, 22(2):165–177, 1994.

[84] U. Wahlby, E. N. Jonsson, and M. O. Karlsson. Assessment of actual significance levels for covariate effects in NONMEM. *J.Pharmacokinet.Pharmacodyn.*, 28(3):231–252, 2001.

[85] J. Warberg. *Human Fysiologi*. Polyteknisk Forlag, Lyngby, 2001.

[86] G. F. Weinbauer and E. Nieschlag. LH-RH antagonists: state of the art and future perspectives. *Recent Results Cancer Res.*, 124:113–136, 1992.

[87] G. Welch and G. Bishop. *An Introduction to the Kalman Filter*. Department of Computer Sciences, University of North Carolina, Chapel Hill, 1997.

[88] P. Welling. *Pharmacokinetics: Processes and Mathematics*. American Chemical Society, Washington D.C., 1986.

[89] A. Westhagen. An Architectural Overview of SPK, 2005. http://depts.washington.edu/rfpk.

[90] R. Wolfinger. Laplace's approximation for nonlinear mixed models. *Biometrika*, 80:791–795, 1993.

[91] I. Yano, S. L. Beal, and L. B. Sheiner. The need for mixed-effects modeling with population dichotomous data. *J.Pharmacokinet.Pharmacodyn.*, 28(4): 389–412, 2001.

[92] Y. Yano, S. L. Beal, and L. B. Sheiner. Evaluating pharmacokinetic/pharmacodynamic models using the posterior predictive check. *J.Pharmacokinet.Pharmacodyn.*, 28(2):171–192, 2001.

[93] L. Zhang, S. L. Beal, and L. B. Sheiner. Simultaneous vs. Sequential Analysis for Population PK/PD Data I: Best-case Performance. *J.Pharmacokinet.Pharmacodyn.*, 30(6):387–404, 2003.

Papers

Paper I

Population Pharmacokinetic Modeling of a Subcutaneous Depot for GnRH Antagonist Degarelix

---

Published in *Pharm. Res.*, **21**(4):574–584 (2004)

Paper II

II

Non-linear mixed-effects pharmacokinetic/ pharmacodynamic modelling in NLME using differential equations

---

Published in *Comput. Methods Programs Biomed.*, **76**(1):31–40 (2004)

Paper III

Pharmacokinetic/ Pharmacodynamic Modelling of GnRH Antagonist Degarelix: A Comparison of the Non-linear Mixed-Effects Programs NONMEM and NLME

III

Published in *J. Pharmacokinet. Pharmacodyn.*, **31**(6):441–461 (2004)

Paper IV

Stochastic Differential Equations in NONMEM: Implementation, Application, and Comparision with Ordinary Differential Equations

IV

Published in *Pharm. Res.*, **22**(8):1247–1258 (2005)

Paper V

Mechanism-based population PK/PD modeling of the hypothalamic-pituitary-gonadal axis following treatment with GnRH agonist triptorelin and GnRH antagonist degarelix

V

Submitted to *Pharm. Res.*, 2005

Index

Clinical drug development, 5

Extended Kalman Filter, 27
  Algorithm, 29
  One-step predictions, 23
  Output predictions, 28
  State predictions, 27
  Update equations, 28

GnRH, 14
  agonist, 17
  antagonist, 18

HPG axis, 13
  Endocrine system, 16
  Gonads, 15
  Hypothalamus, 14
  Pituitary, 15
    Anterior, 15
    Posterior, 15

Non-linear mixed-effects modelling, 20
NONMEM, 24
  FOCE method, 24, 26
  Laplacian approximation, 25

PK/PD, 6
  ADME, 6
PK/PD modelling, 7
  Empirical, 9
  Mechanism-based, 9
  Models, 7
  Physiological, 9
Population PK/PD modelling, 10
Prostate cancer, 13

Stochastic differential equations, 20
  Diffusion term, 21
  Drift term, 21