OPTIMISING PATIENT CARE BY INDIVIDUALISING DRUG DOSAGE

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ABSTRACT

Drug therapy is an important component of the treatment and prevention of diseases. A drug is administered to achieve a treatment target so it is important to quantify the drug dose and dosing regimen prior to administration in order to achieve this target. Following administration, the patient’s response to therapy is assessed and the drug dosage is adjusted accordingly in order to optimise therapy in terms of effectiveness and safety. The process of obtaining a treatment target for a particular drug, calculating a suitable dosage for an individual patient, measuring patient response, and adjusting dosage in order to optimise this response is complex and often inadequately done in clinical practice. This process often requires specialised knowledge in drug pharmacokinetics, pharmacodynamics, and model-based dose individualisation.

In this thesis a treatment target for the anticoagulant drug enoxaparin was explored. The target (anti-factor Xa) was evaluated using a Bayesian dose-individualisation method and data collected retrospectively. The Bayesian forecasting method was then applied prospectively in a randomised clinical trial. Also, the analytical method used to measure enoxaparin concentration (anti-factor Xa) was evaluated in terms of accuracy, precision, stability, and performance in special conditions such as blood sample haemolysis and antithrombinaemia.

The thesis also explored one of the main sources of variability in response between individuals, body composition. A model to predict fat-free mass, as a measure of structural maturation, from age, sex, height, and weight was developed and evaluated. The maturation model was then used successfully to develop a pharmacokinetic-pharmacodynamic model for the anticoagulant drug unfractionated heparin in a paediatric population.
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**Poster Presentations**


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<td>Anti-activated clotting factor X</td>
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<td>AT</td>
<td>Antithrombin</td>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BOV</td>
<td>Between-occasion variability</td>
</tr>
<tr>
<td>BSV</td>
<td>Between subject variability</td>
</tr>
<tr>
<td>BSVp</td>
<td>Predictable between subject variability</td>
</tr>
<tr>
<td>BSVr</td>
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<td>Clearance</td>
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<td>KPD</td>
<td>Kinetic-pharmacodynamic</td>
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<td>Lean body weight</td>
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<td>MAP</td>
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</tr>
<tr>
<td>mg</td>
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<td>Objective function value</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>pcVPC</td>
<td>Prediction-corrected visual predictive check</td>
</tr>
<tr>
<td>PD</td>
<td>Pharmacodynamic</td>
</tr>
<tr>
<td>PI</td>
<td>Prediction interval</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetic</td>
</tr>
<tr>
<td>PKPD</td>
<td>Pharmacokinetic-pharmacodynamic</td>
</tr>
<tr>
<td>PPP</td>
<td>Population pharmacokinetic parameters</td>
</tr>
<tr>
<td>PPPD</td>
<td>Population pharmacokinetic parameters and data</td>
</tr>
<tr>
<td>PPV</td>
<td>Population parameter variability</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised controlled trial</td>
</tr>
<tr>
<td>RE</td>
<td>Relative error</td>
</tr>
<tr>
<td>RMSE</td>
<td>Root mean square error</td>
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<tr>
<td>RSE</td>
<td>Relative standard error</td>
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<tr>
<td>RUV</td>
<td>Random unexplained variability</td>
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<tr>
<td>s</td>
<td>Seconds</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>t\textsubscript{1/2}</td>
<td>Half-life</td>
</tr>
<tr>
<td>TCI</td>
<td>Target concentration intervention</td>
</tr>
<tr>
<td>TDM</td>
<td>Therapeutic drug monitoring</td>
</tr>
<tr>
<td>UFH</td>
<td>Unfractionated heparin</td>
</tr>
<tr>
<td>V</td>
<td>Volume of distribution</td>
</tr>
<tr>
<td>V\textsubscript{c}</td>
<td>Central volume</td>
</tr>
<tr>
<td>VPC</td>
<td>Visual predictive check</td>
</tr>
<tr>
<td>Wt</td>
<td>Total body weight</td>
</tr>
<tr>
<td>X\textsubscript{a}</td>
<td>Activated clotting factor X (pronounced ‘ten a’)</td>
</tr>
<tr>
<td>(\mu\text{mol})</td>
<td>micromol</td>
</tr>
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</table>
## INDICES AND SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>$\sigma_{add}^2$</td>
<td>Variance of additive error</td>
</tr>
<tr>
<td>$\sigma_{prop}^2$</td>
<td>Variance of proportional error</td>
</tr>
<tr>
<td>$\omega^2$</td>
<td>Variance of between subject variability</td>
</tr>
<tr>
<td>A</td>
<td>The amount of drug in a compartment</td>
</tr>
<tr>
<td>C</td>
<td>Concentration</td>
</tr>
<tr>
<td>C50</td>
<td>Drug concentration resulting in half maximal effect</td>
</tr>
<tr>
<td>CL</td>
<td>clearance</td>
</tr>
<tr>
<td>Css,ave</td>
<td>Average steady state concentration</td>
</tr>
<tr>
<td>D</td>
<td>Dose</td>
</tr>
<tr>
<td>E</td>
<td>Effect</td>
</tr>
<tr>
<td>Emax</td>
<td>Maximum effect</td>
</tr>
<tr>
<td>F</td>
<td>Bioavailability</td>
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<tr>
<td>f(.)</td>
<td>Mathematical function</td>
</tr>
<tr>
<td>i</td>
<td>Index for an individual, $i=(1,\ldots,N)$</td>
</tr>
<tr>
<td>j</td>
<td>Index for an observation, $j=(1,\ldots,n)$</td>
</tr>
<tr>
<td>ka</td>
<td>Absorption rate constant</td>
</tr>
<tr>
<td>ke</td>
<td>Elimination rate constant</td>
</tr>
<tr>
<td>ln</td>
<td>Natural log</td>
</tr>
<tr>
<td>N</td>
<td>Total number of individuals</td>
</tr>
<tr>
<td>n</td>
<td>Total number of observations</td>
</tr>
<tr>
<td>p</td>
<td>Index for parameter, $p=(1,\ldots, np)$</td>
</tr>
<tr>
<td>Q</td>
<td>Inter-compartmental clearance</td>
</tr>
<tr>
<td>S0</td>
<td>Baseline effect</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
</tr>
<tr>
<td>V</td>
<td>Volume of distribution</td>
</tr>
<tr>
<td>x</td>
<td>Independent variable</td>
</tr>
<tr>
<td>y</td>
<td>Observation/dependant variable</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Hill/sigmoidicity coefficient</td>
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<tr>
<td>$\epsilon$</td>
<td>Residual unexplained variance</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Random effect (ETA)</td>
</tr>
<tr>
<td>$\theta$</td>
<td>fixed effect parameter</td>
</tr>
</tbody>
</table>
STRUCTURE OF THE THESIS

The thesis is divided into five parts, encompassing eight chapters, and an Appendix section (Table P.1). Part I contains Chapter 1 which covers an introduction to pharmacokinetics, pharmacodynamics, pharmacometrics, and the concepts of dose-individualisation and target-concentration intervention. This chapter will also introduce the two anticoagulants enoxaparin and unfractionated heparin which have been the exemplars of this thesis. The drugs will be discussed in terms of pharmacology, clinical use, and dosing and monitoring challenges.

Part II centres on enoxaparin target development and dosing evaluation and contains two chapters. Chapter 2 outlines the development of a treatment target for enoxaparin from a dataset previously collected by collaborators during a clinical trial. Chapter 3 covers an evaluation of a Bayesian forecasting method for enoxaparin dose-individualisation using the target developed in Chapter 2.

Part III focuses on the application of dose-individualisation of enoxaparin in a clinical setting and contains two chapters. Chapter 4 outlines assay work where an anti-activated Factor X (anti-Xa) assay for the quantification of enoxaparin was investigated in terms of stability, shelf-life, sample haemolysis, and hypoantithrombinaemia. Chapter 5 covers a randomised controlled trial (RCT) of enoxaparin treatment using the anti-Xa assay evaluated in Chapter 4. The RCT compared current dosing guidelines to a Bayesian dose-individualisation in terms of target attainment and clinical outcomes.

Part IV focuses on the influence of covariates on dose-individualisation and contains two chapters. Chapter 6 describes the development and evaluation of a semi-mechanistic model to predict fat-free mass (FFM) in children. Chapter 7 describes the use of FFM to develop a PKPD model of unfractionated heparin in children using a dataset previously collected by collaborators and explores the use of FFM to guide dose-individualisation.
Part V contains Chapter 8 which concludes the thesis with a discussion of the findings and an outline for future research.

Chapter 8 is followed by appendices which contain additional material pertaining to each chapter. This includes clinical trial documents, NONMEM control files, MATLAB code files, and the References section.
# Table P.1: Thesis outline

<table>
<thead>
<tr>
<th>Part</th>
<th>Section</th>
<th>Chapters</th>
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<tr>
<td>I</td>
<td>INTRODUCTION</td>
<td>Chapter 1: Introduction</td>
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<tr>
<td>II</td>
<td>ENOXAPARIN TARGET DEVELOPMENT AND DOSING EVALUATION</td>
<td>Chapter 2: Enoxaparin treatment target development</td>
</tr>
<tr>
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<td></td>
<td>Chapter 3: Evaluation of a Bayesian forecasting method for enoxaparin dose-individualisation</td>
</tr>
<tr>
<td>III</td>
<td>DOSE-INDIVIDUALISATION OF ENOXAPARIN IN ADULTS</td>
<td>Chapter 4: Anti-factor Xa assay evaluation</td>
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<tr>
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<td>Chapter 5: A randomised controlled trial (RCT) of a Bayesian dose-individualisation method for enoxaparin</td>
</tr>
<tr>
<td>IV</td>
<td>USING COVARIATES TO INDIVIDUALISE THERAPY IN CHILDREN</td>
<td>Chapter 6: The development and evaluation of a model to predict FFM in children</td>
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<tr>
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<td>Chapter 7: Using fat-free mass to develop a paediatric PKPD model for unfractionated heparin</td>
</tr>
<tr>
<td>V</td>
<td>DISCUSSION AND FUTURE WORK</td>
<td>Chapter 8: Discussion, conclusions, and future work</td>
</tr>
<tr>
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<td>APPENDICES AND REFERENCES</td>
<td>Appendix 1: additional material for Chapter 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Appendix 2: additional material for Chapter 4</td>
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<td>Appendix 3: additional material for Chapter 5</td>
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<td>Appendix 4: additional material for Chapter 6</td>
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<tr>
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<td>Appendix 5: additional material for Chapter 7</td>
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Part I

INTRODUCTION
Chapter 1: Introduction

Parts of this chapter are based on the following peer-reviewed publications:

**Al-Sallami HS, Cheah S, Han S, Liew J, Lim J, Ng M, Solanki H, Soo R, Tan V, Duffull SB.** *Between-subject variability: should high be the new normal?* European Journal of Clinical Pharmacology 2014; 70:1403–1404

1.1. Introduction to thesis

This thesis addresses the need for and means of dose-individualisation of drug therapy in order to achieve optimal clinical outcomes. A drug is given in order to achieve a therapeutic target which is usually decided a priori. A dosing regimen needs to be suitable to achieve the said target. Once therapy has started, the patient’s response to therapy in terms of effectiveness and tolerability needs to be assessed and the dosage adjusted accordingly. This process is not trivial and is complicated by random variability in response between and within patients which needs to be understood and accounted for in order to optimise therapy. If done poorly or haphazardly, drug therapy can result in over- or under-dosing and subsequent toxicity or subtherapy.

Dose-individualisation, defined as the quantification of a drug dosage that suits the needs of an individual patient, is required for a drug that exhibits a narrow therapeutic window and “large” between-subject variability (what large represents is yet to be defined). This drug will also need to have a reliable and readily accessible assay to quantify its pharmacokinetic and/or pharmacodynamic response. Lastly, this drug needs to have a quantifiable dose-response relationship. It is important to note that dose-individualisation, which requires the incorporation of individual response in this context, is not always feasible or even beneficial. The majority of drugs currently used in clinical practice require some form of dose individualisation that is based on a covariate (e.g. weight or creatinine clearance) without the need for a reiterative calculation of dosage based on individual response. For some drugs, however, the dosage needs to be tailored for each patient based on patient covariates and individual response.

In this thesis, two drugs (enoxaparin and unfractionated heparin [UFH]) are used as exemplars to illustrate the need and the means to individualise therapy. Enoxaparin and UFH are two anticoagulant heparins currently used in clinical practice for the treatment and prevention of thromboses. Current enoxaparin dosing
guidelines are suboptimal and dose-individualisation, informed by the pharmacokinetic-pharmacodynamic properties of the drug, provides better clinical outcomes. In order to illustrate that, a target for enoxaparin plasma concentration (anti-activated factor X, “anti-Xa”) needed to be established. This is a target that is associated with maximal effect with minimal toxicity. In order to achieve this target a dosing method needed to be developed, evaluated, and used in a clinical setting.

Large between-subject variability is an important criterion for dose-individualisation. In pharmacokinetics, variability in time-course of drug concentration is attributed to differences in patient covariates such as size or organ function. Accounting for this variability improves the clinician’s ability to achieve the therapeutic target in a particular patient. Literature evidence in adult patients gives credence to fat-free mass (FFM) as a suitable size descriptor to be used for drug dosing. FFM correlates well with metabolism and drug clearance. Models to predict FFM in adults have been developed and shown to perform well. Similar models in children are needed in paediatric dosing, especially for children at the extrema of body weight. In this thesis, the development and evaluation of a FFM model in children is described. The new model was used to account for variability in the pharmacokinetics of unfractionated heparin in paediatric patients.

This thesis uses the examples of enoxaparin and unfractionated heparin to describe the utility of pharmacometrics in dose-individualisation. Pharmacometric models describe the time-course of drug effect and often apply information from population pharmacokinetic-pharmacodynamic studies to clinical practice. The ability to predict between-subject variability, such as by predicting FFM, increases the predictive performance of these models.
1.2. Pharmacometrics

The selection of an optimal drug dosing regimen requires a quantitative understanding of the time course of drug effects. This dosage is expected to have the highest probability of achieving a desired response in an individual patient or a patient population over the time frame of interest. A response refers to both the expected response from any given dosing schedule as well as the variability in the response profile which arises from differences between individuals and variability within individuals as well as the residual error in the response measurement. Pharmacometrics (PM) is the discipline that explores these properties of drugs and integrates pharmacological models with modelling and optimal trial design.[1]

The pharmacological models pertinent to PM include pharmacokinetic models that describe the time-course of drug concentration in a matrix, pharmacodynamic models that describe the drug concentration-effect relationship, pharmacokinetic-pharmacodynamic models that describe the time-course of drug effect, and disease progression models that describe time-course of disease progression in the presence and absence of drug and placebo. Additionally, PM can link all these models and apply them to competing clinical trial designs in order to understand the impact of various dosing strategies, different patient selection criteria, and different study endpoints on trial outcomes.

The science and discipline of PM has largely evolved from pharmacokinetics.[2] The term pharmacometrics was introduced 33 years ago by Benet and Rowland and defined as the “design, modeling, and analysis of experiments involving complex dynamic systems in the fields of pharmacokinetics and biopharmaceutics”. The area has since progressed rapidly and has been applied to various stages of drug discovery, development, and pharmacotherapy.[4] For the latter, the role of PM model development and application in patient care remains largely underutilised. Many health professionals appreciate the variability in response between individual patients and may have seen first-hand drug-related adverse outcomes experienced by patients due to over- or under-dosing. Clinicians strive to tailor the treatment and
dosing regimens to their patients based on professional experience, intuition, and/or own understanding of the kinetics of dose-response. This, however, is often not enough to optimise patient care due to the often non-linear nature of dose-response and the complex mix of covariates that influence between-subject variability. PM can support prediction of a suitable dosing regimen that suits the individual but this may not be accessible to, understood, or perceived as credible by clinicians.

This thesis will explore whether PM modelling and models can make patient treatment safer and more consistently effective by implementing individualised dosing regimens based on patient covariates and/or response to therapy.

1.2.1. Pharmacokinetics

Pharmacokinetics (PK) describes the time-course of drug concentration in the body.\[^{6, 7}\] It is usual to characterise PK from the perspective of the matrix in which drug is being measured (e.g. plasma). PK comprises two processes: 1) input which describes the time course of drug movement from the site of administration to the site of measurement in the systemic circulation and 2) disposition which describes the time course of drug distribution and elimination from the site of measurement. The underlying PK behaviour of a drug (i.e. PK model) is the combination of these two processes which occur simultaneously. In the simplest pharmacokinetic model, the whole body is assumed to be a single compartment and the drug gets instantaneously distributed throughout the compartment. The rate of change of concentration over time is given by:

\[
\frac{dC}{dt} = - \frac{CL}{V} \times C \quad C(0) = \frac{Dose}{V}
\]

Equation 1.1 One compartment first order elimination PK model
The parameters of this model are clearance ($CL$) and the apparent volume of distribution ($V$). For the purposes of a single dose study in a single individual, parameters can be considered as unknown constants. In a one-compartment model all the body tissues are lumped into a single compartment where the drug is distributed instantaneously. In this example, the apparent volume of distribution of a drug in the compartment is the product of the physical volume of the lumped tissues and the partition coefficient of the drug into those tissues. $CL$ (volume/time) describes the relationship between drug concentration $C$ (mass/volume) in a body compartment and the rate of elimination of the drug (mass/time) from that compartment. The first order rate constant $k$ (1/time) relating rate of drug elimination to the amount of drug in the body is given by $CL/V$. The half-life (h), which is the length of time for the amount in the body to reduce by half, can then be determined as $\ln(2)/k$.

When drugs are dosed chronically on a repeated schedule the concentrations will asymptote to an equilibrium where the amount of drug that enters the body equals the amount of drug that leaves the body during each dosing interval. This is termed PK steady state. In the steady state case,

$$C_{pss, \; avg} = \frac{Dose}{CL \times \tau}$$

Equation 1.2 PK at steady state

where $\tau$ denotes the dosing interval (h). The required dose rate to achieve a specific average steady state concentration can then be predicted.

Most drugs, however, follow more complex PK behaviour than described in equation 1.1 (and depicted in Figure 1.1 “single IV dose”). The concentration-time profile of single and multiple oral doses are also shown in Figure 1.1 for illustration.
1.2.2. Pharmacodynamics

Pharmacodynamics (PD) describes the relationship between drug concentration at the site of action and drug effect.\textsuperscript{[9]} In most cases this process is described by principles of mass action, i.e. one in which a drug binds reversibly with a receptor. The binding then provokes a reaction or series of reactions that eventually culminate in an observable effect. The binding of drug $D$ to receptor $R$ to form an activated drug-receptor $DR$ complex for a single binding site is given by:
Equation 1.3 Drug-receptor binding. The square bracket [ ] represents concentration

\[ k_{on} \] [D] + [R] \xrightarrow{k_{off}} [DR] \]

where \( k_{off} \) is the dissociation rate constant and \( k_{on} \) is the association rate constant.

The affinity of the drug to bind to the receptor, at equilibrium, is expressed as the inverse of the dissociation constant (\( K_d \)):

\[ K_d = \frac{k_{off}}{k_{on}} \]

Equation 1.4 Equilibrium drug-receptor dissociation constant

From Equation 1.4 we can express receptor binding (\( B \)) as:

\[ B = B_{max} \times \frac{C}{Kd + C} \]

Equation 1.5 Drug-receptor binding

where \( B \) is the number of bound receptors, \( C \) is the drug concentration, and \( B_{max} \) is the maximum binding capacity. Under the assumption of a linear transduction model for observable drug effect, the relationship between binding and effect can be described by:

\[ E = k_e \times B \]

Equation 1.6 Drug effect following drug-receptor binding

where \( k_e \) is a proportionality constant that links the receptor occupancy with effect. It is, however, rare for receptor binding information to be available in clinical studies.
In this case we approximate the general mass action model from equations 1.5 and 1.6 as an $E_{\text{max}}$ model:

$$E = S0 + E_{\text{max}} \times \frac{C}{C_{50} + C}$$

Equation 1.7 The $E_{\text{max}}$ drug effect model

where $E_{\text{max}}$ is the maximum drug effect, $S0$ is the baseline of the system in the absence of drug, and $C_{50}$ is the drug concentration that yields 50% maximal drug effect. A generalisation to this model that can yield a different slope for the concentration-effect relationship (either more shallow or steeper) is provided by the sigmoid $E_{\text{max}}$ model:

$$E = S0 + E_{\text{max}} \times \frac{C^{\gamma}}{C_{50}^{\gamma} + C^{\gamma}}$$

Equation 1.8 The sigmoid $E_{\text{max}}$ model

where $\gamma$ is the sigmoidicity parameter which affects the shape of the curve (also termed the Hill coefficient). Values of $\gamma$ greater than 1 will yield a steeper S-shaped curve and less than 1 gives a more shallow approach to the $E_{\text{max}}$ asymptote. The concentration-effect relationship for these models are shown in Figure 1.2. It is important to note that the $E_{\text{max}}$ model predicts the highest achievable response at infinite concentration. These very high concentrations are often not appropriate in clinical practice due to adverse effects.
Figure 1.2 The sigmoid $E_{max}$ model. When $\gamma=1$ the resulting curve is a hyperbola and identical to the $E_{max}$ model. $E_{max} = 1 \ (100\%), E_0 = 0, C_{50} = 0.1$.

1.2.3. Pharmacokinetics-pharmacodynamics

Pharmacokinetics-pharmacodynamics (PKPD) models combine the time course of drug concentrations with binding of drug to the target site(s) and subsequent drug effects. A PKPD model incorporates a link function between the time course of drug concentrations (PK) and pharmacological effect (PD). PKPD models are therefore useful to describe, understand, and predict the extent and time course of drug effects. Incorporating mechanistic behaviour into the PKPD model would improve its ability to predict new situations.

There are two main types of links between PK and PD: (1) immediate effects (also termed direct effects) and (2) delayed effects. Immediate effects occur when the
effects are directly linked with the drug concentration so no additional link models are needed. An example immediate effect model is represented by:

$$\frac{dC}{dt} = -\frac{CL}{V} \times C \quad C(0) = \frac{Dose}{V}$$

$$E(t) = S0 + E_{max} \times \frac{C(t)}{C_{50} + C(t)}$$

Equation 1.9 Immediate effect PKPD model

The PK model provides the time-course of drug concentration $C(t)$ and PD model predicts the drug effect at time $t$ ($E(t)$). This model essentially predicts that the maximum drug effect is reached at the same time as the maximum concentration (Figure 1.3). These models are attractive in their simplicity but for many drugs the link is not immediate.
Chapter 1: Introduction

Figure 1.3 The time course of drug concentration (PK) and drug effect (PD) for an immediate effects model. \( V = 0.6, k = 0.8, k_a = 0.2, Dose = 3, \tau = 4, E_{max} = 150, C_{50} = 0.5, E_0 = 0 \).

Delayed effects occur when the effects appear delayed with respect to the concentration-time profile. One reason for a delay in drug effects may be a delay in the distribution of the drug to the effect site, which can be described using an effect compartment model \cite{[10]} where:

\[
PK: \quad \frac{dC}{dt} = -\frac{CL}{V} \times C \quad C(0) = \frac{Dose}{V}
\]

\[
Link \ model: \quad \frac{dCe}{dt} = keo \times (C - Ce) \quad Ce(0) = 0
\]

\[
PD: \quad E(t) = S0 + E_{max} \times \frac{Ce(t)}{C_{50} + Ce(t)}
\]

Equation 1.10 The effect compartment PKPD model, an example of a delayed effect model
where, $Ce$ is the concentration in the hypothetical effect compartment and $keo$ is the rate constant of loss from this compartment. The rate at which the effect compartment achieves steady state is therefore related only to the rate constant $keo$. This parameterisation of the model assumes that the average concentration in the effect compartment at steady state is the same as in plasma, without loss of generality. The amount of drug in the effect compartment is assumed to be negligible and therefore $Ce$ does not affect the mass balance in the equation for PK. An alternative mechanistic explanation for invoking the effect compartment model is the time required for equilibration of receptor binding.

Another important class of PKPD models that characterise a delay between the concentration and effect profiles specify the drug effect as an inhibitor (or stimulator) of the input (or loss) process(es) of a physiological intermediate. These combinations result in the four basic turnover models (also called indirect response models) that are an appropriate choice if the PD response variable has a natural turnover. [11, 12]

The PD response profiles resulting from inhibition of input and from stimulation of output are similar. However, on multiple dosing or different dose levels the models provide quite different predictions (Figure 1.4). The choice of model is critical for predictions that involve extrapolation. The general form of the turnover model is given by:

$$
PK: \quad \frac{dC}{dt} = -\frac{CL}{V} \times C \\
C(0) = \frac{Dose}{V}
$$

$$
PD: \quad \frac{dE}{dt} = R_{in} \times f(C) - k_{out} \times g(C) \times E \\
E(0) = S0 = \frac{R_{in}}{k_{out}}
$$

**Equation 1.11 A general form of the turnover PKPD model**

where $R_{in}$ is the natural rate of production of the effect (mass/time) and $k_{out}$ is a first-order rate constant of loss (1/time) and $f(C)$ and $g(C)$ are PD models. It is often sufficient for one of these functions to be a PD model and the other to be fixed to 1. In this model it is assumed that the physiological intermediate is at steady state to
get the initial condition $E(0)$. Using the $E_{\text{max}}$ model, two classes of effect (inhibition or stimulation) can occur at either $f(C)$ or $g(C)$ as shown by:

$$PD \ (\text{stimulation of input}) : \quad \frac{dE}{dt} = R_{\text{in}} \times \left( 1 + E_{\text{max}} \times \frac{C(t)^{\gamma}}{C_{50}^{\gamma} + C(t)^{\gamma}} \right) - k_{\text{out}} \times E$$

$$PD \ (\text{stimulation of loss}) : \quad \frac{dE}{dt} = R_{\text{in}} - k_{\text{out}} \times \left( 1 + E_{\text{max}} \times \frac{C(t)^{\gamma}}{C_{50}^{\gamma} + C(t)^{\gamma}} \right) \times E$$

$$PD \ (\text{inhibition of input}) : \quad \frac{dE}{dt} = R_{\text{in}} \times \left( 1 - E_{\text{max}} \times \frac{C(t)^{\gamma}}{C_{50}^{\gamma} + C(t)^{\gamma}} \right) - k_{\text{out}} \times E$$

$$PD \ (\text{inhibition of loss}) : \quad \frac{dE}{dt} = R_{\text{in}} - k_{\text{out}} \times \left( 1 - E_{\text{max}} \times \frac{C(t)^{\gamma}}{C_{50}^{\gamma} + C(t)^{\gamma}} \right) \times E$$

Equation 1.12 Turnover models of a biological intermediate. $R_{\text{in}}$ is the rate of input, $k_{\text{out}}$ is the rate of loss, and $E$ is the turnover response.
Selection of the most appropriate PKPD model should be guided by prior knowledge of the drug pharmacology. Data from more than one dose level greatly helps to distinguish between models (immediate or delayed action and, if delayed, an effect compartment or a turnover model). For a direct effect model the time of peak concentration is also the time of peak effect for all dose levels. The effect compartment model introduces a delay between the peak concentration and peak effect; this could be due to drug transport to the site of action. If this transport is a first-order process (e.g. diffusion), the time delay is independent of dose. All four turnover models exhibit a dose-dependent delay between peak concentration and peak effects. Further details on PKPD models and their evolution have been described in several reviews.\[4, 13, 14\]
PKPD models provide an understanding of the time course of onset, duration and, maximal effect that occurs from any given dose and dosing regimen. From a learning perspective modelling PKPD systems provides the basis to identify the critical features of the biological system that influence drug effects and of the interaction between drug(s) and the system.
1.3. Population analysis

As described before, PKPD models can be developed and used to predict dose-response and guide dosing. However, data arising from human (in fact, any biological organism) studies will incorporate random variability between individuals. This is particularly true with clinical trials involving patients where there is significant variability between patients. The subjects in a clinical trial could have systematic differences in how they handle a drug (e.g. variable size or organ function). Also, process and assay errors can occur which may impact on model selection and parameter estimation. Population analyses are often employed to model data from clinical studies.

In a population analysis of a drug response, a model is developed which describes the structural relationship between dose, time, and effect and also identifies and accounts for the variability in dose-response between individuals. This group, termed a population, is representative of the intended recipients of that drug. The population model can describe the dose-response relationship for each subject in that population. The analysis provides estimation of model mean parameters, the between subject variances (BSV), and the residual unexplained variance (RUV). This provides the basis for dose individualisation as it identifies and accounts for individual patient characteristics (i.e. covariates) that predict differences in drug response.

Population analyses employ several modelling approaches, with some preferred over others [15-18]: the naïve pooled approach; the two stage approach; and the population non-linear mixed effects approach. Mixed effects refer to fixed effect parameters (e.g. mean drug CL in a patient population) and those associated with random effect parameters (e.g. between-subject variance for CL in a patient population). The naïve pooled method assumes that all observations arise from a single individual and hence can be interpreted as that no differences exist between individuals.[19] This results in the inability to estimate random effects parameters, inability to estimate the influence of significant patient covariates, and potentially
biased estimation of fixed effects parameters. For the two stage approach, in stage 1 the data from each individual is modelled separately. In stage 2 the individual parameter estimates are combined in order to calculate the mean and variance of each parameter. The population method combines all observed data and accounts for both population average effects and individual differences simultaneously. This is often carried out using a maximum likelihood method through non-linear mixed effect modelling but can also be done through Bayesian analysis.

1.3.1. Nonlinear mixed-effect modelling (NLME)

Population modelling involves estimating the best combination of parameter values for a model in order to maximise the agreement between a predicted value (e.g. drug concentration at a specific time following a specific dose) and the observed value. A population PK or PKPD model for a drug enables the prediction of drug concentration or drug effect in an individual patient. Due to the ability to utilise pooled data, the population approach can cope with sparse data and suboptimal study designs.

To illustrate NLME, let us use a simple pharmacokinetic example of a drug given as an intravenous bolus to a single subject. The predicted concentration at a given time is a function of explanatory variables such as dose and time after dose and the unknown parameters CL and V as shown in Equation 1.13.

$$ y_j = f(D, x_j, \theta) $$

Equation 1.13 A mathematical representation of a PK model in one individual

where $y_j$ is the predicted concentration at time $j$, $D$ is the drug dose, $x_j$ is the observation time, and $\theta$ is a vector ($P \times 1$) of unknown PK parameters.
However, the predicted concentration for the above subject may not match what was observed. This could be due to measurement error (e.g. assay error), modelling noise (e.g. model misspecification), or process noise (e.g. dosing time erroneously recorded). This variability, termed residual unexplained variability (RUV), needs to be included in this model and would change for each observation. The predicted concentration at a given time can now be described as:

\[ y_j = f(D, x_j, \theta) + \epsilon_j \]

Equation 1.14 A mathematical representation of a PK model with a residual variability component

where \( \epsilon_j \) is the residual error for the \( j \)th observation.

As NLME is used to analyse population data involving a series of individuals, differences in drug-response between these individuals needs to be quantified. Subjects included in a population analysis would have different covariate types or values (e.g. sex, age, weight, height, organ function, concomitant drugs). Accounting for these covariates can explain some of the variability between subjects and provide the basis for dose-individualisation.

For a series of individuals, the predicted concentration at a given time can be described as:

\[ y_{ij} = f(D_i, x_{ij}, \theta_i, \eta_i) + \epsilon_{ij} \]

Equation 1.15 A mathematical representation of a population PK model with a residual variability component

where \( y_{ij} \) is now the \( j \)th observed concentration for the \( i \)th individual and is a function of the known variables dose (\( D_i \)) and time (\( x_{ij} \)); the mean fixed effect model parameters (\( \theta_i \)); and the variability (\( \eta_i \)) between the estimation of each fixed effect
parameter for the $i$th individual from the mean values for the population; $\eta_i$ is assumed to be independent and identically distributed with a mean of zero and variance of $\omega^2$. The residual error $\epsilon_{ij}$ associated with the $j$th observation for the $i$th individual is also assumed to be independent and identically distributed with a mean of zero and variance of $\sigma^2$.

1.3.2. NONMEM

Mixed effects parameters are estimated by optimising a function of the data and the parameters.\[20\] Specialised software programmes are available to perform this estimation for non-linear mixed effects models.\[21\] The programmes share similar estimation methods which are elucidated by Davidian and Giltinan.\[22\] The estimation method includes the computation of the likelihood of the observed data arising given the model. Through iterative algorithms, the best set of parameter estimates which converges (within a certain number of significant digits) at the maximum likelihood is estimated.\[20, 23\] As the integration step required to evaluate the likelihood in non-linear mixed-effect regression is difficult, most methods perform model linearisation (e.g. first-order Taylor approximation)\[20, 24\]

NONMEM\[25\] is the most widely used programme for the analysis of population pharmacokinetic and pharmacodynamic data and was developed in the 1970s by the NONMEM® Project Group at the University of California San Francisco. NONMEM estimates the model parameters using one of several estimation methods: first-order expansion (FO), a first-order expansion about the conditional estimates of the random effects (FOCE), a second-order expansion about the conditional estimates of the random effects (Laplacian), in addition to several other methods (ITS, IMP, IMPMAP, SAEM, BAYES).\[21, 25\] This is done indirectly by minimising the extended least squares objective function which is approximately proportional to minus twice the log-likelihood (-2LL) of the data. Under the assumption of normality, maximum likelihood estimates are obtained at the minimum extended least-squares objective function value (OFV).
Once a population model is fitted to the data, the model performance is evaluated based on statistical significance, predictive performance, and biological plausibility. To show statistically whether one model performs better than another, the likelihood ratio test is usually used. As the OFV is proportional to -2LL, and the likelihood ratio is asymptotically and approximately chi-squared distributed, a decrease in OFV between two nested models of $\geq 3.84$ points denotes a p-value $< 0.05$ with one degree of freedom. The precision of parameter estimates is computed using either the asymptotic standard errors obtained via maximum likelihood estimation or through non-parametric bootstraps. The model’s predictive performance can be assessed through the use of diagnostic plots and visual predictive checks which can show whether model predictions are biased. The predictive performance can also be assessed by using the model to predict into a new data set. Additionally, parameter values and covariate effects in the final model are checked for biological and/or mechanistic plausibility.

NONMEM is written in the programming language FORTRAN. It is used along with several other software programmes such as PREDPP (PREDictions for Population Pharmacokinetics) which is a library of pre-programmed pharmacokinetic models and NM-TRAN which translates NONMEM language into FORTRAN code which can then be compiled with NONMEM to initiate a run. NONMEM was used for data analysis in this thesis (Part IV).
1.4. **Body size/composition as a covariate**

As described above, a population analysis quantifies the between-subject variability which helps to evaluate the relationship between model parameters of interest (e.g. drug CL) and observable patient covariates (e.g. creatinine clearance). It is important to note here that patient covariates account for some of a parameter’s BSV and that some variability would still persist and appears random (see section 1.6.1 below).

Covariate modelling can improve model fit, explain part of the difference between population mean parameters and individual parameter estimates, and improve the mechanistic interpretability of the model. Covariates can therefore provide the basis for dose-individualisation (e.g. basing drug dose on creatinine clearance). Body size has been an important covariate to scale pharmacokinetic parameters (and hence drug dosing).

1.4.1. **Body weight as a scalar for drug dosing**

Total body weight (Wt) has been an important covariate to scale drug doses. This scaling method is based on an assumption that the rate of drug metabolism is proportional to Wt. However, as the body’s metabolic processes occur mainly in non-fat tissues \([26]\), using Wt as a scalar is likely to over-predict drug clearance in some individuals.\(^{[27]}\) This is especially the case in overweight and obese subjects where the proportion of fat is higher per weight.

Various size descriptors (often based on Wt) have been derived and proposed as alternatives to Wt with variable success.\(^{[28]}\) These include: ideal body weight (IBW), body mass index (BMI), adjusted body weight (ABW), allometric scaling, lean body weight (LBW), and fat-free mass (FFM). A brief history and comparison of these various size descriptors can be found here.\(^{[29,30]}\) The sections below will focus on allometric scaling and fat-free mass due to their relevance to this thesis (Part IV).
1.4.2. Allometric scaling

An organism’s physiological parameters correlate with its size. This gave rise to the law of allometry which originally referred to the disproportionate change in morphology relative to a change in body size \cite{31} but was expanded to include disproportionate changes in physiological function relative to size. \cite{32, 33} Consequently, PK parameters can be scaled within and across species using a descriptor of size in accordance to the following equation:

\[ Y = \beta \times X^\alpha \]

**Equation 1.16 The law of allometric scaling in relation to body mass**

where \( Y \) is the parameter of interest; \( X \) is body mass in kg; \( \beta \) is an origin index (intercept); and \( \alpha \) is the scaling exponent. Note that an exponent of 1 indicates an isometric (rather than allometric) relationship.

There is some contention about what value to assign to the exponent parameter (\( \alpha \)) when modelling population PK data. Estimation of this parameter is not always accurate or possible. One school of thought is the use of \( \frac{3}{4} \) which has its origin in interspecies scaling of metabolic rates where values of 0.73-0.74 were calculated.\cite{34-38} West et al argued that this “quarter power” scaling is supported by biological basis.\cite{32} This prompted several pharmacological scientists to propose the use of \( \frac{3}{4} \) when the parameter CL is scaled given the similarity between CL and basal metabolic rate. \cite{38-43} However, others contend that the exponent needs to be estimated for any particular population given the wide range of exponent values estimated \cite{44, 45} and the possible misinterpretation of original metabolic rate research from which the quarter power exponent originated.\cite{46} Also, evidence from interspecies studies suggests that complex differences in drug metabolism and metabolite profiles exist and can’t be accounted for by scaling according to Wt alone.\cite{47} Finally, it has not been established whether Wt or another size descriptor should be used for body mass (\( X \) in equation 1.16).
1.4.3. Fat-free mass

Fat-free mass (FFM) describes the lean component of the body excluding fat.\[^{[48]}\] It is essentially the sum of muscle mass, bones, non-adipose components of internal organs, and extracellular fluid. Previous studies have described lean body weight (LBW) as a measure of non-fat weight. However LBW is not easily amenable to measurement, in contrast to FFM, and it is usual therefore to approximate LBW by FFM.\[^{[30]}\] Of note, in the same individual FFM takes a slightly lower value than LBW as it excludes all fat (including for example the bilayer lipid membranes in cells).\[^{[49]}\]

Various models have been developed to predict FFM following Behnke’s et al evaluation of under-water weighing to quantify body composition in live humans.\[^{[50, 51]}\] Models have been derived from height and weight \[^{[52]}\], skinfold thickness \[^{[53]}\], underwater weighing \[^{[53, 54]}\], total body potassium \[^{[54]}\], and bioelectrical impedance \[^{[55]}\]. Recently, a model for FFM in adults was developed (Equation 1.17) based on Wt, Ht, and sex using bioelectrical impedance and dual-energy x-ray absorptiometry.\[^{[30]}\] The dataset contained a wide range of bodyweights hence the model is expected to have good predictive performance even at the extremes of size.

\[
FFM_{\text{male}} = \frac{(9270 \times Wt)}{6680 + (216 \times BMI)}
\]

\[
FFM_{\text{female}} = \frac{(9270 \times Wt)}{8780 + (244 \times BMI)}
\]

Equation 1.17 FFM model for adult males and females
1.4.3.1. Fat-free mass in children

Body composition changes in relation to age along with changes in body size and shape.[56] A robust model to predict FFM in adults has been developed [30] but was not developed for children. This model incorporate sex, Ht, and Wt as covariates and for it to be used to predict into children, parameters relating to age and/or maturation need to be accounted for.

There is a lack of longitudinal studies to examine changes in body composition with age. One exception is the Fels study which looked at human development from birth into adulthood.[57] It is understood that the maturation of a child is related to his or her growth and development, especially during adolescence. Using data from the Fels study, Reynolds demonstrated that early sexual maturity was related to increased rates of growth in muscle and subcutaneous adipose tissue.[58] James Tanner, a paediatrician, conducted maturation studies including the Harpenden Growth Study which was a longitudinal study of the growth of children from preschool age to maturity.[59-61] To describe growth, Tanner assigned successive stages (Tanner Stages 1 to 5) of development of secondary sex characters at puberty. However, there is a lack of data linking the rate or level of maturation with changes in body composition.

Wt has been and is still used to predict drug doses in paediatric patients but excess fat does not contribute to drug clearance. CL is related to the intrinsic elimination capacity of the various organs and their perfusion. As fat tissue has no intrinsic extraction properties for most drugs, using Wt as a covariate will provide increasingly poor prediction of CL as obesity (i.e. excess fat) increases.

The relationship between size and drug clearance for children is considered to be non-linear.[41] In 1950, body surface area was proposed as a more accurate size descriptor to predict dosing requirements in children than Wt.[62] Various other models were developed and evaluated with various success.[37, 63-67] More recently, FFM calculated using an adult model and a model for organ function maturation
was shown to be superior to both Wt and body surface area for description of
glomerular filtration rate from premature neonates to young adults.\[68\] Finally,
allometrically-scaled weight linked to a metabolic maturation model was proposed
as a suitable method to describe the changes in size and organ function over age.\[37, \]
\[38\]

Several metabolic maturation models have been developed where the metabolic
rate increases as a function of age (Equation 1.18). \[38, 41, 68\] Several asymptotic models
were used including most notably the sigmoid hyperbolic and asymmetric sigmoid
hyperbolic functions. Also, several age descriptors have been used including
postnatal age, postmenstrual (PMA) age, and postconceptional age. Postmenstrual
age (PMA) has been advocated as it accounts for maturation occurring before birth. \[37\]

\[
M = \frac{PMA^\gamma}{PMA^\gamma + TM_{50}}
\]

Equation 1.18 An example of a metabolic maturation model. The model type
is symmetric sigmoid hyperbolic and the age descriptor is the postmenstrual age

where $M$ is metabolic maturation and assumes a number between 0 and 1; $PMA$ is
postmenstrual age in years; $\gamma$ is a sigmoidicity parameter; and $TM_{50}$ is the
maturation half-time (i.e. PMA at which the metabolic maturation is half the size-
adjusted asymptotic/adult value).

It is hypothesised that a FFM model developed for children (rather than
extrapolated or allometrically-scaled from adults) would describe drug clearance
better in this age group. The development, evaluation, and implementation of a FFM
model is the subject of Part IV (Chapters 6 and 7) of this thesis.
1.5. Heparins

The Heparins are glycosaminoglycan polysaccharides used in clinical practice for their anticoagulant effect. Heparin, also called unfractionated heparin (UFH), was discovered in 1916 at Johns Hopkins Medical School by Jay McLean who was a medical student working in Professor William Howell’s laboratory. Professor Howell was interested in the apparent balance in blood between procoagulants and anticoagulants and employed McLean to extract a procoagulant (later known as thromboplastin or tissue factor) from crude thromboplastic tissue. Instead, McLean extracted a fat-soluble compound from canine liver which appeared to possess an anticoagulant effect. A few years later, Howell extracted a water-soluble compound with similar properties and purified it for human use; he later termed it ‘heparin’ in reference to the liver. Of note, both McLean and Howell claimed credit for the discovery of heparin.

Commercially, UFH is extracted from porcine intestinal mucosa as a heterogeneous mixture of disaccharide chains of varying lengths. Since the 1980s, smaller fragments of heparin (low molecular weight heparin, LMWH) were trialled as safer and more convenient alternatives to UFH.

In order to understand how the heparins work as anticoagulants it is important to understand the coagulation process. Coagulation is a complex and important physiological process involved in haemostasis where a balance is maintained between clot formation and dissolution. The coagulation process involves complex interactions of clotting enzymes and proteins and numerous feedback and feedforward reactions. Clotting is achieved when soluble fibrinogen (factor I) is activated to form insoluble fibrin strands (factor Ia). This occurs through two mechanisms: the intrinsic (often activated artificially in the laboratory) and extrinsic (activated through endothelial tissue injury) clotting pathways. The two pathways converge on the crucial step of factor X activation (to Xa) which activates factor II (to IIa) and subsequently factor I (to Ia).
Activated clotting factors, essentially protease enzymes, are inhibited by antithrombin (AT). AT contains a specific arginine-serine peptide sequence to which the activated factors bind forming a stable complex. Heparins bind to AT via a pentasaccharide sequence on the heparin molecule and induce a conformational change in AT making the Arg-Ser peptide sequence more accessible to the proteases. This conformational change accounts for the AT-mediated inhibition of factor Xa (aka anti-Xa effect). The inhibition of factor IIa (aka anti-IIa effect) is thought to occur via a different mechanism. Long heparin molecules (≥ 18 saccharide units, MW > 5400 Da) serve as a catalytic template to which both AT and IIa bind, effectively inhibiting IIa (Scheme 1.1).

\[
\begin{align*}
T + AT & \rightleftharpoons T-AT \\
H + AT & \rightleftharpoons H-AT \\
T + H-AT & \rightleftharpoons T-H-AT \rightleftharpoons T-AT + H
\end{align*}
\]

Scheme 1.1: Reaction sequence underpinning heparin effect.

T = thrombin (or IIa); H = heparin; AT = antithrombin.

In this thesis, enoxaparin and UFH are discussed and used to illustrate how pharmacometric modelling can be used to individualise drug dosage thus optimising patient care. Chapters 2-5 discuss the use of enoxaparin and the development, evaluation, and implementation of a dose-individualisation method in adult patients. Chapter 7 describes the use of patient covariates in the development of a PKPD model of UFH in children.

1.5.1. Enoxaparin

Enoxaparin is a low-molecular weight heparin (LMWH) that is widely used in clinical practice for the treatment and prevention of thrombosis (e.g. myocardial
infarction, deep vein thrombosis, and atrial fibrillation).\textsuperscript{[69]} It acts by binding to AT and enhancing its ability to bind to and deactivate factors Xa and to some extent IIa; this ultimately hampers the formation of a thrombus.\textsuperscript{[79]} It has largely replaced UFH as the parenteral anticoagulant of choice for the management of thrombosis due to its similar or even superior effectiveness without an increase in the incidence of major bleeding events.\textsuperscript{[80-88]} However, the rate of minor bleeding and bruising events was found to be higher than UFH.\textsuperscript{[86]}

Enoxaparin is renally eliminated (fraction excreted unchanged is estimated to be 0.71)\textsuperscript{[89]} and its dose is based on total body weight and renal function.\textsuperscript{[90]} Monitoring of enoxaparin therapy is advocated in the clinical setting especially for patients who are at the extrema of body weight or have a degree of renal impairment in order to reduce the risks of haemorrhage or treatment failure.\textsuperscript{[91]} Since enoxaparin is composed of small heparin fragments of various chain lengths direct measurement and interpretation of blood concentrations is difficult. Instead, an anti-Xa activity assay has been developed and is used for enoxaparin drug monitoring in some clinical settings.\textsuperscript{[92]}

Therapeutic monitoring of enoxaparin through anti-Xa testing is not routinely done in clinical practice. This could be due to various reasons including: the lack of an optimal treatment target, the perceived notion that the dose-response relationship of enoxaparin is predictable across individuals, the perception that enoxaparin is associated with less bleeding events than its predecessor, and the cost/instability of the assay kit. The assay is available only in a small number of hospitals across New Zealand and the assay result would usually take two days to become available to the treating clinician.

When used for the active treatment of thrombosis, enoxaparin is dosed twice daily on a mg/kg of Wt basis and the frequency is halved to once daily when a patient’s CLcr is less than 30 mL/min. Presumably the rationale for these guidelines is that drug CL would increase as body mass increases and also proportionally to
CLcr. However, the guidelines are rather simplistic because: 1) the relationship between CL and Wt is non-linear and, 2) CLcr is a continuous variable so the dichotomy of dosing choices when CLcr is close to the cut-point, 30 mL/min, is likely to result in suboptimal dosing. It has been shown that clinicians often do not adhere to these guidelines especially at the cusp of Wt and CLcr.\[91\] A popular alternative to dosing in the overweight and obese is the arbitrary dose-capping (at 100 mg) which is considered by some as a safer option.\[93\] Needless to say, dose capping may result in underdosing as the absolute CL in the obese patient is higher than in the non-obese but not proportionally to weight.\[94\] An alternative dosing strategy based on FFM has been shown to be a safer option.\[95\] However, the question remains whether covariate-based dosing is sufficient to optimise treatment and whether treatment could be improved through individualised, iterative, response-based dosing.

1.5.2. Unfractionated heparin

Although largely superseded by LMWHs as the anticoagulant of choice, UFH is still used in the acute setting due to its short half-life and easy reversibility through the use of protamine. UFH is the drug of choice to reduce the risk of thromboembolic complications in children during cardiac catheterisation.\[96, 97\]

UFH binds to several proteins in the plasma including: antithrombin, clotting factors, fibrinogen, plasmin, albumin, and lipases.\[98\] UFH is confined mainly in the vascular space and has a distribution volume similar to either the plasma volume or the blood volume depending on what drug assay is used.\[99-101\] The half-life is short and dose-dependent and varies with the assay method used for measurement.\[102\] Some of the first population studies of heparin pharmacokinetics where a single known dose (47-600 IU/kg) was administered and samples were measured at three sampling times post-dose reported a dose-dependent half-life of between 0.7-2.5 h.\[99, 103\] Estes et al were the first to use a computerised method (a Fortran IV programme and IBM System/360). In this work, a one-compartment first order
model was found to best describe the data. The volume of distribution was estimated to be around 0.06 L/kg.\textsuperscript{[99]} UFH concentration was determined through a protamine titration assay which was developed a few years before.\textsuperscript{[104]} UFH does not appear to be metabolised, filtered, or secreted and neither renal nor hepatic impairment appear to reduce its CL. Instead, it is transferred to and possibly degraded by the reticuloendothelial system.\textsuperscript{[99]}

The quest for practical, precise, and universal dosing guidelines for heparin therapy is a few decades old.\textsuperscript{[105-107]} Dose and monitoring requirements tend to vary widely so reliable dosing guidelines are difficult to produce.\textsuperscript{[108]} Many of the difficulties inherent in developing reliable dosing guidelines for UFH are attributed to its molecular heterogeneity (molecular weight is 3 to 30 kD), multiple and variable binding sites, differences among methods for measuring its PK and PD responses, and apparent non-linear elimination.\textsuperscript{[98, 109]}

Various clinical assays are available for measuring the PK and PD responses of UFH. These assays have been divided into two categories: global and specific.\textsuperscript{[110]} Global assays, such as aPTT, measure the time it takes for the blood to clot following the administration of UFH whereas specific assays, such as anti-Xa, measure the extent of inhibition of certain activated clotting factors. The aPTT assay measures the time required for coagulation to occur in a blood sample after the addition of an activating agent (e.g. kaolin) and has a high degree of variability between patients\textsuperscript{[111]} and between reagent batches.\textsuperscript{[112, 113]} Other global assays include the Lee-White whole blood clotting time, thrombin clotting time, and activated coagulation time.\textsuperscript{[114]} The anti-Xa assay measures the optical density of a chromogen released by the enzymatic effect of uninhibited Xa in sample.\textsuperscript{[115]} Other specific assays include anti-Xa clot formation test, polybrene titration, and protamine titration.\textsuperscript{[110]}

Although the PK of UFH has been described by a one-compartment, first-order elimination model \textsuperscript{[99]}, later studies which have used radiolabelled heparin \textsuperscript{[116]} or specific assays such as anti-Xa \textsuperscript{[117]} have demonstrated non-linear elimination. Some
studies have suggested that heparin is cleared through a combination of a rapid saturable mechanism and much slower first-order mechanisms.\textsuperscript{[103, 117-119]} The saturable phase is attributed to binding to endothelial cell receptors and macrophages, where it is depolymerised whereas, the slower, unsaturable mechanism of clearance is largely renal.\textsuperscript{[120]} At therapeutic doses, a considerable proportion of heparin is cleared through the rapid saturable mechanism. These kinetics make the anticoagulant response to heparin nonlinear at therapeutic doses, with both the intensity and duration of effect rising disproportionately with increasing dose.

The use of UFH in clinical paediatric practice is complicated by the lack of clinical outcome studies to determine a therapeutic target. Various dosing regimens exist for a variety of clinical indications and the treatment targets have largely been extrapolated from studies in adults.\textsuperscript{[121]} This target is an aPTT that reflects a heparin level by protamine titration of 0.2 to 0.4 units/mL or an anti-Xa level of 0.35 to 0.7 units/mL. The aPTT therapeutic ranges are universally determined using adult plasma and extrapolating from adults to paediatric patients is likely to be troublesome. For instance, evidence suggests that baseline aPTT values in neonates are often higher compared to adults.\textsuperscript{[122]} Also, the target aPTT (2.5 x baseline aPTT and correlates to an anti-Xa level of 0.35 to 0.7 units/mL) varies significantly with age and heparin dose. \textsuperscript{[123, 124]} Furthermore, infants tend to have lower levels of antithrombin resulting in spurious measurements.\textsuperscript{[125]} These factors make designing practical, precise, and universal dosing guidelines for heparin therapy in children the more difficult.
1.6. The role of pharmacometrics in dose-individualisation

“Variability is the law of life, and as no two faces are the same, so no two bodies are alike” Dr William Osler, 1903

“The only principle of dosage that survives is that the dose must be adjusted to the individual patient, and that nothing can or will supersede clinical experience, and careful study, combined with good judgment” [126] Professor W Dawson, 1940

Pharmacometric analyses allow the quantification of the time-course of drug effect in an individual and across a patient population. This is essential as clinicians aspire towards credible and precise dosing methods to suit the variable needs of their patients. This is clear from the above quotes by William Osler and W Dawson who, like all physicians, have witnessed this variability in their clinics and have often relied on experience and educated guesses to optimise treatment in their non-average patient. I demonstrate in this thesis that a significant portion of this variability can be accounted for through model-based dosing and that dose-individualisation aided by pharmacometrics can optimise patient care.

1.6.1. Variability between individuals

In section 1.3 above, the predicted drug concentration in a patient population at a given time was described by equation 1.15:

$$y_{ij} = f(D_i, x_{ij}, \theta + \eta_i) + \epsilon_{ij}$$

In this model, $\eta_i$ represents the difference between the parameter predictions for the $i$th individual and the typical values for the population ($\theta$) and has a mean of zero and variance of $\omega^2$; and $\epsilon_{ij}$ is the residual error associated with the $j$th observation for the $i$th individual and has a mean of zero and variance of $\sigma^2$. In a well specified model, the residual variance ($\sigma^2$) is comprised mainly of assay error in addition to unpredictable process noise. The between subject variance ($\omega^2$) is the main
contributor to differences between the observed and the predicted biomarker (e.g. drug concentration) between individuals. This variance is comprised of predictable (BSVp) and unpredictable or random (BSVr) components. The latter also comprises between-occasion variability (BOV) which is often small as a large BOV would likely preclude a drug from being clinically useful or even gaining regulatory approval in the first instance if BOV was estimated. BSVp can be reduced by accounting for influential covariates (e.g. size, organ function, disease state, or genetics) on parameter estimates. However, variability in PK parameters across a patient population still remains even after accounting for patient covariates. Variability in a PK parameter is often quantified using the coefficient of variation percentage. A recent review showed that the average CV% for clearance (based on 181 population PK studies) was 40% (IQR 26 – 48)[127] This corresponds to a 5-fold variability in steady state average concentration (Css,ave) which clinically necessities a 5-fold difference in dose-requirements to achieve a target Css across the population (Figure 1.5). The authors suggest a recalibration of current perception of variability so that 25-50% be considered normal. Although this refers to PK variability, which is a key source of variability in drug response, complex PD responses (e.g. coagulation) can present significant BSV in PD parameters.[128]

This begs the question, should all drugs be monitored and dose-individualised? As the purpose of dose-individualisation is to adapt a dosing regimen in order to maximise drug effect and minimise side effects in an individual, a target needs to be defined.
1.6.2. Target concentration strategy

In 1972, Sheiner and Tozer introduced the term target concentration strategy (TCS) in order to improve the outcomes of therapeutic drug monitoring (TDM).\textsuperscript{[129]} TDM refers to a dose individualisation strategy where doses are individualised to maintain plasma concentrations within a defined therapeutic range without actively considering the needs of the individual patient and with the assumption that drug concentrations within the therapeutic range will result in optimal clinical outcomes.\textsuperscript{[130, 131]} It is erroneously assumed that a drug concentration below the lower end of the range will result in treatment failure and that concentrations above the upper end of the range are toxic.\textsuperscript{[132]} In reality, however, the relationship between drug concentration and beneficial and adverse effects is continuous. Therefore, the target concentration should be an optimal concentration (for each individual patient) which maximises that patient’s net benefit (i.e. beneficial target effect with acceptable adverse effects).
In section 1.6.1 above, a question was posed: should all drugs be monitored and dose-individualised? Variability in dose-response between patients is multi-fold and it is not possible to achieve the target range in every single patient due to the BSVr. In 2012, Holford and Buclin proposed the concept of safe and effective variability (SEV).[132] The authors used the criterion employed in bioequivalence studies (90% chance that a bioequivalence statistic lies within 80-125% of the average of the reference formulation) to define the probability of whether a dosing strategy would be safe and effective. SEV can help identify a therapeutic range where a range of target concentrations (e.g. Css) is considered likely to yield a net-benefit.

Based on that, if the achieved concentration in an individual lies within this range, the treatment in the population may be considered safe and effective. If SEV is larger than BSV, then dose-individualisation is not needed. If SEV is smaller than BSV but larger than BSVr, then covariate-based dosing is needed. If SEV is smaller than BSVr, then dose-individualisation is needed on top of covariate-based dosing. Lastly, if SEV is smaller than BOV, then no dosing method could possibly result in a safe and effective target concentration and the drug cannot be used.[132]

In 1995, Holford proposed target concentration intervention (TCI) as a dosing strategy.[133] TCI is defined as a dose individualisation strategy where the target drug response for an individual patient is related to a PK or PD biomarker (e.g. drug concentration or anti-Xa) that will achieve this target. TCI evolved from TDM and was proposed as a more patient-centred approach to dose individualisation.[130] TCI has been successfully implemented in clinical practice in adult and paediatric patient populations.[134-136]
1.6.3. Dose-individualisation

Dose individualisation involves the selection of a drug dosing regimen that suits the needs of an individual patient in order to increase drug effectiveness and minimise its adverse effects. Dose individualisation is not a new concept: in the 1700s William Withering used the intensity of diuresis to reduce digitalis dose and mitigate its toxicity.

Four drug dosing methods exist: 1) a population method where the average drug CL is used to predict dose requirements and therefore the same standard dose is given for all patients; 2) a covariate method where patient variables such as size and organ function are used to predict the group clearance and therefore all individuals with the same set of covariates will be given the same dose (e.g. enoxaparin where the group is defined on the basis of weight and renal function); 3) an informal adaptive feedback method where a dose is given (often a covariate-based dose) and at some stage a response measurement is made and the dose is adjusted heuristically (e.g. blood pressure and ACE inhibitors); and 4) a formal adaptive feedback method where computerised algorithms are used to incorporate the value of the response variable and the dose is adjusted via a computer algorithm (e.g. TCIWorks for gentamicin).

In reality, very few drugs are dosed based on method (1) as this implies that no covariate would change dose. Almost every drug, except perhaps drugs that are administered to a local site, are dose-individualised based on covariates (e.g. paracetamol dose per Wt in children). Similarly, very few drugs are dosed based on method (4) perhaps not surprisingly as drugs that need formal adaptive control may receive limited interest in the market due to complexity in their dosing.
For Dr Osler and Professor Dawson, drug dosing and dose adjustment was based on the clinician’s experience and ‘gut feeling’, perhaps not dissimilar to dosing method (3) above. However, variability in dose-response, the sources of much of this variability, and the treatment targets can now be quantified much better with the help of PKPD modelling. Surely this can translate to better dosing and improved patient outcomes. In Chapters 2 to 5 of this thesis, the treatment target and dose-individualisation of enoxaparin using a computerised algorithm will be explored. In Chapter 6, a model to predict a useful size descriptor (FFM) will be developed and evaluated. Finally, in Chapter 7, the FFM model will be used in order to predict heparin (UFH) disposition in children and the utility of this model in dose-prediction will be explored.
Part II

ENOXAPARIN TARGET DEVELOPMENT AND DOSING EVALUATION
Chapter 2: Enoxaparin target development

Parts of this chapter are based on the following peer-reviewed publication:

**Al-Sallami HS, Barras MA, Green B, Duffull SB.** *Routine plasma anti-Xa monitoring is required for low molecular weight heparins.* Clinical Pharmacokinetics 2010; 49(9):567-571
Part II encompasses enoxaparin target development and dosing evaluation and contains two chapters. Chapter 2 outlines the development of a treatment target for enoxaparin from a dataset previously collected by collaborators during a clinical trial. Chapter 3 covers an evaluation of a Bayesian forecasting method (TCIWorks) for enoxaparin dose-individualisation using the target developed in Chapter 2.
2.1. Introduction

Low-molecular-weight heparins (LMWHs) are anticoagulants used in the treatment and prevention of thromboembolic diseases. When compared with unfractionated heparin (UFH), LMWHs are at least as effective in the treatment of acute coronary syndromes\cite{80,81} and venous thromboembolism,\cite{82} with a similar incidence of bleeding.\cite{80-82} LMWHs have been described as having several advantages over UFH, including (i) linear pharmacokinetics; (ii) a more predictable dose-response relationship; (iii) a longer elimination half-life; (iv) superior subcutaneous bioavailability; and (v) a lower incidence of thrombocytopenia and osteopenia.\cite{139,140} However, it is unclear whether these purported benefits translate into improved clinical utility in the short term. In this article, we contest the notion that the ‘predictable pharmacokinetics’ of LMWHs negate the need for routine plasma monitoring of anti-Xa activity. Although we use enoxaparin as an example, we believe the context applies equally to all LMWHs. Different LMWHs contain different distributions of molecular weight fractions, but current evidence does not show significant differences in the pharmacokinetic/pharmacodynamic profiles of these drugs. Most of the information on pharmacokinetics/pharmacodynamics arises from enoxaparin, and we therefore believe that this should, at least for the time being, be generalised to all LMWH versions.

We now consider the term ‘predictability’. In its broadest sense, predictability can be defined as the combination of accuracy and precision, where accuracy refers to the ability to obtain an unbiased event on average, and precision is a measure of reproducibility. Within our present context, precision refers to the ability to achieve a specified target concentration or response in different individuals where there is between-subject variability (BSV) associated with pharmacokinetic and pharmacodynamic parameters. Does enoxaparin fulfil these criteria? It is recognized that the standard treatment dose of enoxaparin (1 mg/kg twice daily, based on total bodyweight [Wt]), is accurate (unbiased) in that it achieves, on average, appropriate outcomes for a population – for example, a reduction in the risk of myocardial
Chapter 2: Enoxaparin treatment target development

Infarction. This has been demonstrated in clinical studies.\cite{83,141} In terms of precision, however, it must be considered whether standard doses achieve the proposed target anti-Xa activity across a range of individuals. BSV in the apparent volume of distribution (V) and clearance (CL) of enoxaparin is, however, sufficiently high (33\% and 34\%, respectively)\cite{89,94} to cast doubt on the predictability of plasma enoxaparin concentrations. Note that the original purported advantages are related specifically to BSV for items (ii) and (iv) above. In addition, it is noted that the concept of linear pharmacokinetics [item (i)] does not mean ‘predictability’. This is not unique to enoxaparin—for example, gentamicin has linear pharmacokinetics but high BSV relative to its small therapeutic index, which necessitates pharmacokinetics-based monitoring.\cite{142}

Consider that pharmacokinetics-based monitoring is indicated when the standard drug dosage has a high probability of an unwanted response, such as treatment failure or adverse effects. The ability to achieve a target response from a standard dose of a drug is a function of its BSV and therapeutic index. Assuming that within-subject variability is low, if BSV is significantly large compared with the therapeutic index, then pharmacokinetics-based monitoring is indicated. As LMWHs have significant BSV, we hypothesize that conventional dosing will not achieve the target response more often than current dosing guidelines for UFH. Therefore, LMWHs should be considered candidates for pharmacokinetics-based monitoring.

The aim of this study is to determine whether routine monitoring of anti-Xa concentration during enoxaparin treatment is warranted. To test our hypothesis, therapeutic targets for enoxaparin and UFH are defined. Models for pharmacokinetics and pharmacodynamics are used to simulate dosing outcomes and thus compare the ability of each drug to achieve a therapeutic target. In addition, independent empirical evidence from clinical trials is considered.
2.2. Targets

Dosing of UFH is monitored by measuring the activated partial thromboplastin time (aPTT) in plasma. The initial dose of UFH is empirical and the dose is then adjusted on the basis of aPTT values to achieve a desirable range of 1.5–2.5 times the normal physiological value.[143]

The current licensed dosage of enoxaparin for the treatment of acute coronary syndromes is 1 mg/kg of Wt twice daily, with a dosage reduction to once daily if the patient’s creatinine clearance ($CL_{cr}$) is < 30 mL/min.[90] The concentration of enoxaparin is quantified by measuring anti-Xa activity in plasma.[143] The correlation between anti-Xa activity and clinical outcomes is not clear cut, but dose-finding studies of enoxaparin, such as the TIMI (Thrombolysis in Myocardial Infarction) 11A trial, have demonstrated that an increase in the dosage from 1 mg/kg twice daily to 1.25 mg/kg twice daily is associated with a corresponding increase in both anti-Xa activity and the incidence of major bleeding (from 1.9% to 6.5%).[144] This phase II study suggested that anti-Xa activity between 500 and 1000 IU/L was found to maintain effectiveness and minimize bleeding events. However, it is unclear from this study whether the peak activity, the minimum activity during a dose interval or some other measure of exposure needs to be within this range. Given a terminal elimination half-life of 4–6 hours, it is almost impossible to guarantee that any individual will achieve both peak and trough activity within this range. The range, therefore, provides some therapeutic interest but is not a practical target for monitoring. In a large cohort study by Montalescot et al.[145], estimated peak (between 4 and 6 hours post-dose, referred to henceforth as C5) anti-Xa activity of < 500 IU/L was associated with more than a 3-fold increase in mortality due to therapeutic failure, compared with peak anti-Xa activity of > 500 IU/L.

In a recent randomised-controlled trial (RCT), Barras et al.[95] showed that individualization of enoxaparin dosing on the basis of a patient’s lean bodyweight and renal function decreased bleeding (relative risk [RR] = 0.12, 95% CI 0.01, 0.89) and bruising events (RR = 0.3, 95% CI 0.12, 0.71) when compared with conventional,
product-label dosing. From data arising from that RCT (see Appendix 1, A1.1 for a description of the data), we compared the maximum anti-Xa activity (i.e. the peak), the minimum anti-Xa activity (i.e. the trough) and the area under the activity/time curve (i.e. the AUC) between patients who experienced a bleeding event and those who did not. Trough activity showed the biggest difference between the two groups. This supposition was recently supported in the literature on another Xa inhibitor.[146]

We then plotted the RR reduction in bleeding against the minimum anti-Xa activity over the dose interval (trough activity). It was seen that trough anti-Xa activity of 500 IU/L or less was associated with half the bleeding risk (Figure 2.1). A reasonable, and accessible, target for activity is therefore proposed, where the peak activity exceeds 500 IU/L and the trough activity falls below 500 IU/L under a twice-daily dosing schedule. This C5 and trough target should ideally be reached within the first 48 hours and also at steady state.

Figure 2.1 Relative risk (RR) reduction vs the minimum plasma concentration (C_min) of enoxaparin. C_min is the concentration at 12 h after dosing in a twice daily dosing regimen.
2.3. *Evidence from model-based simulations:*

Using the targets defined above, models for pharmacokinetics and pharmacodynamics are now used to compare the ability of enoxaparin and UFH to achieve the therapeutic target.

2.3.1. *Simulation of unfractionated heparin:*

We simulated aPTT-time profiles of 10,000 virtual patients who received a constant infusion of UFH at a dose of 1500 IU/h for 48 hours. See Appendix 1, A1.2 for MATLAB code. We assumed a Michaelis-Menten pharmacokinetic model (Equation 2.1) with an empirical pharmacodynamic model linking the concentration to the aPTT (Equation 2.2):

\[
\frac{dA}{dt} = \frac{R - V_{\text{max}}}{k_m + \frac{A}{V}} \times \frac{A}{V}
\]

*Equation 2.1 A PK model for unfractionated heparin*

where \( \frac{A}{V} \) is the UFH plasma concentration; \( R \) is the infusion rate (1500 IU/h); \( V_{\text{max}} \) is the maximum rate of elimination; \( k_m \) is the Michaelis-Menten constant.

\[
aPTT = aPTT_0 \times \exp(M) \times A
\]

*Equation 2.2 A PD model for unfractionated heparin*

where \( aPTT \) is the activated partial thromboplastin time and dependent on \( A \); \( aPTT_0 \) is the baseline \( aPTT \) and is assumed to be 26–36 seconds; and \( M \) is the slope of \( A \) versus the aPTT, with a mean ± SD value of 1.5 ± 0.5.
The model was based on the work of Mungall and Floyd\cite{147} where the warfarin component of the model was removed. Table 2.1 provides a summary of parameter estimates of the model and their relationship to the covariates.

### Table 2.1 Parameter estimates for the unfractionated heparin model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>CV% of BSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_m (IU/L)$</td>
<td>450</td>
<td>22.2</td>
</tr>
<tr>
<td>$V_{max} (IU/h)$</td>
<td>3121</td>
<td>31.4</td>
</tr>
<tr>
<td>$V (L)$</td>
<td>4.9</td>
<td>3</td>
</tr>
</tbody>
</table>

BSV= between-subject variability; CV= coefficient of variation

#### 2.3.2. Simulation of enoxaparin:

Concentration-time profiles of 10,000 virtual patients were simulated for a standard dosing regimen of 1 mg/kg of Wt twice daily for 6 days. See Appendix 1, A1.3 for MATLAB code. Patients with a $CL_{cr} < 30$ mL/min were excluded. We assumed a two-compartment model with first order input and linear elimination, as described by Green et al.\cite{89,94} (Table 2.2). The model is a multiple univariate model, where the covariance is assumed to be zero. The residual variability was excluded in order to represent the true but unknown concentration values. The virtual patients were generated from groups of patient characteristics – such as height, bodyweight, sex and serum creatinine – that closely resembled those from the RCT performed by Barras et al.\cite{95}
Table 2.2 Parameter estimates for the enoxaparin model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>CV% of BSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CL(L/h)$</td>
<td>0.858</td>
<td>32.7</td>
</tr>
<tr>
<td>$V_1(L)$</td>
<td>4.567</td>
<td>34.4</td>
</tr>
<tr>
<td>$k_a(h^{-1})$</td>
<td>0.255</td>
<td>25.2</td>
</tr>
<tr>
<td>$V_2(L)$</td>
<td>29.6</td>
<td>29.9</td>
</tr>
<tr>
<td>$Q(L/h)$</td>
<td>0.62</td>
<td>69.8</td>
</tr>
</tbody>
</table>

$CL$ = total body clearance; $k_a$ = absorption rate constant; $Q$ = intercompartmental clearance; $V_1$ = apparent volume of distribution in the central compartment; $V_2$ = apparent volume of distribution in the peripheral compartment.

2.3.3. Simulation results:

Among the UFH group, 48% achieved a steady-state average aPTT target within 48 hours of treatment (with 26% over target and 26% under target) [Figure 2.2]. Similarly, the enoxaparin dose achieved the target concentration (peak > 500 IU/L and trough <500 IU/L) in 54% of patients during the course of treatment (with 23% over target and 23% under target; no subjects failed to achieve both targets simultaneously) [Figure 2.3a and 2.3b]. As expected, the average patient achieved the target, indicating that the target is, on average, appropriate and that the dosing regimen is not biased. However, it is clear that current dosing recommendations do not provide reproducible (precise) results across the population.
Figure 2.2 Simulation of the activated partial thromboplastin time (aPTT) vs time after administration of unfractionated heparin. The 5th, 50th and 95th percentiles are shown. The middle profile fulfils the target criteria, where aPTT values are within 1.5–2.5 times the baseline value (represented by horizontal dashed lines). The top profile exceeds the range and is thus associated with a higher risk of bleeding. The bottom profile is associated with a higher risk of treatment failure.
Figure 2.3 Simulation of anti-Xa activity vs time (a) after dose 3 and (b) after dose 12 (at steady state) of enoxaparin. The 5th, 50th and 95th percentiles are shown. The desired target is an estimated peak activity between 4 and 6 h post-dose (C5) of > 0.5 IU/mL (equivalent to 500 IU/L, represented by the dashed horizontal line) and a trough of < 0.5 IU/mL.
2.4. *Empirical evidence:*

Enoxaparin is at least as effective as UFH in the treatment of thrombosis, and both drugs are associated with a similar incidence of bleeding.\cite{80-82} A systematic review of six RCTs (totalling 21,946 patients) assessed effectiveness and bleeding outcomes in acute coronary syndrome patients randomized to receive either enoxaparin or UFH.\cite{148} The two groups were compared in terms of mortality and major bleeding, defined according to the TIMI criteria.\cite{83} At 30 days, mortality was similar in both groups (3.0% vs 3.0%; odds ratio [OR] 1.00; 95% CI 0.85, 1.17) and there was no significant difference in major bleeding (4.7% vs 4.5%; OR 1.04; 95% CI 0.83, 1.30).

Both drugs are effective anticoagulants; however, current dosing strategies fail to achieve desired biomarker targets in approximately 50% of subjects.\cite{149} To account for this, various bodyweight-based nomograms have been developed to help achieve the target aPTT within the first 24 hours of therapy;\cite{143} subsequent dose individualization is, however, based on assessment of aPTT results. We see that in clinical studies where aPTT results have been measured, the percentage of success (approximately 44%) is similar to that predicted in these simulations.\cite{149} This indicates that, for UFH, monitoring of the aPTT contributes minimal additional advantages. There is a wealth of information regarding the use of computerized Bayesian forecasting methods and the benefits of achieving the biomarker target,\cite{150} compared with empirical dose adjustment methods. In these cases, empirical dose individualization was often found to be little better than no monitoring at all. It is predicted that these Bayesian techniques would dramatically improve dose individualization with UFH. One example of Bayesian software is TCIWorks, which is currently used in clinical practice for dose individualization of various aminoglycosides.\cite{151} Similarly, Bayesian pharmacokinetic dose individualization of LMWHs would ensure that the biomarker target is achieved more often. It is important to note, however, that the anti-Xa target identified in this article for enoxaparin may not be suitable for other LMWHs.
2.5. Conclusions

It is clear from the simulation-based and empirical evidence that the current dosing of enoxaparin is no safer and the dose-exposure-response relationship no more predictable than those of UFH. Since aPTT monitoring is considered routine with UFH, based on the criteria for assessing the need for therapeutic monitoring, anti-Xa activity monitoring must be required for LMWHs.
Chapter 3: Evaluation of a Bayesian forecasting method for enoxaparin dose-individualisation

Parts of this chapter are based on the following manuscript currently under review:

3.1. TCIWorks

Enoxaparin is a low molecular weight heparin used in the treatment of thrombosis. The current approved treatment dose of enoxaparin is based on total body weight (Wt) and its dosing frequency is based dichotomously on creatinine clearance (CLcr). Recent evidence has shown this dosing strategy to be suboptimal and Bayesian dose-individualisation has been proposed as a safer and more effective alternative.

Dose-individualisation, based on an individual’s response, is indicated for drugs that meet certain criteria. These include a narrow therapeutic range, a large between-subject variability, a well-defined dose-concentration relationship, and a good correlation between drug concentration and the intensity or probability of a pharmacodynamic effect. Enoxaparin meets all of these criteria. The plasma concentration of enoxaparin can be quantified indirectly by measuring anti-factor Xa activity (anti-Xa). A peak anti-Xa concentration of > 5 mg/L and a trough of < 5 mg/L has been proposed previously by the authors as a therapeutic target for enoxaparin treatment (where 5 mg/L is equivalent to 500 IU/L). Also in this work it was established that dosing based on covariates (such as weight and CLcr) alone was unlikely to achieve the target in more than 46% of patients. A higher attainment of the target may be achieved using a Bayesian dose-individualisation method, for example by implementing a population PK model for enoxaparin in the Bayesian dose-individualisation software TCIWorks (www.tciworks.info). Recent work has described the use of TCIWorks for warfarin and its predictive performance. It was found that TCIWorks provided a good prediction of INR after inclusion of the third INR response.

3.2. Objectives

To evaluate the predictive performance of a computerised Bayesian dose-individualisation method for enoxaparin.
3.3. Methods

3.3.1. Data

The data used in this analysis have been described previously by Barras et al.\textsuperscript{[95]} and the patient demographics are summarised in Table 3.1. Briefly, this study involved randomising 122 patients on enoxaparin treatment to a conventional versus a covariate-based individualised dosing method (based on patient lean body weight\textsuperscript{[30]} and CLcr). Patients were followed up for 30 days and monitored for effectiveness and safety endpoints. One hundred and nine patients had one or more anti-Xa measurements performed 1-4 hours after dose. The sampling times were based on a published optimal design study.\textsuperscript{[156]} The anti-Xa test was performed on plasma samples using STA-Rotachrom (Diagnostica Stago, Asnieres, France) with a lower limit of assay detection of 0.1 IU ml-1 and an interassay precision (% CV) of 5.9%. The assay measures the inhibition of Xa by quantifying the absorbance of a sample, at 405 nm wavelength, which is converted to enoxaparin concentration with the use of an absorbance/concentration standard curve. These values along with patient demographics and dosing history were entered into TCIWorks.

\begin{table}
\centering
\begin{tabular}{|l|c|}
\hline
Age (years) & 61 (23 – 91) \\
Sex (M:F) & 66:43 \\
Weight (kg) & 77 (43 – 120) \\
Height (cm) & 170 (150 – 190) \\
CLcr (mL/min) & 75 (26-174) \\
\hline
\end{tabular}
\caption{Patient demographics and other clinical characteristics of patients in the study\textsuperscript{[95]} (n = 109). Data is presented as median (range) unless otherwise specified.}
\end{table}
3.3.2. Enoxaparin model

The model for enoxaparin in TCIWorks is based on two publications and is a two-compartment model with first-order input and linear elimination. [89, 94] Green and Duffull [94] performed a population pharmacokinetic-pharmacodynamic analysis in 96 patients stratified into normal weight, overweight, and obese and who received treatment doses of enoxaparin as part of their normal care. Approximately three anti-Xa concentrations were measured for each patient. The mean age, weight, height, and CLcr of this cohort were 71 years, 85 kg, 173 cm, and 99.6 mL/min, respectively. Green et al[89] performed a population pharmacokinetic analysis in 38 patients with a median age 78 years, mean CLcr of 32 mL/min, and mean weight of 69 kg who received treatment doses of enoxaparin as part of their normal care. Approximately ten anti-Xa concentrations were measured for each patient. The final model parameter values, between-subject variability (BSV), and residual unexplained variability (RUV) components (provided in Table 3.2) are coded into TCIWorks. This was a two-compartment model with first order input and linear elimination. The model included CLcr and Wt as covariates on CL and Wt as a covariate on Vc.
Table 3.2 Parameter estimates for the enoxaparin model in TCIWorks

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (L/h)</td>
<td>0.858 (32.7)</td>
</tr>
<tr>
<td>Vc (L)</td>
<td>4.567 (34.4)</td>
</tr>
<tr>
<td>Vp (L)</td>
<td>29.6 (29.9)</td>
</tr>
<tr>
<td>Q (L/h)</td>
<td>0.62 (69.8)</td>
</tr>
<tr>
<td>ka (h⁻¹)</td>
<td>0.255 (25.2)</td>
</tr>
<tr>
<td>( \sigma_{\text{additive (mg/L)}} )</td>
<td>0.524</td>
</tr>
<tr>
<td>( \sigma_{\text{proportional (%)}} )</td>
<td>20</td>
</tr>
</tbody>
</table>

CV= coefficient of variation (i.e. BSV); CL= total clearance; ka = absorption rate constant; Q= intercompartmental clearance; Vc = volume of distribution in the central compartment; Vp = volume of distribution in the peripheral compartment; \( \sigma^2 \) = RUV.

Model parameters were derived from the studies by Green et al [89, 94]

3.3.3. TCIWorks

Models are defined in TCIWorks as a set of ordinary differential equations (ODEs). The ODEs are solved numerically using a fourth order Runge-Kutta method. The least squares objective function (OFV) is defined as a maximum a posteriori (MAP) function and the minimization of this OFV is achieved using a simplex estimation algorithm. The MAP OFV combines prior knowledge of the probability density function of the pharmacokinetic parameters and the likelihood of the individual patient data (e.g. observed drug concentrations).[157, 158] Point estimates of the posterior parameter values minimise the following Bayesian objective function value (\( OFV_{MAP} \):
\[ OFV_{MAP} = \sum_{j=1}^{n} \left( \frac{y_j - g(\theta, x_j)}{\sigma^2_{h(g(\theta, x_j), a, b)}} \right)^2 + \sum_{p=1}^{p} \left( \frac{\ln \theta_p - \ln \mu_{\theta_p}}{\omega^2_{\theta_p}} \right)^2 \]

Equation 3.1 The maximum a posteriori objective function value used in TCIWorks

where \( OFV_{MAP} \) is the MAP objective function; \( y_j \) is the \( j^{th} \) observed concentration in an individual patient, for whom there are \( n \) observations; \( g(\theta, x_j) \) is the model predicted \( j^{th} \) observed concentration from a \( p \times 1 \) vector of individual parameter estimates \( \theta \) and \( x \) denotes covariates; \( \sigma^2_{h(g(\theta, x_j), a, b)} \) is the variance associated with the \( j^{th} \) observed concentration where \( h(g(\theta, x_j), a, b) = g(\theta, x_j)^2 \times a + b, \quad a = \sigma^2_{proportional} \) and \( b = \sigma^2_{additive} \); \( \ln \theta_p \) is the natural log of the \( p^{th} \) parameter in the reference population; \( \ln \mu_{\theta_p} \) is the natural log of the mean of the reference population value of the \( p^{th} \) parameter; and \( \omega^2_{\theta_p} \) is the log-normal between-subject variance associated with the \( p^{th} \) parameter.

### 3.3.4. Predictions

Two prediction methods for each observation were compared: (1) the prior predictions (calculated from patient covariates) for each of the 1\(^{st}\), 2\(^{nd}\), 3\(^{rd}\), and 4\(^{th}\) observations and (2) the posterior predictions of the next observation for the 2\(^{nd}\), 3\(^{rd}\), and 4\(^{th}\) observations.

For example a patient has \( n \) number of observations. The patient’s covariates were entered into TCIWorks and the anti-Xa predictions for the 1\(^{st}\) to \( n^{th} \) observations from the prior model were recorded. Then, for \( j > 1 \), \( j-1 \) observations were entered and along with the patient’s covariates the anti-Xa prediction for the \( j^{th} \) observation was recorded.
3.3.5. Predictive performance

The Sheiner and Beal [159] method for determining bias and imprecision was used to assess whether the anti-Xa observations differed significantly from model predicted values. Imprecision was determined by the root mean-squared prediction error (RMSE) which indicates how closely the model predictions match the observations; the smaller the RMSE, the more precise the prediction (Equation 3.2). Bias was determined using the mean prediction error (ME) and was used to quantify the tendency to over- or under-estimate the measured concentrations. A smaller ME indicates less bias (Equation 3.3).

\[ RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (y_i - g(\hat{\theta}, x_i))^2} \]

Equation 3.2 Root mean-squared prediction error, a measure of imprecision

\[ ME = \frac{1}{N} \sum_{i=1}^{N} (y_i - g(\hat{\theta}, x_i)) \]

Equation 3.3 Mean prediction error, a measure of bias

where \( y_i \) and \( g(\hat{\theta}, x_i) \) are the observed and predicted anti-Xa concentration for the \( i^{th} \) individual, respectively; and \( N \) is the total number of individuals.

To compare the prior and posterior prediction methods, the 95% confidence intervals (CI) for the ME were used. If the 95% CI did not include zero, the method with the smaller absolute ME was deemed to be less biased. The imprecision of both methods was compared by analysing the ratios of variances using the F-test (assuming normality); where the variance \( (\sigma^2) \) is calculated according to Equation 3.4. The ratio of the larger variance over the smaller variance was compared to 1 using \( n \) degrees of freedom (where \( n \) is the number of observations available at each analysis).
\[
\sigma^2 = MSE - ME^2
\]

Equation 3.4 Variance as a function of imprecision and bias

where \( MSE \) is the mean squared error.

### 3.3.6. Probability of achieving target anti-Xa concentration:

The ability of TCIWorks to predict target anti-Xa concentration was assessed by calculating the probability that a target is achieved given the bias and imprecision of the prediction method. The target is a successful peak (anti-Xa between 5 and 10 mg/L) and a successful trough (anti-Xa between 0 and 5 mg/L) for twice daily dosing.\(^{[152]}\) As the prior predictions are not influenced by the observations, the weighted mean of all predictions was used. A standard normal \( Z \) table was used where \( Z \) is calculated as:

\[
Z = \frac{X - \mu}{\sigma}
\]

where \( X \) is the lower or upper boundary for the target anti-Xa concentration (5 or 10 mg/L for the peak and 0 or 5 mg/L for the trough); \( \mu \) is the median target peak (7.5 mg/L) or the median target trough (2.5 mg/L); and \( \sigma \) is the standard deviation obtained from Equation 3.4. Assuming that a dosing regimen is determined that on average exactly achieves the target goal (for example peak of 7.5 mg/L and trough of
2.5 mg/L) then it is straightforward to calculate the probability of target attainment across the population. The probability of a successful peak was calculated as:

\[ \Pr (5 \leq Z \leq 10) = \Pr (Z \leq 10) - \Pr (Z \leq 5), \]

and the probability of a successful trough was calculated as:

\[ \Pr (0 \leq Z \leq 5) = \Pr (Z \leq 5) - \Pr (Z \leq 0). \]
3.4. Results

There were a total of 238 anti-Xa measurements in the dataset: 109 first observations (mean = 4.1 mg/L), 98 second observations (mean = 8.6 mg/L), 26 third observations (mean = 6.9 mg/L), and 5 fourth observations (mean = 8 mg/L).

3.4.1. Bias

The ME values and 95% CI are presented in Table 3.3. The prior predictions were negatively biased with a weighted mean error of -2.1 mg/L. The posterior predictions were initially negatively biased which became non-significant as more observations became available. The MEs of the posterior predictions were -2.2, -0.6, and -0.6 mg/L for the second, third, and fourth observations, respectively.

Table 3.3 Mean error (mg/L) estimates and the corresponding 95% CI for the prior and posterior predictions

<table>
<thead>
<tr>
<th>Observation</th>
<th>ME (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prior</td>
</tr>
<tr>
<td>1</td>
<td>-1.5 (-1.9 to -1.1)</td>
</tr>
<tr>
<td>2</td>
<td>-2.9 (-3.5 to -2.3)</td>
</tr>
<tr>
<td>3</td>
<td>-1.6 (-2.5 to -0.7)</td>
</tr>
<tr>
<td>4</td>
<td>-2.3 (-3.9 to -0.7)</td>
</tr>
</tbody>
</table>
3.4.2. Precision

Both prior and posterior predictions were imprecise but the imprecision of the posterior predictions decreased as the number of observations increased (3.3 to 1.8 mg/L). Table 3.4 shows the RMSE values of the prior and posterior predictions. The weighted mean RMSEs for the prior predictions was 3.2 mg/L. The variance ratio of prior/posterior estimations and the corresponding F statistic are also shown.

Table 3.4 Root of mean squared error (mg/L) estimates for the prior and posterior predictions. The variance ratios for observations two, three, and four are shown.

<table>
<thead>
<tr>
<th>Observation</th>
<th>RMSE</th>
<th>Variance ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prior</td>
<td>Posterior</td>
</tr>
<tr>
<td>1</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>4.2</td>
<td>3.3</td>
</tr>
<tr>
<td>3</td>
<td>2.8</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td>2.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* indicates statistical significance (P < 0.05)
3.4.3. Probability of achieving target:

The TCIWorks enoxaparin dosing method resulted in a higher probability of achieving target anti-Xa concentration than the prior method (Table 3.5). This is most evident for prediction of observations 2 and 3.

Table 3.5 Probability (in %) of target anti-Xa concentration using the Prior and Posterior prediction methods.

<table>
<thead>
<tr>
<th>Updated model</th>
<th>Probability of achieving target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior</td>
<td>69</td>
</tr>
<tr>
<td>Posterior with 1 observation</td>
<td>69</td>
</tr>
<tr>
<td>Posterior with 2 observations</td>
<td>90</td>
</tr>
<tr>
<td>Posterior with 3 observations</td>
<td>85</td>
</tr>
</tbody>
</table>
3.5. Discussion

The Bayesian estimation of anti-Xa performed relatively well with bias that became insignificant after the 3rd sample was included in the MAP algorithm. Imprecision reduced after the second observation was incorporated into the MAP algorithm. The prior model suffered from significant bias and imprecision. This model reflects current clinical practice where enoxaparin dosing is based only on patient Wt and CLcr. These covariates, while important, do not sufficiently account for the variability in dose-response. The model was based on two population studies; one with a higher proportion of obese individuals than the current study [94], and the second with a higher proportion of patients with renal impairment.[89] This may explain the consistent bias associated with the prior model in this study.

Enoxaparin, along with other low-molecular weight heparins, is widely used in the treatment and prevention of thromboembolic conditions. It has similar effectiveness to unfractionated heparin (UFH) in the treatment of acute coronary syndromes [80, 81] and venous thromboembolism,[82] with a similar incidence of bleeding.[80-82] It has the advantage of having linear pharmacokinetics, longer half-life, superior subcutaneous bioavailability, and a lower incidence of thrombocytopenia and osteopenia.[139, 140] However, like UFH it has high between-subject variability in dose-response and a narrow therapeutic range which warrant dose-individualisation.[152] The current treatment dose of enoxaparin is 1 mg/kg twice a day based on Wt with a reduction to 1 mg/kg once a day for patients with a CLcr of less than 30 mL/min.[90, 160] However, clinicians find these guidelines lack credibility especially around guideline transition points.[91] For example, the doubling of dose in a patient whose CLcr increases from 29 mL/min to 30 mL/min. In this work it was found that significant divergence in prescribing behaviour was observed at weight values close to 90 kg and CLcr between 20–40 mL/min.[91]

Bayesian forecasting methods for dose individualisation have been shown to provide accurate and precise dose predictions for several drugs using a small number of samples.[161-163] This analysis showed that using Bayesian dose
individualisation method to individualise enoxaparin treatment is successful in achieving the anti-Xa target and the probability of achieving the target is higher than current covariate dosing. The clinical impact of this method in terms of effectiveness and safety is yet to be determined.

It is of note that TCIWorks does not provide a dose recommendation. Instead, it provides the user with an opportunity to trial potential future doses based on previous individual response. In this setting the user, ultimately a clinician, would be able to choose a dose that balances the therapeutic need vs. the risk in an individual patient.
3.6. Conclusions

The Bayesian dose individualisation method provided accurate and precise predictions of anti-Xa concentration. The use of a Bayesian dosing method for enoxaparin, where individual patient observations are incorporated into the prior model, proved superior in predicting the target concentration than current dosing practice. There was limited additional benefit in obtaining more than three observations during dose-individualisation.
Part III

DOSE-INDIVIDUALISATION OF ENOXAPARIN IN ADULTS
Chapter 4: Anti-factor Xa assay evaluation

Parts of this chapter are based on the following manuscript currently in press:

Part III of the thesis describes the clinical application of enoxaparin dose-individualisation and contains two chapters. Chapter 4 describes an evaluation of an anti-Xa assay for clinical use. The available assay presented constraints for use in a clinical setting where anti-Xa results are needed in real time and patient enrolment is unpredictable. The assay was evaluated in terms of freeze-thaw stability, shelf-life, sample haemolysis, and hypoantithrombinaemia. Chapter 5 describes a clinical trial where the dose-individualisation method described in Chapter 3 is compared against conventional dosing.
4.1. Introduction

Enoxaparin is a low-molecular weight heparin (LMWH) that is widely used in clinical practice for the treatment and prevention of thrombosis (e.g. myocardial infarction, deep vein thrombosis, and atrial fibrillation).[69] It acts by binding to antithrombin-III (AT) and enhancing its ability to bind to and deactivate the clotting factors; this ultimately hampers the formation of a thrombus.[79]

Enoxaparin is renally eliminated (fraction excreted unchanged is estimated to be 0.71)[89] and its dose is based on total body weight and renal function.[90] Monitoring of enoxaparin therapy is advocated in the clinical setting especially for patients who are at the extrema of body weight or have a degree of renal impairment in order to reduce the risks of haemorrhage or treatment failure.[152] Since enoxaparin is composed of small heparin fragments of various chain lengths, direct measurement and interpretation of blood concentrations is difficult. Instead, an anti-Xa activity assay has been developed and is used for enoxaparin drug monitoring in some clinical settings.[92] The principle of the anti-Xa assay is shown in Scheme 4.1. Factor Xa not inactivated by the LMWH-AT complex is free to react with the added substrate (Suc-Ile-Glu(g-Pip)-Gly-Arg·pNA; S-2732) resulting in the formation of p-nitroanilide (pNA). The appearance of pNA, determined spectrophotometrically at a wavelength of 405 nm, is inversely proportional to the concentration of enoxaparin in a plasma sample.
LMWH + AT ⇔ LMWH-AT  \hspace{1cm} (1)

LMWH-AT + Xa ⇔ LMWH-AT-Xa + Xa_free  \hspace{1cm} (2)

Xa_free + chromogenic substrate → peptide + p-nitroanilide  \hspace{1cm} (3)

Scheme 4.1: Reaction sequence underpinning the anti-Xa assay. Steps (1) and (2) occur in a patient’s blood sample. Step (3) occurs when the substrate is added to the patient’s plasma in the laboratory test. The appearance of pNA is measured spectrophotometrically at 405 nm.

The following four anti-Xa assay kits are available for laboratory use: COATEST® LMWH Heparin/Heparin; BIOPHEN Heparin; ROTACHROM® Heparin; and ACTICHROME® Heparin. These kits use the same principle (Scheme 4.1) to measure anti-Xa activity of LMWH. The COATEST® LMWH Heparin/Heparin assay kit is readily available in New Zealand and was used in this study (see Appendix 2, Figure A2.1 for assay package insert). COATEST® (Chromogenix Instrumentation Laboratory, Milan, Italy; Art. No. 821363) allows both manual and automated determination of anti-Xa in a single-stage procedure making it suitable for analysis of clinical samples. This is important for clinicians as anti-Xa test results inform the treatment dose and are required before the next dose of enoxaparin is administered (i.e. within 12 to 24 hours). Additionally, AT is not added to samples in this assay which raises the question whether AT deficiency (hypoantithrombinaemia) may influence the assay result.\[164\]
4.2. **Objectives**

The objective of this study was to investigate some of the limitations of the routine use of the assay for the measurement of enoxaparin concentration in plasma samples in patients treated with enoxaparin.
4.3. Materials and Methods

4.3.1. Materials

COATEST® assay kits and AT 10 IU vials were purchased from Abacus ALS (Auckland, New Zealand). Standard human plasma vials were purchased from Siemens Healthcare Diagnostics (Auckland, New Zealand). Glacial acetic acid was purchased from Sigma-Aldrich New Zealand Ltd (Auckland, New Zealand). Enoxaparin (Clexane®; Sanofi, Paris, France) 20 mg/0.2 mL injections were purchased from the Southern District Health Board (Dunedin, New Zealand). Deionized water, filtered through 0.22 µm was used for reconstitution of standard human plasma, factor Xa, the chromogenic substrate, and antithrombin. The LMWH standards dalteparin (COATEST: Chromogenix Instrumentation Laboratory, Milan, Italy) and enoxaparin (Clexane: Sanofi-Aventis, Sydney, Australia) were used for quality control standards. Pooled blank plasma samples (collected in 3.2% sodium citrate) were used for the assay validation and the haemolysis experiments and were collected from consenting healthy volunteers (see Appendix 2, A2.2 for volunteer Information Sheet and Consent Form). Plasma was prepared by centrifugation (2000×g, 20 min, 20 °C). Standard human plasma (Dade Behring Marburg GmbH, Marburg, Germany) was used for preparation of analytical standards for the standard curve and the stability and hypoantithrombinaemia experiments.

4.3.2. Preparation of analytical standards and reagents

Anti-Xa (IU/mL) was measured by adding 25 µL of a plasma sample to 200 µL of the chromogenic substrate S-2732® (in buffer) and 200 µL of factor Xa in a 1.5 mL polystyrene cuvette. Two competing reactions then start: the inhibition of Xa by the LMWH-AT complex, and the Xa-catalysed release of pNA from the chromogenic substrate. After eight minutes incubation, 200 µL of acetic acid (20% v/v) was added to stop generation of further pNA and the endpoint absorbance was measured. A control cuvette was prepared at the same time containing 400 µL of buffer instead of the substrate and Xa. The final volume of each cuvette was 625 µL. The absorbance
was measured using a spectrophotometer (Pharmacia Biotech Ultrospec 2000) at a wavelength of 405 nm.

4.3.3. Time-course of the Xa-substrate reaction

The time course of the reaction was determined to verify the optimal reaction time of the assay. Two methods were used: a) analytical standards spiked with 0.1 IU/mL dalteparin were stopped by addition of 200 µL of 20% acetic acid solution after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 minutes then absorbance at 405 nm was measured, b) analytical standards spiked with 0.1 and 1.0 IU/mL were placed in the spectrophotometer without addition of the stopper solution and absorbance was recorded at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 minutes. In method (b) the volume of the stopper solution (i.e. 200 µL) was offset by doubling the volumes of all other reagents (plasma, substrate, and Xa); the final volume of the cuvette was 850 µL.

4.3.4. Standard Curve validation

The dalteparin standard was mixed with standard human plasma to produce five analytical standards of 0.1, 0.3, 0.5, 0.8, and 1.0 IU/mL. These were measured spectrophotometrically at 405 nm. Generalised least squares regression analysis was used to determine the parameters of the standard curve. The regression analysis was performed using Microsoft Excel (Version 2010). Using the Solver add-on in Excel, the model parameters were estimated by minimising the weighted residual sum of squares. The weight for each observation was proportional to the reciprocal of the error variance for that observation.
4.3.5. Assay inaccuracy and precision

Plasma samples spiked with 0.2, 0.4, 0.5, 0.7, and 1.0 IU/mL LMWH were prepared in duplicates and measured. These samples were prepared and analysed throughout a 12 month period during a clinical trial and served as quality control. Precision was determined using the coefficient of variation (CV%) of the replicate measurements (Equation 4.1).\[165\] Inaccuracy was assessed by comparison of the concentration calculated from the standard curve with the solution nominal concentration (Equation 4.2).\[166\]

\[
CV(\%) = \frac{SD}{Mean} \times 100
\]

Equation 4.1 Coefficient of variation (CV%) of replicate measurements, a measure of precision

where \( CV \) is the Coefficient of Variation; \( SD \) is the standard deviation of the replicate measurements; and \( Mean \) is the average measurement of the replicates.

\[
Inaccuracy(\%) = \frac{Conc_{measured} - Conc_{nominal}}{Conc_{nominal}} \times 100
\]

Equation 4.2 Inaccuracy of measured concentrations

where \( Conc_{measured} \) is the sample’s standard LMWH concentration as determined from the standard curve and \( Conc_{nominal} \) is the nominal LMWH concentration.
4.3.6. **Stability of Xa on freezing**

Aliquots of the reconstituted Xa solution were frozen (−20 °C) then thawed at 1, 2, 3, 4, 5, and 6 months and used to measure anti-Xa activity of plasma samples spiked with 0.5 IU/mL dalteparin. A further vial of reconstituted Xa was frozen and thawed six times (i.e. 6 freeze/thaw cycles) and used to determine anti-Xa activity in plasma samples spiked with 0.5 IU/mL. The thawed solution was used to measure anti-Xa concentration in an analytical standard spiked with 0.5 IU/mL dalteparin. For both parts of this experiment, aliquots of the 0.5 IU/mL analytical standard were frozen (−20 °C) and thawed before use at 1, 2, 3, 4, 5, and 6 months.

4.3.7. **Effect of red blood cell haemolysis**

To test the effect of haemolysis, blood (in a citrated tube) was collected from a consenting healthy male volunteer and haemolysed by mixing with a hypotonic solution (water). Increasing amounts of haemolysed blood (in increments of 2.5%) were added to plasma spiked with 0.5 IU/mL dalteparin and anti-Xa concentration was measured in triplicates. Absorbance versus % haemolysis was plotted and deviation from the nominal absorbance (with 0% haemolysis) was calculated using Equation 4.2.

4.3.8. **Effect of AT concentration on the anti-Xa assay**

Standard human plasma (SHP), 50:50 AT:SHP solution, and 50:50 water:SHP solution were spiked with 0.6 IU/mL dalteparin and the anti-Xa concentration was measured. Deviation from the nominal LMWH concentration was calculated using Equation 4.2.
4.4. Results

4.4.1. The time-course of the Xa-substrate reaction

The reaction and resulting colour was found to reach a plateau at about 8 minutes (Figure 4.1). The recommended time for the reaction is 8 minutes (COATEST® LMW Heparin product information 2003).

Figure 4.1 The time course of the Xa-substrate reaction. Upper plot: samples were spiked with 0.1 IU/ml (o) dalteparin and stopped at various time points by addition of 20% acetic acid between 0 and 11 min. Lower plot: samples were spiked with 0.1 IU/ml (o) and 1 IU/ml (Δ) dalteparin. Duplicate results are shown.
4.4.2. Standard curve for anti-Xa concentration

A standard curve (Figure 4.2) was produced using five concentration points (in triplicates): 0.1, 0.3, 0.5, 0.8, and 1.0 IU/mL. Parameters of the curve (intercept and slope) were estimated using generalised least square (Equation 4.3).

\[
Absorbance = 1.14 \times e^{-1.07\times\text{Conc}}
\]

Equation 4.3 Regression equation of the anti-Xa assay standard curve

where Absorbance is at a wavelength of 405 nm and Conc is the low-molecular weight heparin’s concentration. The standard error for the two parameters was 0.09 (for the intercept) and 0.29 (for the slope).
Figure 4.2 Anti-Xa standard curve for low-molecular weight heparin (dalteparin standard). Reaction time was 8 min. An exponential equation (equation 4.3) was fitted.

4.4.3. Assay inaccuracy and precision

The performance of the standard curve was tested at various time points and using different concentrations along the standard curve and different assay kits. It was found to have acceptable accuracy and precision (Table 4.1).
Table 4.1 The inaccuracy and precision of the anti-Xa assay. Samples, in duplicates, were spiked with various concentration of dalteparin. Multiple assay kits were used.

<table>
<thead>
<tr>
<th>Nominal (IU/mL)</th>
<th>Kit #</th>
<th>Measured (IU/mL)</th>
<th>Inaccuracy (%)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>1</td>
<td>0.19</td>
<td>-6.70</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>1</td>
<td>0.17</td>
<td>-13.05</td>
<td>4.98</td>
</tr>
<tr>
<td>0.4</td>
<td>1</td>
<td>0.41</td>
<td>3.53</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>1</td>
<td>0.41</td>
<td>3.30</td>
<td>0.15</td>
</tr>
<tr>
<td>0.5</td>
<td>2</td>
<td>0.56</td>
<td>12.72</td>
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</tr>
<tr>
<td>0.5</td>
<td>2</td>
<td>0.55</td>
<td>10.04</td>
<td>1.70</td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
<td>0.52</td>
<td>3.66</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
<td>0.54</td>
<td>7.42</td>
<td>2.52</td>
</tr>
<tr>
<td>0.7</td>
<td>1</td>
<td>0.75</td>
<td>7.46</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>1</td>
<td>0.77</td>
<td>9.81</td>
<td>1.53</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>0.92</td>
<td>-8.19</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>0.90</td>
<td>-10.36</td>
<td>1.69</td>
</tr>
<tr>
<td>1.0</td>
<td>4</td>
<td>0.87</td>
<td>-13.09</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>4</td>
<td>0.96</td>
<td>-4.40</td>
<td>6.73</td>
</tr>
</tbody>
</table>

4.4.4. Stability of Xa on freezing

There was little difference in Xa performance 30, 60, 90, and 150 days after aliquots were frozen (Figure 4.3). The aliquots at day 120 were inconclusive as the frozen spiked plasma standard contained a clot which would have consumed a higher amount of dalteparin than the rest of samples. The aliquots at day 180 showed an apparent loss in Xa potency.

In the freeze/thaw part of this experiment (Figure 4.4), there was little variability in Xa performance 30, 60, 90, and 150, and 180 days after freezing. The aliquots at day 90 had the highest variability but this can be attributed to a clot.
forming in the spiked plasma sample. Although variable, there appears to be a trend for Xa loss of activity (causing spuriously higher LMWH concentration than the nominal concentration).

Figure 4.3 Stability of Xa on freezing. Samples were spiked with 0.5 IU/ml of dalteparin. Aliquots of Xa were frozen and thawed on days of measurement. Measurements on day 120 are spuriously low probably due to clot formation in the plasma aliquot.
Figure 4.4 Stability of Xa on freeze/thaw. Samples were spiked with 0.5 IU/ml of dalteparin. Xa was repeatedly frozen and thawed on days of measurement. Measurements on day 90 are spuriously low probably due to clot formation in the plasma aliquot.
4.4.5. Effect of red blood cell haemolysis on the anti-Xa assay

Haemolysis as low as 2.5% was found to interfere with the assay (Figure 4.5). The concentration of LMWH in a sample would appear to decrease with little haemolysis then increase again as haemolysis increases.

![Figure 4.5 Measured anti-Xa concentration vs sample haemolysis (%). Samples were spiked with 1.0 IU/ml of dalteparin.](image)
4.4.6. Effect of antithrombin concentration on the anti-Xa assay

There was no difference in sample absorbance with a 50% reduction in AT content of a spiked sample. This was corroborated by the lack of change in absorbance when 50% of plasma was replaced with AT (Figure 4.6).

Figure 4.6 Measured anti-Xa concentration vs sample antithrombin content. Samples were spiked with 0.6 IU/ml of dalteparin.
4.5. Discussion

The anti-Xa activity assay is a useful method to quantify the concentration of enoxaparin and other LMWHs in plasma samples. Therapeutic monitoring of these anticoagulants is useful in order to optimise their effectiveness and minimise their adverse effects.[152] In New Zealand, routine testing of anti-Xa concentration is limited and only a few centres provide this service. This makes it difficult for clinicians to use anti-Xa results to inform treatment dosage. This project was undertaken as part of setting up an anti-Xa assay to support a clinical trial involving the dosing and monitoring of enoxaparin. Once the assay kit (COATEST®) was set up and used, requests to test its capabilities and limitations in clinical practice ensued and this gave rise to the range of experiments explained in this paper.

The standard curve was estimated using generalised least square regression (GLS). This is slightly different than the usual ordinary least square method (OLS) which assumes that the observations used to construct the standard curve have the same variance (i.e. variances are homoscedastic) and the errors of the observations are uncorrelated. GLS allows the variance of the residuals to vary across all observations and also allows for the residuals to be correlated. This better reflects the process by which the data were generated and confers more influence to observations with a small variance than observations with a large variance on the fitted curve.[167]

The incubation period recommended after Xa is added to the substrate and the spiked plasma was eight minutes. As the assay was envisaged to be used in the clinic, a question was raised whether measurement outside the recommended time would cause significant deviation in the results. The assay involved an enzymatic reaction with an initial exponential increase in velocity then a gradual decrease leading to a plateau in activity. Between seven and eight minutes the rate of the reaction was very small and without adding the acetic acid solution the reaction would continue for at least 12 minutes albeit with a very low rate (Figure 4.1).
One issue that rendered the assay kit expensive was the shelf-life of Xa. The manufacturer would only recommend 30 days and did not endorse an extended shelf-life due to lack of data. The rest of the reagents, apart from the buffer, had shelf-lives of 180 days. It was evident that Xa lost activity when stored at 2-8 °C. The two experiments with freezing and freeze-thaw stability have shown that aliquots of Xa could be stored for up to 150 days without loss in activity. This is expected to reduce the costs of providing this service in clinical practice.

The haemolysis experiment was intended to shed some light on whether it is still acceptable to measure anti-Xa in plasma samples that are lightly haemolysed. Enoxaparin and other LMWHs are used in a patient population where regular blood drawing is hindered by advanced age, obesity, frailty, and a history of multiple venipuncture. Haemolysis in vivo is expected to result in a change in heparin content and activity. Results of this experiment also show that haemolysis affected the measured LMWH concentration.

Lastly, there was concern about whether patients with a degree of hypoantithrombinaemia would provide inaccurate anti-Xa results. AT is expected to vary between patients and low AT would allow more Xa in the sample to cleave the substrate-pNA bond hence resulting in spurious absorbance readings. Teien et al showed that the addition of purified AT into the plasma samples increased the accuracy of the assay. However, the relatively smaller AT experiment in the current paper showed that even 50% antithrombinaemia is not likely to affect these results significantly. This lack of effect could be attributed to the high LMWH concentration used in this experiment (0.6 IU/mL). The use of purified AT may be justified when significantly lower LMWH concentrations are measured. The concentration used in this experiment is what is expected to be measured during enoxaparin treatment.
4.6. Conclusions

A commonly used anti-Xa assay kit has been validated and its capabilities and limitations in clinical practice tested through a set of experiments. Results are hoped to elucidate on the practicalities around anti-Xa testing and may be useful for laboratory personnel and clinicians dealing with LMWH treatment and monitoring.
Chapter 5: A randomised controlled trial of a Bayesian dose-individualisation method for enoxaparin

Parts of this chapter are based on the following manuscript currently in press:

5.1. Introduction to Chapter 5

This chapter describes a randomised controlled trial comparing a Bayesian dose-individualisation method for enoxaparin against standard dosing. Due to issues with recruitment, the trial was terminated before reaching its target sample size. Most patients who were potentially eligible for inclusion were either revascularised (angioplasty) or were discharged on home enoxaparin (administered either by the patient or a district nurse).

The medical collaborator for this trial (Dr J Schollum, Nephrology Department, Dunedin Hospital) possessed the randomisation code and implemented the dose changes when applicable.

5.2. Background

Enoxaparin is a low-molecular weight heparin (LMWH) widely used in clinical practice for the treatment and prevention of thrombosis.\cite{69} It is renally eliminated\cite{89} and its dose is based on total body weight (Wt) and renal function (creatinine clearance, CLcr).\cite{90} As was illustrated in Part II of this thesis, monitoring of enoxaparin therapy is warranted in the clinical setting especially for patients who are at the extrema of body weight or have a degree of renal impairment in order to reduce the risks of haemorrhage or treatment failure.\cite{152} Plasma concentrations of enoxaparin are quantified by measuring anti-Xa activity.\cite{92}

When used for the active treatment of thrombosis, enoxaparin is dosed twice daily based on mg/kg of total body weight (Wt) and the frequency is halved to once daily when creatinine clearance (CLcr) is less than 30 mL/min. This is potentially suboptimal because the relationship between CL and Wt is non-linear and CLcr is a continuous variable. Obese patients who receive a mg/kg of Wt dose are likely overexposed to the drug.\cite{94} Also, the dichotomous halving of the daily dose once CLcr is less than 30 mL/min is likely to result in underexposure of the drug in renally-impaired patients.\cite{94} Indeed, a pilot study showed that clinicians do not find
these guidelines credible for patients at the cusp of size (Wt > 90 kg) or renal function (CLcr < 30 mL/min). An alternative covariate-based dosing strategy based on fat-free mass (FFM) has been shown to decrease the risk of bleeding while maintaining therapeutic benefit. However, the question remains whether treatment could be improved through individualised, iterative, response-based dosing.

Iterative, pharmacokinetically-guided dosing methods are well documented in the literature with one notable study showing a significant improvement in remission in children treated for acute lymphoblastic leukaemia with pharmacokinetically-guided individualised chemotherapy, with no increase in toxicity. The method relies on the measurement of a blood drug concentration (or the concentration of a biomarker) in conjunction with computerised Bayesian regression methods to estimate the likely exposure of each patient to the drug. Then, based on predefined acceptable exposure levels, the dose for each patient is adjusted accordingly to meet his/her clinical needs.

In Chapter 2 of this thesis an enoxaparin concentration target was identified: peak anti-Xa concentration (taken 4-6 hour post dose) > 500 IU/L and a minimum anti-Xa concentration (taken at the trough) < 500 IU/L. In Chapter 3 a dose-individualisation software (TCIWorks) which utilises a Bayesian statistical algorithm and a population model for enoxaparin was evaluated and found to have a higher probability in achieving target anti-Xa when compared to standard dosing. In Chapter 4 the anti-Xa assay was evaluated for real-time clinical research; the assay was investigated in terms of stability, shelf-life, sample haemolysis, and hypoantithrombinaemia. In this chapter, enoxaparin dosing to target through the use of TCIWorks will be compared to standard dosing in terms of effectiveness and safety.
5.3. Objectives

To compare pharmacokinetically-guided dosing regimen of enoxaparin against standard dosing in a prospective randomised controlled trial.

5.4. Methods

5.4.1. Design

This was a randomised, controlled, double-blind, parallel group study. Patients were recruited from Dunedin Hospital and randomly assigned to either a pharmacokinetically-guided dosing regimen or a standard treatment. The patients and investigators were blinded to study allocation. The prescribing doctor was not blinded to treatment allocation.

5.4.2. Patients

All patients admitted for the treatment of a registered indication for enoxaparin (ACS, PE, DVT) who were likely to require treatment for at least 48 hours were eligible for inclusion into the study. Patients were screened for eligibility and then asked to provide informed consent (see Appendix 3, A3.1 for participant Information Sheet and Consent From). The amount of the first dose was at the discretion of the prescribing doctor and followed local and national enoxaparin dosing protocols.[90]

Patients were enrolled at the start of their enoxaparin treatment and were not eligible for inclusion if they: were pregnant; less than 18 years old; had received warfarin in the past 7 days or heparin therapy (UFH or LMWH) in the last day; had an INR > 1.2 or an APTT > 60 seconds at the time of recruitment; or an estimated CLcr less than 10 mL/min (using the Cockcroft and Gault equation[171]). A computerised randomisation code was generated in MATLAB® (R2009a, The MathWorks, Inc., Natick, Massachusetts) to allocate patients into either the individualised or standard treatment arm of the study.
Haemoglobin and platelet counts were measured to aid objective assessment of bleeding complications. In addition, patient length of hospitalisation was recorded as a marker for the patients’ health status and concomitant medication and relevant biochemistry was also recorded throughout the course of treatment.

5.4.3. Dosing regimens and plasma sampling

The standard dosing regimen for all patients was that determined by the prescribing doctor. To maintain trial blindness, the same blood sampling strategy was performed on the standard dosing group as per the individualised-dosing group.

For the individualised dosing regimen, the initial dosage was one chosen by the prescribing doctor. One blood sample was taken at any time after the first dose and before the next dose is given. The exact timing of the dose and the blood sample was recorded. Each blood sample was transferred to the School of Pharmacy laboratories for preparation and analysis (see Chapter 4 for assay details) and the resultant anti-Xa concentration was entered into TCIWorks along with appropriate patient covariates (age, sex, weight, height, and dosing history). A dose recommendation is then communicated to the medical collaborator who possessed the randomisation sequence. The dose recommendation was implemented if the patient was in the individualised group or ignored if in the standard group. Subsequent blood samples were then drawn after two further doses if the patient’s anti-Xa concentrations did not meet the target or every 2 days of treatment thereafter.
5.4.4. Randomisation and allocation concealment

A random sequence (120 numbers in blocks of 10) was generated using MATLAB. The sequence was given to a research physician who oversaw the prescribing process. The sequence remained concealed until the analysis was complete.

5.4.5. Endpoints

The primary and secondary endpoints were based on safety. The primary endpoint was the total number of patients with a bleeding event. A bleeding event incorporated any major or minor bleed. A major bleed was defined by a decrease in haemoglobin greater than 30 g/L or evidence of an internal anatomical bleed such as retroperitoneal, intracranial or intraocular (as used in the TIMI 11B trial[83]). Minor bleed was defined as a bleed other than a major bleed, e.g. epistaxis, haematuria, an injection or venepuncture site bleeding, and haematemesis.

The secondary endpoint was defined as a composite of any primary endpoint and any single major bruise that had a surface area of greater than 20 cm² and was distal to the site of injection and venipuncture sites for blood draws (as used in a post hoc analysis of the ESSENCE study[172]).

In addition, the percent success of attaining target concentration was compared between the dosing regimens.

To assess endpoints, the prevalence, size, and location of bleeding or bruising were recorded on a pre-designed data collection form (see Appendix 3, A3.2 for Clinical Report Form template). This was performed via a total body assessment of the patient and review of patient medical notes. The review was scheduled at enrolment into the study and daily until enoxaparin therapy was ceased. In addition patients were followed up for 30 days in order to monitor evidence of therapeutic outcomes and events such as revascularisation, re-thrombosis, or repeat emboli.
5.4.6. Power

The power calculations were based on findings from the randomised controlled trial by Barras et al\cite{95} where individualised dosing (based on covariates) was compared to standard dosing. In this study the average prevalence of bruising was 20\% and all bleeding (major and minor) was 8\%. To attain a power of 80\% it was estimated that 120 patients would be required to show a 10\% absolute reduction in bruising and 5\% absolute reduction in bleeding, i.e. a 50\% relative decrease of these events. This corresponds to 60 patients in the dose-individualisation arm and 60 in the standard treatment arm. Recruitment occurred with replacement, such that if a patient drops out before 48 hours then they were replaced by the next patient that was assigned a new randomisation code. All data were included in the final analysis as per intention to treat analysis.

In an observational pilot study conducted two years previously, 59 patients with similar diagnoses who received enoxaparin were recruited in six months.\cite{91} Therefore, it was anticipated that 12-18 months would be sufficient to recruit the 120 patients required for this RCT.

5.4.7. Statistical Analysis

Analyses of end points were performed on an intention-to-treat basis. Baseline patient characteristics were compared using a one-way analysis of variance with dosing effect for continuous data and a chi-square test for categorical data. All tests were two-sided with a 5\% level of significance (\(\alpha = 0.05\)).

The relative risk (RR) ratio between the two arms of the study was computed for the safety endpoint. The study duration for each patient was from the time of the patient’s first dose of enoxaparin to midnight on the day of the final dose of enoxaparin.
All statistical analyses were performed using STATA v11 (StataCorp. 2009. *Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP).

### 5.4.8. Ethics

The study was approved by the Lower South Regional Ethics Committee (Project Key: LRS/07/11/041) and the Otago District Health Board. The study was registered through the Australian New Zealand Clinical Trials Registry (Trial ID: ACTRN12608000573358). Māori consultation was also undertaken.
5.5. Results

Twenty patients were recruited between January 2009 and December 2010. Of these, 9 were in the standard group and 11 were in the individualised group. All relevant data were obtained for all patients except the 30-day follow-up data which was missing for 1 patient in the standard group and 2 patients in the individualised group.

5.5.1. Patients

Baseline demographic data and treatment information are shown in Table 5.1. Continuous variables are described as mean (standard deviation) and were compared between the two treatment groups by one-way analysis of variance. Categorical variables are described as percentages and were compared with chi-square statistics. All tests were two-sided with a 5% level of significance ($\alpha = 0.05$). There were no statistically significant differences between the two groups but it is unclear whether this is due to lack of difference or the study being underpowered.
## Table 5.1 Baseline and dosing characteristics

<table>
<thead>
<tr>
<th></th>
<th>Standard (n = 9)</th>
<th>Individualised (n = 11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age (SD), years</td>
<td>66 (8.6)</td>
<td>60.5 (16.7)</td>
<td>0.387</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>5 (56)</td>
<td>3 (27)</td>
<td>0.199</td>
</tr>
<tr>
<td>Male</td>
<td>4 (44)</td>
<td>8 (73)</td>
<td>0.199</td>
</tr>
<tr>
<td>Mean height (SD), cm</td>
<td>167.4 (9.9)</td>
<td>171.3 (9.1)</td>
<td>0.367</td>
</tr>
<tr>
<td>Mean weight (SD), kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wt</td>
<td>79.2 (10.6)</td>
<td>95.9 (23.5)</td>
<td>0.065</td>
</tr>
<tr>
<td>FFM</td>
<td>51.6 (9.8)</td>
<td>61.3 (12.1)</td>
<td>0.071</td>
</tr>
<tr>
<td>Mean BMI (SD), kg/m²</td>
<td>28.5 (4.9)</td>
<td>32.4 (6.5)</td>
<td>0.152</td>
</tr>
<tr>
<td>Mean CLcr (SD), mL/min</td>
<td>55.8 (16.6)</td>
<td>66.3 (26.3)</td>
<td>0.314</td>
</tr>
<tr>
<td><strong>Diagnosis, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACS</td>
<td>5 (56)</td>
<td>3 (27.3)</td>
<td>0.199</td>
</tr>
<tr>
<td>PE</td>
<td>4 (44)</td>
<td>6 (55)</td>
<td>0.653</td>
</tr>
<tr>
<td>Surgery (on long term warfarin)</td>
<td>0 (0)</td>
<td>2 (18.2)</td>
<td>0.178</td>
</tr>
<tr>
<td><strong>Enoxaparin treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean treatment duration (SD), days</td>
<td>3.6 (1.9)</td>
<td>3.9 (2.1)</td>
<td>0.740</td>
</tr>
<tr>
<td>Mean dose/day (SD), mg</td>
<td>80 (13)</td>
<td>85 (18)</td>
<td>0.516</td>
</tr>
<tr>
<td>Mean total dose/patient (SD), mg</td>
<td>550 (245)</td>
<td>630 (335)</td>
<td>0.563</td>
</tr>
<tr>
<td><strong>Other medicines, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiplatelets</td>
<td>3 (33)</td>
<td>1 (9)</td>
<td>0.178</td>
</tr>
<tr>
<td>Warfarin</td>
<td>4 (44)</td>
<td>8 (73)</td>
<td>0.423</td>
</tr>
</tbody>
</table>

ACS = acute coronary syndrome; CLcr = creatinine clearance[^171]; FFM = fat-free mass[^30]; IBW = ideal body weight[^173]; PE = pulmonary embolism; Wt = total body weight
5.5.2. Target achievement

A total of eight patients did not achieve the anti-Xa target (Table 5.2). Patients who had a peak anti-Xa under 500 IU/L were considered under target and those who had a trough anti-Xa above 500 IU/L were considered over target. Dose adjustments (decrease in 7 patients and increase in 1 patient) were requested and, depending on which group the patient is in, were actioned by the prescribing physician.

<table>
<thead>
<tr>
<th>Table 5.2 Failure to achieve anti-Xa target</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Standard</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Number of patients off target, n (%)</td>
</tr>
<tr>
<td>over, n</td>
</tr>
<tr>
<td>under, n</td>
</tr>
</tbody>
</table>

5.5.3. Safety endpoints

A total of four bleeding events occurred during enoxaparin treatment, three in the standard dosing group and one in the Individualised group (Table 5.3). One event was minor bleeding (melaena) which occurred in the standard dosing group. Two secondary events (bruises > 20 cm²) occurred in the standard dosing group. One patient in the Individualised group had a secondary event following angioplasty (bruise around the site of needle entry in the femoral artery). No major bleeding events were observed in this study.
Table 5.3 Safety endpoints. RR = relative risk

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Individualised</th>
<th>RR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with a bleeding event, n (%)</td>
<td>3 (33)</td>
<td>1 (9)</td>
<td>0.27 (0.03 – 2.19)</td>
<td>0.178</td>
</tr>
<tr>
<td>Minor, n</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bruise, n</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.5.4. Treatment effectiveness

There were no recurrent thromboembolic events in either arm during the treatment stage or at 30 days. Two patients in the Individualised arm and one patient in the standard arm of the study were lost to follow up.
5.6. Discussion

In this randomised, controlled, double-blind clinical trial, standard dosing of enoxaparin which is based on Wt and CLcr was compared to individualised dosing where the patient’s response along with covariates were used to inform dose selection. The study was terminated before reaching its target sample size and the results are therefore not able to be interpreted inferentially.

For this study to be carried out, a treatment target for enoxaparin needed to be identified (Chapter II), a dosing method (TCIWorks) needed to be trialled and evaluated (Chapter III), and an anti-Xa assay needed to be evaluated for clinical use (Chapter IV). This study was carried out for almost 2 years but recruitment fell short. This is largely due to ACS patients being catheterised within 24 hours of admission and VTE patients discharged on home enoxaparin while the dose of oral anticoagulants was stabilised. All ACS patients in this cohort were either awaiting urgent coronary bypass surgery or were admitted when the catheterisation laboratory was not available. The rest of the patients had either symptomatic PE which required longer hospital admission or were not suitable for home enoxaparin. Theoretically, many more patients could have been recruited in the community (after discharge on home enoxaparin). However, ethical, legal, and logistical issues would have made this difficult.

It has been known for a few years that current dosing guidelines for enoxaparin are too simplistic and may explain the reported rate of bleeding and bruising which is similar or worse than its predecessor unfractionated heparin. The relationship between CL and Wt is non-linear hence a fixed dose per Wt is likely to result in an overdose in the obese. Also, CLcr is a continuous variable so the dichotomy at 30 mL/min is likely to result in suboptimal dosing in some patients. It has been shown that clinicians often do not adhere to these guidelines especially at the cusp of Wt and CLcr.[91] When more reasonable covariate-based dosing was used, the rate of bleeding has been reported to significantly decrease.[95]
Chapter 5: A randomised controlled trial of a Bayesian dose-individualisation method for enoxaparin

This presented the question: would a more individualised method for enoxaparin dosing result in a further reduction in adverse events?

In this RCT, the relative risk of minor bleeding or bruising in the Individualised arm was lower than in the standard arm albeit not statistically significant. The study was underpowered which is reflected in the wide 95% CI of the relative risk (0.03 – 2.19). It remains a conjecture whether an adequately powered study would have shown significantly lower RR of bleeding with Individualised dosing.

Therapeutic monitoring of enoxaparin through anti-Xa testing is not routinely done or advocated in clinical practice. This could be due to various reasons including: the lack of an optimal treatment target, the perceived notion that the dose-response relationship of enoxaparin is predictable across individuals, the incorrect perception that enoxaparin is associated with less bleeding events than its predecessor, and the cost/instability of the assay kit. The assay is available only in two hospitals across New Zealand and the assay result would usually take 2-3 days to become available to the treating clinician.

The variability in dose-response of enoxaparin necessitates the dosage regimen to be individualised. Covariate-based dosing has shown benefit in dosing enoxaparin while response-based dose-individualisation has shown benefit in dosing a few other drugs. [134-136] Applying this dosing method to enoxaparin dosing should theoretically improve clinical outcomes but empirical evidence for this remains to be seen.

A total of four bleeding events occurred during enoxaparin treatment, three in the standard dosing group and one in the Individualised group (Table 5.3). One event was minor bleeding (melaena) which occurred in the standard dosing group. That patient’s anti-Xa was off target (anti-Xa > 500 IU/L). Two secondary events (bruises > 20 cm²) occurred in the standard dosing group but neither patient had an anti-Xa that was above target. One patient in the Individualised group had a
secondary event following angioplasty (bruise around the site of needle entry in the femoral artery). No major bleeding events were observed in this study.
Part IV

USING COVARIATES TO INDIVIDUALISE THERAPY IN CHILDREN
Chapter 6: The development and evaluation of a model to predict fat-free mass in children

Parts of this chapter are based on the following manuscript currently in press:

Part IV of this thesis focuses on the influence of covariates on dose-individualisation and contains two chapters. Chapter 6 describes the development and evaluation of a semi-mechanistic model to predict fat-free mass (FFM) in children. Chapter 7 describes the use of FFM to develop a PKPD model of unfractionated heparin in children using a dataset previously collected by collaborators. It also explores the use of FFM to guide dose-individualisation.
6.1. Background

Human body mass, shape, and function change with age and metabolic state.[174] In the discipline of pharmacometrics, interest in body composition stems from the quest for a size descriptor that provides the best prediction of differences in pharmacokinetic parameters such as clearance (CL) between individuals.[28] Total body weight (WT) has often been used to scale drug doses but this has been found to over-predict drug CL in some cases in obese adults.[27, 175] Finding a more suitable size descriptor is particularly important given the rise of obesity in both developed and developing countries. The body’s metabolic processes occur mainly in lean tissues [26] and this has turned the focus towards understanding the influence of fat free mass (FFM).

FFM describes the lean component of the body excluding fat.[48] Thus FFM is the sum of muscle mass, bones, non-adipose components of internal organs, and extracellular fluid. Previous studies have described lean body weight (LBW) as a measure of non-fat weight. However LBW is not easily amenable to measurement, in contrast to FFM, and it is usual therefore to approximate LBW by FFM.[30] Of note, in the same individual FFM takes a slightly lower value than LBW as it excludes all fat (e.g. the bilayer lipid membranes in cells).[49]

Body composition changes in relation to age along with changes in body size and shape.[56] WT has been and is still used to predict drug doses in paediatric patients but excess fat does not contribute to drug clearance and hence a size metric that includes WT may provide increasingly poor prediction of CL as obesity increases. FFM calculated using an adult model[30], has been shown to be superior to both WT and body surface area for description of glomerular filtration rate from premature neonates to young adults.[68] A FFM model developed in children is likely to describe functional maturation better. There is currently a paucity of large, mechanism-based models to predict FFM in children. Current models were either developed using small samples[176, 177], covering a small age range[176, 177], or were fully empirical thus limiting their external validity.[178]
The relationship between size and drug clearance for children, and adults, is considered to be non-linear.\cite{41} In 1950, body surface area was proposed as a more accurate size descriptor to predict dosing requirements in children than WT.\cite{62} More recently allometric scaling has been shown to provide a more appropriate scale measure for individuals with normal body composition from children to adults.\cite{38, 41} For overweight and obese adults, various size descriptors (e.g. ideal body weight, adjusted body weight, body surface area) have been proposed to aid appropriate dosing of drugs. Previous work on FFM prediction has resulted in models that predict FFM in male and female adults (Equations 6.1 and 6.2).\cite{30} The FFM prediction model incorporates sex, height, and weight as covariates. These models were developed in 373 patients (168 male, 205 female) with a wide range of body weight (40.7 to 216.5 kg) and BMI values (17.1 to 69.9 kg/m²). Observed FFM was measured using bioimpedance and dual-energy X-ray absorptiometry (DXA).\cite{179}

\[
\text{Adult } FFM_{\text{male}} = \left[ \frac{(9270 \times WT)}{6680 + (216 \times BMI)} \right]
\]

Equation 6.1 A model for fat-free mass in adult males

\[
\text{Adult } FFM_{\text{female}} = \left[ \frac{(9270 \times WT)}{8780 + (244 \times BMI)} \right]
\]

Equation 6.2 A model for fat-free mass in adult females

where $FFM$ is in kg, $WT$ is total body weight in kg; $BMI$ is body mass index in weight per height squared (kg,m$^2$); and the constants are fixed effect parameters estimated for males and females. These models may need to account for body structural maturation over age (in addition to differences in sex and changes in height and weight) in order to provide appropriate predictive performance of FFM in children.
A variety of methods have been introduced to determine human body composition.\textsuperscript{[180, 181]} DXA indirectly measures the fat mass of soft tissue in a defined field by using two different frequency low-energy X-ray beams passing through the body. The densities of fat and lean tissues are different hence the two beams are attenuated to differing degrees. The attenuation ratio, which is the ratio of beam attenuation at the lower energy to that at the higher energy, can be used to calculate fat free mass. Various other methods exist such as underwater weighing, potassium counting, and isotope dilution but these either require substantial cooperation from the subjects and/or are associated with exposure to radiation, making them difficult to use in children.\textsuperscript{[182]} While the use of DXA is common in paediatric research due to its ease of use in children, it is not routinely used in the clinic for dose individualisation.

6.2. Objectives

To develop and evaluate a model to predict fat free mass in children.
6.3. Methods

6.3.1. Data

Two datasets containing demographic data and FFM measurements obtained by dual-energy x-ray absorptiometry (DXA) were used in this analysis. Data was collected and ethics approval (by the New Zealand Lower South Ethics Committee) and written consents were obtained by researchers at the Bone Health and Body Composition Research Group (University of Otago, Dunedin, New Zealand). Demographic data and covariate distribution are summarised in Table 6.1. DXA measurements were performed and analysed with a Lunar DPX-L scanner (software package 4.7; Lunar, Madison, WI).[183] The two data sets were collected over different time periods and were used here for model building (index) and model testing (test). The Index dataset, collected between 2000 and 2005, contained 926 measurements for 496 females and 853 measurements for 515 males and the Test dataset, collected between 2006 and 2009, contained one measurement each for 90 females and 86 males (Table 6.2).
Table 6.1 Demographic characteristics and FFM measurements of study population. Values are expressed as median (range). See Appendix 4, A4.1 for histograms of subject covariates. Tanner Score is a categorical variable in the form of an integer between 1 and 5. BMI = body mass index; BMC = Bone Mineral Content

<table>
<thead>
<tr>
<th>Sex</th>
<th>Female</th>
<th></th>
<th>Male</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dataset</td>
<td>Age (y)</td>
<td>Weight (kg)</td>
<td>Height (cm)</td>
</tr>
<tr>
<td></td>
<td>Index</td>
<td>(n = 496)</td>
<td>(12.3 to 120.1)</td>
<td>(87.7 to 188)</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>(n = 90)</td>
<td>(3 to 29.3)</td>
<td>(3 to 29.7)</td>
</tr>
<tr>
<td></td>
<td>Index</td>
<td>(n = 515)</td>
<td>(4.8 to 18.9)</td>
<td>(13.2 to 116.5)</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>(n = 86)</td>
<td>(4.9 to 18)</td>
<td>(14.5 to 122.5)</td>
</tr>
</tbody>
</table>
Table 6.2 A summary of observations in each dataset for females and males

<table>
<thead>
<tr>
<th>Sex</th>
<th>Female</th>
<th></th>
<th>Male</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dataset (n = 496)</td>
<td>Test (n = 90)</td>
<td>Index (n = 515)</td>
<td>Test (n = 86)</td>
<td></td>
</tr>
<tr>
<td>Number of observations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single observation</td>
<td>926</td>
<td>91</td>
<td>853</td>
<td>86</td>
</tr>
<tr>
<td>Two observations</td>
<td>229</td>
<td>90</td>
<td>314</td>
<td>86</td>
</tr>
<tr>
<td>Three observations</td>
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<td>64</td>
<td>Nil</td>
</tr>
<tr>
<td>Four observations</td>
<td>150</td>
<td>Nil</td>
<td>131</td>
<td>Nil</td>
</tr>
<tr>
<td>Five observations</td>
<td>2</td>
<td>Nil</td>
<td>6</td>
<td>Nil</td>
</tr>
</tbody>
</table>

6.3.2. Model development

Three models were considered for prediction of FFM in children. Two of these models were developed from the data described above: (1) a fully empirical model (M1) based solely on the data and (2) a maturation model (M2) that incorporates information from FFM values in adults as an asymptote for older children. In addition, the adult model (which was developed independently and termed M3 in this work) was applied directly to the FFM data from children.

6.3.2.1. Empirical model (M1)

The fully empirical model (M1) was developed to provide a best description of the index data set given the available covariates. Since empirical models are likely to have poor predictive performance when extrapolating to new data sets that have patient characteristics at the extreme or outside the range of covariates in the data used to develop the model, it is not intended for this model to be used in clinical practice.
The model was developed using STATA v11 (StataCorp. 2009. *Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP). The model was allowed to include up to 10 covariates with second level interaction terms. The model was a mixed-effects multiple linear regression (of the general form given by Equation 6.3) using maximum likelihood. Fixed effects (covariates) were regressed against the dependent variable (observed FFM obtained from DXA) and added in a step-wise forward selection procedure based on a statistically significant reduction (P < 0.05) in the log-likelihood (LL). Random between-subject variability (BSV) and residual unexplained variability (RUV) were also accounted for in the regression using additive models. The general form of the model is given here:

\[ FFM_{ij} = x_{ij}\theta + \eta_i + \varepsilon_{ij} \]

**Equation 6.3 A linear mixed-effects multiple regression model for fat-free mass**

where FFM is the \( j \)th observed value of fat free mass for the \( i \)th subject, \( x_{ij} \) is a covariate, \( \theta \) is a \( p \times 1 \) vector of model parameters, \( \eta_i \) is the \( i \)th subject’s between subject random effect which is normally distributed with a mean of zero and a variance of \( \sigma^2 \), and \( \varepsilon_{ij} \) is the difference of the observed and individual predicted observation and is normally distributed with a mean of zero and a variance of \( \sigma^2 \). Covariates considered, with interactions, were sex, age, height, weight, fat mass, % fat, bone mineral content, total body calcium, and Tanner score. The latter was assessed by the parent/guardian of the children or the adolescents themselves using standard descriptions and pictures.
6.3.2.2. Maturation model (M2)

Various structural maturation models were developed to estimate FFM as a function of age. These models were based on published literature in which asymptotic models were used to describe maturation as a function of age. \[38, 41\] The performance of these models was compared to the fully empirical FFM model (M1) and the published adult FFM model (M3).

The maturation models considered in this project were hyperbolic, sigmoid hyperbolic, asymmetric hyperbolic, exponential, and Weibull. A generic model structure is described below (Equation 6.4). The data were modelled in NONMEM v7.2 \[184\] (with Wings for NONMEM v720 \[185\]). We use the term ‘maturation model’ to describe a model which asymptotes to a mature (i.e. adult) FFM value as postnatal age increases. This distinguishes it from a fully empirical function (e.g. M1) that does not contain asymptotes when post-conception age approaches zero and as postnatal age approaches adulthood. Statistical analysis and diagnostic plots were used for model selection. The Test dataset was used for external evaluation.

\[
FFM_{ij} = f(\theta_i, x_{ij}, \eta_j) \times \text{AdultFFM}_{ij} + \epsilon_{ij}
\]

Equation 6.4 A non-linear, mixed-effects regression model for fat-free mass

where \(f(\theta_i, x_{ij})\) is a nonlinear function that asymptotes towards 1. It has fixed effects \(\theta\) estimated from observed covariate \(x_{ij}\) with between subject variability (BSV) \(\eta_i\) which is normally distributed with a mean of zero and a variance of \(\omega^2\), \(\text{AdultFFM}\) is the fat free mass calculated using the adult model but based on the covariate values for the \(i^{th}\) subject (Equation 6.1) on the \(j^{th}\) occasion, and \(\epsilon_{ij}\) is the difference of observed from individual prediction and is normally distributed with a mean of zero and a variance of \(\sigma^2\).
NONMEM is a nonlinear mixed effects modelling software which allows the user to account for BSV and RUV as well as parameter differences influenced by fixed effects. BSV was evaluated using additive (normal distribution) and exponential (log-normal distribution) random effects. RUV was evaluated using additive, exponential, and combined error models. Covariates considered include sex, age, height, weight, and Tanner Score. These covariates correlate with structural maturation and are relatively easy to measure.

Model selection was based on the likelihood ratio test. The objective function value (OFV) of NONMEM is proportional to minus twice the log-likelihood (-2LL). A decrease in OFV between two nested models of $\geq 3.84$ points denotes a $p$-value $< 0.05$. For non-nested models the Akaike information criterion was used.

6.3.2.3. Adult model (M3)

A published adult model (M3) of FFM \cite{30} was considered as the base model for M2. Since M1 and M2 were developed based on the data used here then it was expected that M3 would not perform as well as M1 or M2 but would provide a useful standard to compare the performance of the other models. M3 is given by equations 6.1 and 6.2 above and is individualised to each individual by using their values of height, weight and sex. Use of this model would predict the value of FFM for any given set of covariates had the child been considered as an adult.

6.3.3. Evaluation of model performance

Goodness-of-fit plots (e.g. observed versus population predicted FFM) were used to evaluate the models. Semi-parametric marginal visual predictive checks (VPCs) \cite{186} were constructed to explore the predictive ability of the final models with reference to age. 1000 datasets were simulated. Patients, and their covariates, were non-parametrically bootstrapped. Their covariates were retained with the patient ID in order to compute the FFM given any set of parameters. The parameters and
residual error were parametrically bootstrapped from their final covariate centred mean and between subject variance and residual variance, respectively. Bootstrap data were then constructed at each (selected) individual’s values of their covariates. For the index dataset, the 2.5th, 50th, and 97.5th percentiles from the simulated FFM data were plotted against age (divided into 10 bins), with the observed data superimposed. As the test dataset is considerably smaller compared to the index dataset, the 10th, 50th, and 90th percentiles from the simulated FFM data were plotted against age (ranked and divided into 5 bins).

All models (M1, M2, and M3) were also compared in terms of their mean error (ME) and root mean square error (RMSE). In order to eliminate the influence of correlated observations on ME and RMSE due to repeated measures, one thousand nonparametric bootstraps were generated from observed and predicted values. In this bootstrap observations were bootstrapped as well as individuals. In any one bootstrapped dataset no individual would have more than 1 observation to avoid repeated measures. In each data set each time that the patient ID was re-chosen the same sample was chosen. The choice of repeated samples was random between data sets. The mean ME and RMSE with the 95% confidence interval were obtained and compared between models.
6.4. **Results**

6.4.1. **Empirical model (M1)**

The fully empirical FFM model (M1) contained nine terms with interactions. Equation 6.5. This model is intended to provide a reference for an empirical "best" description of the data.

\[
FFM = -12.75 + (0.21 \times Ht) + (0.38 \times 10^{-4} \times Ht \times BMC) + (1.20 \times Sex) \\
+ (0.80 \times Sex \times TS) + (0.26 \times 10^{-4} \times Wt \times BMC) \\
+ (0.59 \times 10^{-2} \times Sex \times Age^2) - (0.86 \times 10^{-6} \times BMC^2 \times (1 - Sex)) \\
+ (0.81 \times 10^{-1} \times Wt \times (1 - Sex))
\]

**Equation 6.5 A fully empirical FFM linear mixed-effects model (M1)**

where BMC is bone mineral content, TS is Tanner Score, and Sex assumes the value 0 for females and 1 for males.

Fixed and random effect parameter estimates for the M1 model are summarised below. Table 6.3
Table 6.3 Parameter estimates and 95% confidence intervals for the fully empirical model (M1). Female sex = zero and male sex = 1; Ht = height in cm; TS = Tanner Score, an integer between 1 and 5; Age was in years, and BMC = bone mineral content in grams.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-12.75 (-14.36 to -11.14)</td>
</tr>
<tr>
<td>$\omega^2$</td>
<td>3.32 (2.91 to 3.79)</td>
</tr>
<tr>
<td>Ht coefficient</td>
<td>0.21 (0.20 to 0.23)</td>
</tr>
<tr>
<td>HtBMC coefficient</td>
<td>$0.38 \times 10^{-4}$ (0.33 $\times 10^{-4}$ to 0.43 $\times 10^{-4}$)</td>
</tr>
<tr>
<td>Sex coefficient</td>
<td>1.20 (0.55 to 1.85)</td>
</tr>
<tr>
<td>SexTS coefficient</td>
<td>0.80 (0.59 to 1.00)</td>
</tr>
<tr>
<td>WtBMC coefficient</td>
<td>$0.26 \times 10^{-4}$ (0.19 $\times 10^{-4}$ to 0.33 $\times 10^{-4}$)</td>
</tr>
<tr>
<td>SexAge$^2$ coefficient</td>
<td>0.0059 (0.0041 to 0.0077)</td>
</tr>
<tr>
<td>$BMC^2(1-\text{Sex})$ coefficient</td>
<td>$-0.86 \times 10^{-6}$ (-1.03 $\times 10^{-6}$ to -0.70 $\times 10^{-6}$)</td>
</tr>
<tr>
<td>Wt(1-\text{Sex}) coefficient</td>
<td>0.081 (0.059 to 0.104)</td>
</tr>
<tr>
<td>$\sigma^2$ coefficient</td>
<td>1.89 (1.71 to 2.10)</td>
</tr>
</tbody>
</table>

$\omega^2$ is the BSV for the Intercept; $\sigma^2$ is the residual variance
6.4.2. Maturation model (M2)

The final maturation model was a sigmoid hyperbolic model with age and sex as covariates. Equation 6.6. See Appendix 4, A4.2 for noteworthy model building steps.

\[ M2 = \left[ \alpha + \frac{(\beta - \alpha)}{1 + \left( \frac{Age}{A50} \right)^\gamma} \right] \times M3 \]

Equation 6.6 A sigmoid hyperbolic model to describe FFM maturation (M2)

where \( \alpha \) and \( \beta \) are the lower and upper bounds, respectively, of the sigmoid hyperbolic function; \( A50 \) is the FFM maturation half-time; \( \gamma \) is the sigmoidicity coefficient; and \( M3 \) is the adult FFM model. The parameters \( \alpha \), \( \gamma \), and \( A50 \) were estimated separately for each sex. \( \beta \) was fixed to 1 so that the asymptote was equal to the adult value predicted for each sex.

The model is presented in full for males and females in Equations 6.7 and 6.8.

\[
FFM(\text{males}) = 0.88 + \left( \frac{(1 - 0.88)}{1 + \left( \frac{Age}{13.4} \right)^{-12.7}} \right) \times \left( \frac{9270 \times WT}{6680 + (216 \times BMI)} \right)
\]

Equation 6.7 A model for fat-free mass in male children

\[
FFM(\text{females}) = 1.11 + \left( \frac{(1 - 1.11)}{1 + \left( \frac{Age}{7.1} \right)^{-1.1}} \right) \times \left( \frac{9270 \times WT}{8780 + (244 \times BMI)} \right)
\]

Equation 6.8 A model for fat-free mass in female children
Fixed and random effect parameter estimates for the M2 model are summarised below. Table 6.4

Table 6.4 Parameter estimates and 95% confidence intervals for the maturation model (M2)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter estimate</th>
<th>Bootstrap mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>1.11</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$ **</td>
<td>1*</td>
<td>1*</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>1.1</td>
<td>12.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_{50} , (y)$</td>
<td>7.1</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\omega^2_\beta$</td>
<td>0.0113</td>
<td>0.0061</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>0.0017</td>
<td>0.0036</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*values were fixed

$\omega^2_\beta$ is the BSV for the fixed parameter $\beta$; $\sigma^2$ is the residual variance

Goodness of fit plots (observed vs. population predicted FFM) for the Index dataset (Figure 6.1) show an improved data fit with M2 models compared to M3 particularly for males. The fully empirical model M1 has, as expected, visually the best fit. See Appendix 4, A4.3 for residual plots.
Figure 6.1 Observed vs. predicted FFM using the three models. The upper row is M1, middle row M2, and lower row M3 for females and males for the index dataset. The line of identity is also shown.

Figure 6.2 shows VPC plots for all models investigated for females and males. The plots indicate that the maturation model (M2) provides better predictions than the adult model (M3). For the VPC, 1000 datasets were simulated and the 2.5th, 50th and 97.5th percentiles from the simulated FFM data were plotted against age, with the observed 2.5th, 50th and 97.5th superimposed. See Appendix 4, A4.4 for covariate (BMI) VPC plots.
6.4.3. External evaluation

The models were externally evaluated by predicting into a test dataset. The diagnostic plots for the test dataset (Figure 6.3) show a slightly improved data fit with model M2 compared to M3 particularly for males. M1 and M2 appeared similar.
Figure 6.3 Observed vs. predicted FFM using the three models. The upper row is M1, middle row M2, and lower row M3 for females and males for the test dataset. The line of identity is also shown.

Figure 6.4 shows the VPC plots for all models investigated for females (4a) and males (4b) using the Test dataset. The plots indicate that the maturation model (M2) provides better predictions than the adult model (M3).
Finally, the bias (ME) and precision (RMSE) were compared for the three models (Table 6.5). Mean and 95% CI were computed from 1000 bootstraps generated from observed and predicted values from each model. This was done in order to eliminate the influence of correlation of observations on ME and RMSE due to repeated measures. Again, the maturation model (M2) had less bias and imprecision compared to the adult model (M3).
Table 6.5 Mean error (ME), root mean squared error (RMSE) of the maturation model (M2) compared to the fully empirical and the adult models for the Index and Test datasets. Mean and 95% confidence interval values were calculated from 1000 bootstraps of observed and predicted FFM.

<table>
<thead>
<tr>
<th>Model</th>
<th>Index dataset</th>
<th>Test dataset</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME*</td>
<td>RMSE*</td>
</tr>
<tr>
<td></td>
<td>(0.03 to 0.16)</td>
<td>(1.03 to 1.23)</td>
</tr>
<tr>
<td>M1</td>
<td>0.09</td>
<td>1.12</td>
</tr>
<tr>
<td>M2</td>
<td>0.24</td>
<td>1.58</td>
</tr>
<tr>
<td>M3</td>
<td>0.29</td>
<td>3.76</td>
</tr>
</tbody>
</table>

* values are presented as mean (95% CI)
6.5. **Discussion**

This study provides age-driven asymptotic models to predict FFM maturation in children. These models were allowed to asymptote to a mature (i.e. adult) value with age. There were no subjects below the age of 3 years in the datasets to develop the models so these models should not be extrapolated to infants and neonates.

The final model which provided the best prediction of FFM incorporates a previously described adult model\[^{30}\] and a sigmoid hyperbolic function which asymptotes towards adult FFM as age reaches adult values. The model assumed that the adult model was unbiased. A similar asymptotic model was proposed by Anderson & Holford in order to describe size and functional maturation of glomerular filtration rate\[^{68}\] and drug clearance from premature neonates through to adulthood.\[^{41}\] The authors argued that although allometric scaling accounts for the nonlinear relationship between body size and function, predicting drug clearance in children from adult values requires a model which accounts for functional maturation. An appropriate measure of size is essential in estimating pharmacokinetic parameters and consequently drug doses.

A large dataset was used to develop the model. Subjects spanned a large range of weight (12.3 to 120.1 kg) and BMI values (12.6 to 41.3 kg/m\(^2\)). Approximately 28.6% and 32.9% of males and females, respectively, can be considered overweight or obese (defined as BMI above the 85\(^{th}\) BMI-for-age percentile). A smaller dataset was used to provide an external evaluation of model performance. Although relatively small, this dataset contained demographics that spanned a similar range of weight (14.5 to 122.5 kg) and BMI values (13.4 to 44.9 kg/m\(^2\)). To note, based on the demographics of the Dunedin region, the majority of subjects in this dataset are Caucasian so model performance in other ethnicities is uncertain.

The fully empirical model (Equation 6.4) that was developed in order to provide the best fit is too impractical to be used clinically. The adult model was used to provide a naive description of the data, in the absence of a paediatric model, and
also to provide the asymptotic values for the maturation model. It is of note that, based on the VPCs and ME and RMSE values provided in Table 6.5, the adult model (M3) provides a reasonable description of the FFM of female children from 3 years of age. The model is less precise than the maturation model but was not different in the magnitude of its prediction error from the maturation model in the female test dataset (ME = 0.21 kg, 95% CI -0.32 to 0.74 vs -0.25 kg, 95% CI -0.40 to -0.09, respectively), although the sign is reversed. However the adult model shows significant bias in the male test dataset compared to the maturation model (ME = -3.24 kg, 95% CI -4.19 to -2.36 vs -0.77 kg, 95% CI -1.15 to -0.39, respectively). The maturation model developed here is more precise over both sexes and the range of ages and can essentially be used to predict FFM in children (over 3 years) and adults (up to 80 years old). Our results show that predicted FFM in girls is similar to that predicted for women but predicted FFM in boys is less than that predicted for men.

Estimation of between subject variability proved difficult despite the relatively large dataset. Although, in this case, a nuisance parameter, estimation of BSV is important to allow inference to be drawn regarding the fixed parameters. Over half the subjects (n=543) in this dataset had a single observation which may explain the difficulties in estimating the BSV. Unbalanced repeated measures data were associated with a preponderance of singleton measures and only one instance of five observations and repeated measures were more common in the older age group (one subject had her fifth measurement at age 16 years and the eight subjects with four observations had the last measurement at a median age of 19 years). Since the value of BSV is of little clinical importance then any bias in its estimate will be of limited interest. In order to mitigate the influence of correlation between repeated measures on model predictions, nonparametric bootstraps were created. Each bootstrap contained the total number of subjects but with only one observation with the corresponding prediction in a given bootstrapped dataset randomly selected. Through this method, the 95% CI around bias and precision estimations were calculated.
6.6. Conclusions

A model to predict FFM from age, weight, BMI, and sex has been developed for use in male children. The existing adult model is suitable for use in female children. Since the maturation model includes the adult model then either can be used for female children. These models should be considered when calculating paediatric drug doses if dosing recommendations exist that utilise FFM.
Chapter 7: Using fat-free mass to develop a paediatric PKPD model for unfractionated heparin

Parts of this chapter are based on the following manuscript currently under preparation:

**Al-Sallami HS**, Newall F, Monagle P, Ignjatovic V, Cranswick N, Duffull SB. *Development of a population PKPD model of unfractionated heparin in paediatric patients.*
7.1. Background

Unfractionated heparin (UFH) is an anticoagulant used for the treatment and prevention of thrombosis (e.g. myocardial infarction and venous thromboembolism). It comprises a heterogeneous mixture of glycosaminoglycans of various chain lengths (average molecular weight of 15 kDa) normally derived from porcine or bovine intestines.\[69\] UFH binds to antithrombin III (AT) via a pentasaccharide sequence and induces a conformational change which enhances AT binding to activated clotting factors such as Xa. Additionally, long UFH molecules (≥ 18 saccharide units, MW > 5 kDa) serve as a catalytic template to which both AT and factor IIa bind, effectively inhibiting IIa.\[187\]

As an extension of its established role in adult patient populations, UFH is widely used in paediatric patients mainly due to its long history of clinical use and ease of reversibility.\[188, 189\] It has a relatively short half-life which makes it more appropriate for use in critically ill children, who may be at a greater risk of bleeding. Current guidelines on the use of antithrombotic agents in children recommend that UFH be used as a first-line intervention to treat arterial and venous thromboses.\[121\] UFH is also recommended for thromboprophylaxis (e.g. cardiac angiography, cardiopulmonary bypass, and haemodialysis).

Thromboembolism, and subsequently clinical studies, in paediatric patients is rare and most recommendations are based on extrapolation from adult data. However, there is evidence that such extrapolation may be inappropriate due to significant pharmacokinetic and pharmacodynamic differences between neonates, infants, children, and adults.\[122, 190-194\]

There are standard dosing nomograms for UFH in children.\[195\] The current recommendation is for a UFH bolus to be no greater than 100 units/kg; bolus doses to be withheld or reduced in the presence of significant bleeding risks.\[121\] Given the variability of dose requirements for UFH, plasma monitoring is advocated but
applying the activated partial thromboplastin time (aPTT) target range from adults to paediatric patients is not appropriate. Instead, a heparin concentration (determined by protamine titration) of 0.2 to 0.4 units/mL or an anti-Xa concentration of 0.35 to 0.7 units/mL is advocated.[196]

Variability in the dose-response of UFH necessitates consideration of the individual risk factors for bleeding and the perceived risk of thrombosis.[121] Accounting for the sources of this variability can potentially optimise treatment.

7.2. Objectives

To develop and evaluate a PKPD model to predict the dose-response relationship of UFH in paediatric patients. Also, to explore the use of FFM to guide dose-individualisation of UFH in this population.
7.3. Methods

7.3.1. Data

The data used in this analysis has been described previously by Newall et al.\[194\]
Briefly, sixty-four children requiring cardiac angiography who received a single intravenous bolus dose of unfractionated heparin (UFH) participated in this study (Table 7.1). Patients received no antiplatelet or anticoagulant therapy in the 10 days preceding the scheduled procedure. Blood samples were collected at baseline and at 15, 30, 45 and 120 minutes post-UFH administration. UFH concentrations (231 measurements) were determined using a modified protamine titration method.\[197\]
The aPTT was measured (164 measurements) using the PTT-A® kit (Diagnostica Stago) with the upper limit modified to measure up to 999 seconds; inter-assay coefficient of variation was 3.1%. Additionally, 126 aPTT values were considered above the upper limit of quantification (> 999 seconds).

Table 7.1 Demographic characteristics of study population. Values are expressed as mean (range). N is number of subjects.

<table>
<thead>
<tr>
<th></th>
<th>64</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>64</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>30:34</td>
</tr>
<tr>
<td>Age (y)</td>
<td>6.7 (0.5 to 15.5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>23.6 (6.7 to 68.6)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>115.7 (65 to 176)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.1 (11.5 to 24.7)</td>
</tr>
<tr>
<td>UFH Dose (IU)</td>
<td>2020 (600-5000)</td>
</tr>
<tr>
<td>UFH dose per weight (IU/kg)</td>
<td>91 (47.9 to 105.4)</td>
</tr>
</tbody>
</table>
7.3.2. Model building

Data from all 64 patients were included in the model-building process. Two patients had no recorded value of height and their height was imputed using multivariate linear regression using the distribution of age, sex, and weight in the dataset.

Linear and non-linear elimination one- and two-compartment disposition models were considered to describe the concentrations of UFH. Emax, sigmoid Emax, linear, and log linear models were considered to describe the concentration-effect relationship. PD parameters were estimated sequentially using the population pharmacokinetic parameters and data (PPP & D) estimation method.[198]

7.3.2.1. Covariate selection

Age, sex, total body weight (Wt), and fat-free mass (FFM) were considered as covariates on model parameters. The model for FFM is that described in Chapter 6. The covariates were added to the model using forward inclusion backward elimination.

7.3.3. Model selection

The analysis was performed in NONMEM v7.2[184] (with Wings for NONMEM v720 [185]) using the first-order conditional estimation method with interaction and combined residual error (RUV) model. Between-subject variability (BSV) was implemented using exponential models. Model selection was guided by (1) the decrease in the objective function value (OFV, the minimisation criterion in NONMEM); (2) visual goodness-of-fit analysis; and (3) the estimated uncertainty in parameter estimates as reported by the 95% confidence interval of parameter estimates based on nonparametric bootstrapping.
Model selection was based on the likelihood ratio test. The OFV of NONMEM is proportional to minus twice the log-likelihood (-2LL). The difference in the OFV between two nested models is $\chi^2$-distributed, meaning that a difference of 3.84 corresponds to $P = 0.05$ for one degree of freedom.

### 7.3.4. Evaluation of model performance

The final PK and PKPD models were evaluated using visual predictive checks (VPCs). One thousand datasets were simulated and the 5th, 50th, and 95th percentiles from the simulated response data and the 95% confidence interval around the percentiles were plotted against time with the same percentiles of the observed data superimposed. Additionally, the 95% confidence intervals of parameter estimates in the final model were calculated using nonparametric bootstraps. One thousand bootstrap samples were simulated and used to estimate model parameters. Runs with terminated minimisation were excluded and replaced with additional successful runs.

### 7.3.5. Simulation of current dosing guidelines

Current dosing guidelines for UFH infusion for the treatment of thromboses were used to simulate the dose-response and success rate in attaining the therapeutic target (defined as aPTT between 50-90 seconds at steady state) was calculated using FFM-based, Wt-based, and age-and-Wt-based dosing. Ten thousand virtual patients were simulated in MATLAB (version 2013b, MathWorks, Natick, MA) using parametric resampling based on a large children dataset. A joint multivariate normal distribution of sex, age, weight, and height was constructed and randomly sampled. The covariates were then used as a part of the covariate model to generate the population parameters. The fixed and random parameters of the final heparin PKPD model were used for the simulation.
The current guidelines for UFH infusion in children recommend a bolus dose based on a patient’s weight followed by a continuous infusion of 20 IU/kg for children between 1-12 years and 18 IU/kg for children between 12-18 years.\textsuperscript{121} As the model was developed using single high (mean = 91 IU/kg) prophylactic doses of UFH, the dosing guidelines were simulated at steady state where the influence of a bolus dose is negligible.

The final PKPD model was used to simulate the dose-response in ten thousand virtual patients who were administered age-adjusted UFH infusion. See Appendix 5, 5.2 for MATLAB code. The dose was 28 IU/kg if the subject was under 1 year old, 20 IU/kg if between the ages of 1 and 12 years, and 18 IU/kg if over 12 years old. The success rate for achieving a target aPTT of 50-80 seconds at steady state was calculated with the assumption that no dose-adjustments occurred. The simulation showed that the majority of patients had aPTT above 80 seconds with a mean aPTT of 380 seconds. Additionally, an optimal FFM-based dose was calculated through a linear regression of target aPTT vs dose curve in order to explore the dose rate based on the PKPD model further.
Chapter 7: Using fat-free mass to develop a paediatric PKPD model for unfractionated heparin

7.4. Results

Sixty-four paediatric patients provided PK and PD data following a single high dose of UFH during a cardiac catheterisation procedure. None of the patients were being treated for thromboses at the time of the study.

7.4.1. Population pharmacokinetics

A one compartment model with linear elimination with a combined (additive and exponential) residual error model provided the best fit for the dose-concentration data (Table 7.2). Size (Wt and FFM) on both CL and V had significant influence on model performance and resulted in a 90 point reduction in the OFV. FFM as a covariate on CL and Wt as a covariate on V as well as allowing the CL and V to co-vary using a block covariance matrix resulted in the lowest variance of CL and V, lowest additive error (without increasing the proportional error), and lowest OFV. Bootstrap estimates of final model parameters were similar to model estimates and the precision (95% CI) was reasonable except for the additive error bootstrap estimate which had a wide 95% CI (Table 7.2). Diagnostic plots in the form of VPC show the model has performed well when 1000 datasets were parametrically bootstrapped (Figure 7.1).
Table 7.2 Final PK model parameter estimates. Bootstrap results are presented as mean and 95% confidence interval.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Covariate model</th>
<th>Parameter estimate</th>
<th>Bootstrap (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta_{CL}$ (L/h/15 kg)</td>
<td>$TVCL = \theta_{CL} \times FFM/15$</td>
<td>0.603</td>
<td>0.601 (0.528 to 0.684)</td>
</tr>
<tr>
<td>$\omega_{CL}$ (%)</td>
<td></td>
<td>0.50</td>
<td>0.50 (0.40 to 0.61)</td>
</tr>
<tr>
<td>$\theta_v$ (L/20 kg)</td>
<td>$TVV = \theta_v \times Wt/20$</td>
<td>0.751</td>
<td>0.745 (0.656 to 0.840)</td>
</tr>
<tr>
<td>$\omega_v$ (%)</td>
<td></td>
<td>0.40</td>
<td>0.39 (0.29 to 0.48)</td>
</tr>
<tr>
<td>Corr (CL, V)</td>
<td></td>
<td>0.75</td>
<td>0.74 (0.40 to 0.99)</td>
</tr>
<tr>
<td>D1 (h)</td>
<td></td>
<td>0.1*</td>
<td></td>
</tr>
<tr>
<td>$\sigma_{prop}$ (%)</td>
<td></td>
<td>0.17</td>
<td>0.16 (0.11 to 0.22)</td>
</tr>
<tr>
<td>$\sigma_{add}$ (U/L)</td>
<td></td>
<td>90</td>
<td>93 (1 to 204)</td>
</tr>
</tbody>
</table>

* The infusion rate D1 was fixed
7.4.2. Population pharmacokinetics-pharmacodynamics

A linear model provided the best fit for the concentration-effect data using the PPP&D sequential estimation method (Table 7.3). When asymptotic models (such as Emax and sigmoid Emax) were attempted the estimates for Emax and C50 become very large and implausible and the correlation between these two parameters was high. Censored PD data (above the upper limit of quantification) were accounted for using Beal’s M3 likelihood estimation (modified to account for right-censoring of data). See Appendix 5, A5.1 for NONMEM code. Between-subject and residual variability were also estimated. The PKPD model performed well using visual predictive checks (Figure 7.2) especially once censored data were accounted for.
Table 7.3 Final PKPD model parameter estimates. Bootstrap results are presented as mean and 95% confidence interval. E0 is baseline aPTT in seconds. SLP is the slope of the linear model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Covariate model</th>
<th>Parameter estimate</th>
<th>Bootstrap (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta_{CL}$ (L/h/15 kg)</td>
<td>TVCL = $\theta_{CL} \times FFM/15$</td>
<td>0.603*</td>
<td>0.50* (34.6 to 36.7)</td>
</tr>
<tr>
<td>$\omega_{CL}$ (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\theta_{V}$ (L/20 kg)</td>
<td>TVV = $\theta_{V} \times Wt/20$</td>
<td>0.751*</td>
<td>0.40* (0.58 to 0.78)</td>
</tr>
<tr>
<td>$\omega_{V}$ (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corr (CL, V)</td>
<td></td>
<td>0.745*</td>
<td>0.1* (0.26 to 0.33)</td>
</tr>
<tr>
<td>D1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E0 (s)</td>
<td>35.6</td>
<td>35.6 (0.0038 to 0.0051)</td>
<td></td>
</tr>
<tr>
<td>$\omega_{E0}$ (s)</td>
<td>0.004</td>
<td>0.0044 (0.001 to 0.012)</td>
<td></td>
</tr>
<tr>
<td>SLP</td>
<td>0.67</td>
<td>0.67 (0.50 to 0.76)</td>
<td></td>
</tr>
<tr>
<td>$\omega_{SLP}$</td>
<td>0.64</td>
<td>0.63 (0.26 to 0.33)</td>
<td></td>
</tr>
<tr>
<td>$\sigma_{prop}$ (%)</td>
<td>0.30</td>
<td>0.30 (0.001 to 0.012)</td>
<td></td>
</tr>
<tr>
<td>$\sigma_{add}$ (U/L)</td>
<td>0.005</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

* Fixed
7.4.3. Simulation of heparin infusion

An optimal dose that would result in approximately 57% success in attaining a target aPTT of 65 seconds at steady state was found to be 2.4 IU/kg. This dose, however, is much smaller than what is observed in clinical practice which suggests the model cannot be extrapolated to the smaller infusion doses.
7.5. Discussion

This is the first study to describe a PKPD model of unfractionated heparin in children. The model describes the data reasonably well despite having a third of the aPTT data above the upper limit of quantification (> 999 s). To account for these censored values, a modified version of Stuart Beal’s M3 method was used.\[202\] The method improves the model fit by calculating the likelihood that a datum falls outside the limit of quantification.\[203\]

The model used FFM as a better measure of the influence of size on drug clearance. Wt has often been used to scale drug doses but this has been found to over predict drug clearance in obese adults.\[27\] Finding a more suitable size descriptor is particularly important given the rise of obesity in both developed and developing countries.

The PKPD model was based on data collected from 64 children who received a large (~ 92 IU/kg) single bolus dose during cardiac catheterisation. Both PK and PD models were found to be linear which somewhat contradicts current evidence of UFH pharmacology. Evidence from the literature suggests that UFH is cleared through a mixture of saturable mechanism, through binding to endothelia cells and macrophages, and first-order mechanism through the kidneys.\[204\] As a result, the dose-response of UFH is considered non-linear with the half-life increasing from ~ 30 minutes after a single dose of 25 IU/kg to ~ 60 minutes after a dose of 100 U/kg.\[103, 117, 119\]

In our PKPD model, the average CL was found to be 0.6 L/h (per 15 kg FFM) and the average V was 0.75 L (per 20 kg Wt). This results in a half-life of approximately 52 minutes. It is likely that our model describes an incomplete segment of heparin dose-response and would over predict aPTT when a small dose is given (and possibly under predict aPTT with higher doses). This became evident when the model was applied to the infusion dosing guidelines which utilise doses of 18-28 IU/kg. The average aPTT predicted was 380 seconds instead of the 50-80
seconds observed in clinical practice. Data covering a larger range of dose and concentration would have made this model more clinically applicable.
7.6. Conclusion

A PKPD model to describe the time-course of UFH effect was developed in a paediatric population which received a high single prophylactic bolus dose. FFM was shown to describe drug disposition well and could potentially be used in dose calculation after appropriate evaluation. However, the model was linear and resulted in overprediction of aPTT when smaller UFH infusion doses were simulated.
PART V

DISCUSSION AND FUTURE WORK
Chapter 8: Discussion, conclusions, & future work
8.1. Discussion

In this thesis, the need for and means of dose-individualisation of drug therapy in order to achieve optimal clinical outcomes were addressed. A drug is given in order to achieve a therapeutic target which is often decided a priori independently of the dosing regimen. The selection of the dosing regimen by the prescriber can have a profound impact on the patient’s clinical outcomes. Underdosing may result in treatment failure yet expose the patient to medicines unnecessarily and waste healthcare resources. Overdosing may cause significant morbidity and mortality. The choice of dosing regimen requires the prescriber to have a quantitative understanding of the drug effect for a given dose, the time course over which the effects are expected to occur, and how variability between patients impacts on dose requirements. A population model can be developed and used to guide dose-individualisation.[205]

Between-subject variability in response (BSV), which determines variability in dose requirements, is important and complex. Variability in the dose-response of enoxaparin results in bleeding event rates that are equal or worse than its predecessor, unfractionated heparin. This variability could be accounted for through target-concentration intervention (TCI). For TCI to work, a therapeutic target needs to be identified (Chapter 2) and a forecasting dose-individualisation method needs to be developed and evaluated (TCIWorks was evaluated in Chapter 3). In Chapter 5, a randomised-controlled trial (RCT) where individualisation of enoxaparin dose was compared to conventional dosing showed an apparent decrease in the relative risk of minor bleeding or bruising albeit not statistically significant. The latter was likely due to the lack of statistical power.

Some of the dose-response variability between patients could be accounted for by covariate-based dosing (e.g. dose per weight or organ function); this was the subject of Chapters 6 and 7 of this thesis. Assuming that the source of overall variability is largely determined by variability in drug disposition, a model for
paediatric fat-free mass (FFM) was developed and evaluated (Chapter 6). FFM is believed to correlate linearly with drug clearance. The model was developed using a large dataset of FFM measurements and a model for structural maturation based on age which asymptotes into a published and evaluated adult model of FFM. In Chapter 7, the model was used to develop and evaluate a heparin PKPD model in paediatrics. FFM was superior to total body weight in terms of model fit.

A model-based approach to dose-individualisation is both logical and useful.[206, 207] However, it requires considerable knowledge, skills, and resources in order to apply it to clinical practice. For that reason, it should be targeted to drugs and situations where the benefit is clear as often covariate-based dosing is sufficient. In this case the concept of safe and effective variability (SEV) becomes useful. [132] SEV can help identify a therapeutic range where a range of target concentrations (e.g. steady state drug concentration) is considered optimal. Based on that, if the achieved concentration in an individual lies within this range, the treatment in the population may be considered safe and effective.

In order to illustrate the relationship between SEV, BSV, and dosing let us consider SEV and BSV as variances and the total BSV as the sum of random (BSVr) and predictable (BSVp) between-subject variance. If SEV is larger than BSV, then dose-individualisation is not needed. This means that if the whole of the variability between subjects is smaller than the level of variability considered to be safe and effective then one dose fits all (e.g. some locally administered drugs and some vaccines).[208] If SEV is smaller than BSV but larger than the unpredictable portion of BSV (BSVr), then covariate-based dosing is needed (e.g. paracetamol dose based on patient’s weight). If SEV is smaller than the unpredictable portion of BSV, then TCI is needed on top of covariate-based dosing (e.g. gentamicin dosing based on patient’s weight and CLcr in addition to TCI). Lastly, if SEV is smaller than BOV, then no dosing method could possibly result in safe and effective target concentration and the drug cannot be used.[132] Figure 8.1.
Figure 8.1 A schematic representation of the relationship between safe and effective variability (SEV) and between-subject variability (BSV) in dose-response. BSVr = unpredictable/random BSV; BOV = between-occasion variability; TCI = target concentration intervention.
Although the concept of TCI is relatively new, the principles of dose-individualisation are not. Clinicians see patients individually and the treatment is based on factors specific to the individual patient (e.g. disease state, comorbidity, concomitant therapy). Clinicians would then follow dosing-guidelines which are mostly developed in order to account for the needs of the “averaged” patient. These guidelines are part of the effort towards obtaining the best evidence for treatments, but this evidence is usually confined to empirical evidence such as randomised controlled trials (RCTs). These clinical trials are often conducted in order to test hypotheses that are rarely based on disease or drug mechanisms and are often difficult to apply to individual patients.\cite{209}

Before clinical evidence and dosing guidelines can be tailored towards a patient’s individual needs, the variability in dose-response needs to be properly understood. Drugs are often described as having predictable pharmacokinetics and/or pharmacodynamics thus having fixed or simple dosing regimens and potentially not requiring routine monitoring (e.g. through drug concentration monitoring)\cite{210-214}. Essentially, predictability in drug response (e.g. time course of drug concentration) refers to low variability between individuals. In population analysis, this variability is reflected in parameter variability across individuals and can be quantified by the coefficient of variability (CV\%). Recent work has shown that, based on 181 population pharmacokinetic studies of 95 drugs, the CV\% of drug clearance averages around 40\%.\cite{127} This is the level of variability expected after statistically significant covariates have been accounted for in the final model and reflects the random/unpredictable component of the BSV of a parameter. This information will help clinicians ensure that treatment individualisation is informed by a patient’s response and the variability in the concentration-effect of the drug.

Another advantage population analysis can provide to clinicians is quantification of dose-response and the role of patient covariates on the dose-response variability. Clinicians fundamentally know that the dosing guidelines of a drug will not be appropriate for every individual patient. Professor W Dawson in
1940 stated that “The only principle of dosage that survives is that the dose must be adjusted to the individual patient, and that nothing can or will supersede clinical experience, and careful study, combined with good judgment” [126] However, acknowledging the need to individualise the dose does not explain how the dose can be individualised. Dr William Osler in 1903 believed that “if it were not for the great variability among individuals, medicine might as well be a science, not an art”. That is, the mechanism by which a dosing regimen is individualised largely based on art, experience, good judgment rather than science. The “science” Sir William Osler was referring to is likely clinical trials where the environment is controlled and patient groups at risk of over- or under-exposure are excluded. However, population analysis helps learning about dose-response in a patient population in addition to confirming a hypothesis.[215] It is about the science of quantifying the dose-response and the variability in this dose-response. It is about quantifying the effect of patient covariates on dose-response thus informing the dose requirements of a certain patient group. Finally, it is about quantifying the variability in dose-response which remains unexplained by patient covariates thus informing TCI-based dose-individualisation. Therefore, Sir William Osler’s quote could be rephrased to “If it were not for the great variability among individuals, the practice of medicine might as well be based on guidelines”.
8.2. Limitations and future direction

Overall, an important limitation in the modelling projects presented in this thesis is not accounting for BOV (a.k.a. inter-occasion or intra-individual variability). Individual PKPD parameters may vary over time in a patient. Some of this variability can be linked to altered physiological processes between occasions such as age or creatinine clearance but most of the variability appears random. The latter source of variability is termed BOV.

If BOV is present and is not estimated, it adds to the residual error of the model. RUV accounts for measurement, dosing, and sampling errors and model misspecification and is assumed to be independent. If BOV is present as part of the RUV, rather than estimated, then biased model estimates (in both structural and variance parameters) may arise. The extent of bias is dependent on the magnitude of BOV and BSV.

8.2.1. Enoxaparin dose-individualisation in adults

In chapter 2, an anti-Xa target for enoxaparin therapy was identified. The correlation between anti-Xa and clinical outcomes is not clear-cut but early enoxaparin dose-finding studies have demonstrated that anti-Xa between 500 to 1000 IU/L will maintain effectiveness and minimise bleeding events. However, given a terminal half-life of 4 to 6 hours it is almost impossible to guarantee that any individual will achieve both a peak and trough concentration in this range using once or twice daily dosing. So in order to identify a target that can be reached using this dosing regimen, data from the clinical trial by Barras et al was used to compare anti-Xa peak, trough, and area under the curve (AUC) between patients who experienced a bleeding event and those who did not. A trough anti-Xa concentration ≤ 500 IU/L was associated with half the bleeding risk. Of note, all the events in this study were minor bleeding and bruising. Additionally, a large cohort
study estimated that a peak anti-Xa < 500 IU/L was associated with 3-fold increase in mortality due to therapeutic failure. \cite{145} For this target to be achieved clinically, the dose-individualisation software TCIWorks was used in a randomised controlled trial (RCT) to compare the conventional covariate-based dosing to the individualised dosing method (Chapter 5). However, the RCT was underpowered which makes it difficult to verify whether individualised dosing of enoxaparin would result in optimal treatment. A future study would employ strategies to enrol more patients. Namely, involving multiple cardiac units and enrolling patients who are discharged for home enoxaparin.

8.2.2. Scaling drug doses in children

Children have been labelled therapeutic orphans and are often excluded from PK and PD studies. \cite{218} Because of that, dosing guidelines are often extrapolated from adult studies sometimes resulting in harm.\cite{219} Total body weight (WT) has been and is still used to predict drug doses in paediatric patients but excess fat does not contribute to drug clearance. The relationship between size and drug clearance for children is non-linear.\cite{41} There have been numerous attempts to scale adult doses to children with size descriptors other than WT such as body surface area \cite{62} and allometric scaling (with the exponent either fixed at 0.75 or estimated).\cite{37, 38, 63-67}

In Chapter 6, a model to predict FFM in children was developed and evaluated. The model is comprised of two portions: a sigmoidal maturation term based on post-natal age and an asymptote which is essentially FFM as calculated using the adult model by Janmahasatian et al.\cite{30}

There are two areas concerning this model where further work is required. First, the model is not supported by data in children less than 3 years old. A future study would use FFM data from birth to old age and model these simultaneously resulting in a single continuous model of FFM over age. Second, the main purpose of this model was to be used to scale drug doses after appropriate evaluation. For this
to occur an evaluation of the model’s ability to predict drug CL in a child is needed. However, for this to be done properly where FFM is compared to other size descriptors, a study design exploring the number and range of size required to show a discernible difference between these size descriptors needs to be developed.

Further, as clearance pathways develop in the foetus before birth, the use of postnatal age as a descriptor of maturation may be inadequate and postmenstrual or postconceptional age may prove a better predictor of the metabolic rate. This needs to be explored and is especially needed when dealing with premature neonates.

8.2.3. Unfractionated heparin dose-individualisation in children

In Chapter 7, a PKPD model for unfractionated heparin was developed from a single-dose paediatric study. The model provided a statistically better fit when FFM (based on the model developed in Chapter 6) was used as a covariate in clearance. The PK data were measured using protamine titration where UFH is quantified by the amount of protamine it takes for a sample to reach normal clotting time. The PD data were measured using the activated partial thromboplastin time (aPTT) which is a global clotting time test. The PK part of the model was fitted using a one compartment, intravenous bolus model with linear elimination. The PKPD part was a linear model and fitted the data well as evident from the VPC plots. However, when the model was used to extrapolate into different (much lower) dosing regimens the predictions in terms of dose/infusion rate requirements did not match those normally seen in clinical practice. This begged the question, was a linear model suitable for a drug which has often exhibited a non-linear dose-response relationship?

The data came from a clinical study where children received a single high dose (90 IU/kg) of UFH prior to cardiac catheterisation. This made it less likely for non-linear elimination to be seen. A future study would involve modelling data
where a wide range of doses were given resulting in a wider concentration range thus capturing the Michaelis-Menten phenomenon if it existed.

Another limitation is whether protamine titration is an adequate measure of UFH concentration. Protamine is a mixture of arginine-rich polypeptides (average MW 4500 Da) used routinely in clinical practice to neutralise the anticoagulant effect of UFH. Protamine competes with antithrombin for binding to heparin. It appears that protamine only binds to long heparin molecules which may explain why it only partially reverses the activity of low molecular weight heparin (LMWH). Protamine reverses the anti-IIa activity of both UFH and LMWH which is instigated mainly by long heparin molecules, whereas it poorly reverses the anti-Xa activity of LMWH.[220, 221]

This begs the question, does protamine titration truly reflect UFH concentration or just the concentration of the long chains? To verify this, a study where both protamine titration and anti-Xa activity are measured following a dose of UFH should be undertaken.

Finally, the aPTT assay is the most widely used assay to quantify UFH effect. It measures the time required for coagulation to occur in a blood sample after the addition of an activating agent (e.g. kaolin). The assay has a high degree of variability between patients[111] and between reagent batches.[112, 113] Also, baseline, response, and target aPTT in children have not been well established.[188, 222] A future study could look to compare other global or specific coagulation assays[110] in describing the concentration-response relationship of UFH.

Once a PKPD model, a treatment target, and a reliable assay are obtained, a dose-individualisation method should be implemented and trialled with the goal of consistent and prompt target achievement, reduction/efficiency in the number of dose-adjustments, and maintenance or improvement in the effectiveness and safety of UFH therapy.
8.3. Conclusions

PKPD population modelling and simulation were used to explore and inform dose-individualisation, covariate-based and/or TCI-based, of the anticoagulants enoxaparin and unfractionated heparin in children.

A treatment target was identified for enoxaparin therapy and a Bayesian forecasting method for dose-individualisation was evaluated and then implemented in a small clinical trial. A decrease, although not statistically significant, in the risk of bleeding and bruising was observed.

A model for fat-free mass in children was developed and evaluated. The model describes age-based structural maturation and asymptotes to a previously published adult mode. The model performed well during evaluation and can potentially be used in scaling paediatric drug dosing after proper evaluation. FFM was found to be superior to total body weight in describing the time-course of drug effect of unfractionated heparin in a paediatric population.
APPENDICES

The following appendices contain additional material related to the individual thesis chapters.
Appendix 1: Additional material for Chapter 2
### A1.1 Anti-Xa target identification for enoxaparin therapy

**Table A1.1 Enoxaparin RCT (Barras et al, 2008) patient characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Bleeding or bruising event ≥ 20 cm²</th>
<th>Any bleeding or bruising event</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Event (n = 27)</td>
<td>No Event (n = 76)</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Demographic variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age – y⁻¹</td>
<td>69.3 (15.0)</td>
<td>59.2 (15.9)</td>
<td>0.005*</td>
</tr>
<tr>
<td>Sex – no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>14 (52)</td>
<td>27 (36)</td>
<td>0.16</td>
</tr>
<tr>
<td>Male</td>
<td>13 (48)</td>
<td>49 (64)</td>
<td>0.16</td>
</tr>
<tr>
<td>Weight – kg⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wt</td>
<td>73.3 (20.3)</td>
<td>76.4 (16.9)</td>
<td>0.44</td>
</tr>
<tr>
<td>LBW</td>
<td>53.4 (12.5)</td>
<td>58.18 (12.4)</td>
<td>0.09</td>
</tr>
<tr>
<td>Wt ≥ 100 kg – no. (%)</td>
<td>3 (11)</td>
<td>7 (9)</td>
<td>0.14</td>
</tr>
<tr>
<td>CLCR (Wt) – ml min⁻¹</td>
<td>78.6 (40.9)</td>
<td>99.6 (39.3)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Warfarin – no. (%)</td>
<td>10 (37)</td>
<td>25 (32)</td>
<td>0.46</td>
</tr>
<tr>
<td>Antiplatelet drugs – no. (%)</td>
<td>14 (52)</td>
<td>30 (39)</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Exposure variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C min – IU ml⁻¹</td>
<td>0.54 (0.26)</td>
<td>0.45 (0.23)</td>
<td>0.11</td>
</tr>
<tr>
<td>C max – IU ml⁻¹</td>
<td>1.18 (0.61)</td>
<td>1.07 (0.30)</td>
<td>0.20</td>
</tr>
<tr>
<td>C max 0.24 – IU ml⁻¹</td>
<td>1.02 (0.58)</td>
<td>0.95 (0.24)</td>
<td>0.24</td>
</tr>
<tr>
<td>AUC 0.24 – IU ml/hr</td>
<td>14.5 (5.3)</td>
<td>14.1 (3.9)</td>
<td>0.66</td>
</tr>
<tr>
<td>cAUC – IU ml/h</td>
<td>56.6 (40.9)</td>
<td>29.2 (24.8)</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>
A1.2 MATLAB code for the unfractionated heparin simulation

Run file (hep_go_model.m)

```matlab
clear all
clc
format short g

nrep=10000;

get_param

% Hep PD model
THETA_PD=[1.5];
ETA_PD=normrnd(0,(0.5/1.5)^2,nrep,1);
M=THETA_PD*exp(ETA_PD); %M=slope of [hep] vs APTT

aPTT0=30; %seconds (as per ACC/AHA guide; range in the NZMJ98 article is 26-36 seconds)

T=48; %time to end of simulation in h

t = [0:1:T]; % time at which [hep] & aPTT are measured

c=length(t);

x0=0; % x0=initial concentration

R = 1500; % R=hep infusion rate in U/hr

options=odeset('RelTol',1e-3);

Data=[];
for j=1:nrep
    sol=ode45(@hep_function,[0 T],x0,options,R,V(j),Vmax(j),Km(j));
    C = deval(sol, t)./V(j)/1000;
    aPTT(j,:) = aPTT0*exp(M(j))*C;
    aPTT_48(j)=aPTT(j,c);
end

Success_worst= (sum(aPTT_48>=45 & aPTT_48<=75))/nrep*100;
Success_best= (sum(aPTT_48>=((26*1.5) & aPTT_48<=(36*2.5)))/nrep*100;
percentage_over=(sum(aPTT_48>(36*2.5)))/nrep*100;
percentage_under=(sum(aPTT_48<(26*1.5)))/nrep*100;
```
Appendix 1: Additional material for Chapter 2

**get_param.m**

```matlab
THETA = [0.450*1000 3121 0.07*70];
% Km = [hep] in U/L when elimination is max/2,
% Vmax U/hr=max rate of elim,
% V = volume of dist (L)

MEANETA=[0 0 0];

OMEGA=[(0.1/0.45)^2 0 0
       0 (980/3121)^2 0
       0 0 0.09];
% No weight distribution was used as steady state [hep] appears invariable with weight

ETA = mvnrnd(MEANETA,OMEGA,nrep);

Km = THETA(1)*exp(ETA(:,1));
Vmax = THETA(2)*exp(ETA(:,2));
V = THETA(3)*exp(ETA(:,3));
```

**hep_function.m**

```matlab
function dydt=hep_function(t,y,R,V,Vmax,km)

dydt=[R-(Vmax.*y./V)./(km+y./V)];
```
A1.3 MATLAB code for the enoxaparin simulation

Run file (enox_sim.m)

```matlab
clear all
clc
format short g

nrep = 10000;

% if wt_type==1 then wt=wt else wt=lbw
wt_type=1;
dpkg=1;
dose_per_kg=1; % flag variable when = 1 then dose is given per kg when = 0 then dose = dpkg

time_of_interest=5;
target_concentration = 0.5;

% upper and lower bounds for covariates
upper = [195 150 1000 90]; % [height weight src age]
lower = [150 45 50 30];

lower_clcr=30;

% The following script file returns cov_m and cov_f with nrep rows and 4 columns (ht, wt, scr, age)
cov_distn_model
% covariate distribution model for ht, wt, src, and age for males and females in RCT by Barras et al

% covariates
make_cov

% observation times
TT = [0:0.25:12];
R=zeros(1, length(CLCR));
a=find(CLCR<lower_clcr);
CLCR(a)=[];
LBW(a)=[];
wt(a)=[];
nrep=nrep-length(a);

di = 12;
DN = [3 12];
```
% the enox model

for ij=1:length(DN)
    dn=DN(ij);

    go_model_v2

    C5=F(:,find(TT==time_of_interest));
    C5_keep(:,ij)=C5;

    Cmin=F(:,length(TT));
    Cmin_keep(:,ij)=Cmin;

    SCmin(:,ij)=Cmin<=target_concentration;
    SCmax(:,ij)=C5>=target_concentration;
    CONC(ij,:,:)=F;
end

success=[SCmin SCmax];
success_dose1=[SCmin(:,1) SCmax(:,1)];
success_dose2=[SCmin(:,2) SCmax(:,2)];

success_peak=[SCmax(:,1) SCmax(:,2)];
success_trough=[SCmin(:,1) SCmin(:,2)];
f_peak_success = sum(sum(success_peak')'==2)/nrep;
f_trough_success = sum(sum(success_trough')'==2)/nrep;

f_overall_success = sum(sum(success')'==4)/nrep;
f_dose1_success = sum(sum(success_dose1')'==2)/nrep;
f_dose2_success = sum(sum(success_dose2')'==2)/nrep;
f_overall_failure = sum(sum(success')'==0)/nrep;
f_dose1_failure = sum(sum(success_dose1')'<2)/nrep;
f_dose2_failure = sum(sum(success_dose2')'<2)/nrep;
% covariate distribution model

data=xlsread('Height_weight_Sex2.xls');

useful_data=data(:,3:7);
useful_data(:,3)=log(useful_data(:,3));

chicks=useful_data(find(useful_data(:,5)==1),1:4);
blokes=useful_data(find(useful_data(:,5)==2),1:4);

vc_f=cov(chicks), i.e. variance-covariance matrix for females = covariance height, weight, src, and age
vc_m=cov(blokes);

% generate multivariate deviates
cov_f = mvnrnd(mean(chicks),vc_f,nrep*3);
cov_m = mvnrnd(mean(blokes),vc_m,nrep*3);

cov_f(:,3)=exp(cov_f(:,3));
cov_m(:,3)=exp(cov_m(:,3));

% check to see that all deviates are legal
lower=repmat(lower, nrep*3, 1);
upper=repmat(upper, nrep*3, 1);
for ij=1:4
  a=find(cov_f(:,ij)==0);
cov_f(a,:)=[];
b=find(cov_m(:,ij)==0);
cov_m(b,:)=[];
end

% return to script the matrix variables
for ij=1:4
  a=find(cov_f(:,ij)==0);
cov_f(a,:)=[];
b=find(cov_m(:,ij)==0);
cov_m(b,:)=[];
end

% cov_f=cov_f(1:nrep/2,:);
cov_m=cov_m(1:nrep/2,:);
flag=ones(nrep/2,1);
cov_m(:,5)=flag; % males are now denoted as 1
cov_f(:,5)=(1-flag); % females are now denoted as 1-1=0
Appendix 1: Additional material for Chapter 2

cov_{mf}=[cov_f(1:nrep/2,:) ; cov_m(1:nrep/2,:)]; % To use as a single dataset which contains m and f.
LBW and CrCl are calculated differently.

% output back to main programme (note how the variables are now linked to Sex)
ht = cov_{mf}(:,1);
w = cov_{mf}(:,2);
scr = cov_{mf}(:,3);
age = cov_{mf}(:,4);
sex = cov_{mf}(:,5);

emp_ht = useful_data(:,1);
emp_wt = useful_data(:,2);
emp_scr = data(:,5);
emp_age = useful_data(:,4);
emp_sex = useful_data(:,5);

cov_distn_model.m

% Calculation of BMI (weight/height(in meters)^2) for males and females
BMI = wt./(ht./100).^2;

% Calculation of LBW for males and females
LBW = (9.27*10^3*wt./(6.68*10^3+(216*BMI))).*sex+ (9.27*10^3*wt./(8.78*10^3+(244*BMI))).*(1-sex);

% Calculation of CrCl for males and females
CLCR = ((1.23*(140-age).*LBW)./scr).*sex+ ((1.04*(140-age).*LBW)./scr).*(1-sex);

emp_BMI = emp_wt./(emp_ht./100).^2;
emp_LBW = (9.27*10^3*emp_wt./(6.68*10^3+(216*emp_BMI))).*emp_sex+ 
(9.27*10^3*emp_wt./(8.78*10^3+(244*emp_BMI))).*(1-emp_sex);
emp_CLCR = ((1.23*(140-emp_age).*emp_LBW)./emp_scr).*emp_sex+ 
((1.04*(140-emp_age).*emp_LBW)./emp_scr).*(1-emp_sex);

go_model.m

THETA = [0.858 4.567 0.255 29.6 0.62]; % from enox model (Cl, Vc, Ka, Vp, Q)

% Calculation of ETA
MEANETA = [0 0 0 0 0];
OMEGA = [0.327^2 0 0 0 0; 
0 0.25^2 0 0 ;
0 0 0.299^2 0 ;
0 0 0 0.698^2 ];
ETA = mvnrnd(MEANETA,OMEGA,nrep);

% Individual parameters
cl = (THETA(1)*CLCR/6+0.2*LBW/70).*exp(ETA(:,1));
\[ v_2 = (\text{THETA}(2) \times \text{LBW}/70) \times \exp(\text{ETA}(\cdot,2)); \]
\[ k_a = \text{THETA}(3) \times \exp(\text{ETA}(\cdot,3)); \]
\[ v_3 = \text{THETA}(4) \times \exp(\text{ETA}(\cdot,4)); \]
\[ q = \text{THETA}(5) \times \exp(\text{ETA}(\cdot,5)); \]

if dose_per_kg==1
    d = dpkg*wt;  % dpkg is a variable, changed as desired
else
    d = dpkg*ones(length(wt),1);
end

% Reprametrisation
k_{12} = q ./ v_2;
k_{21} = q ./ v_3;
k_{10} = c_l ./ v_2;
zzz = k_{12} + k_{21} + k_{10};
yyy = k_{21} * k_{10};
alpha = 0.5 * (zzz + sqrt(zzz * zzz - 4 * yyy));  % alpha = distribution rate constant, beta = elimination rate constant
beta = 0.5 * (zzz - sqrt(zzz * zzz - 4 * yyy));

aa = d .* k_a .* (k_{21} - alpha) ./ (v_2 .* (beta - alpha) .* (ka - alpha));
bb = d .* k_a .* (k_{21} - beta) ./ (v_2 .* (alpha - beta) .* (ka - beta));
cc = d .* k_a .* (k_{21} - ka) ./ (v_2 .* (alpha - ka) .* (beta - ka));

R1 = (1 - exp(-d .* alpha .* di')) ./ (1 - exp(-alpha .* di'));  % R1 = accumulation due to alpha, R2 = accumulation due to beta, R3 = accumulation due to ka
R2 = (1 - exp(-d .* beta .* di')) ./ (1 - exp(-beta .* di'));
R3 = (1 - exp(-d .* ka .* di')) ./ (1 - exp(-ka .* di'));

% Calculation of Concentrations: dx/dt = -ka.x, dx1/dt = ka.x + k_{21}.x_2 - k_{12}.x_1
% (x_1 = amount in Central, x_2 = amount in Peripheral, x = amount in Gut/subcut layer, ka = absorption rate constant)

for j = 1:length(TT)
    F(:, j) = (aa .* exp(-alpha .* TT(j))).*R1+(bb .* exp(-beta .* TT(j))).*R2+(cc .* exp(-ka .* TT(j))).*R3;
end
Appendix 2: Additional material for Chapter 4
Appendix 2: Additional material for Chapter 4

A2.1 Anti-Xa assay for enoxaparin monitoring

Fig. A2.1: The COATEST® LMW Heparin/Heparin assay package insert.

Calculation

\[
\text{Absorbance} = \frac{(1/C) \times (2.303 \times \text{Sample OD})}{(1/C) \times (2.303 \times \text{Standard OD})}
\]

Limitations

- The method is not specific for LMW heparin and can be affected by other substances that absorb at the wavelength used.
- The results can be influenced by the stability of the sample and the storage conditions.

Expected results

- The expected absorbance for enoxaparin standards should be within the range of 0.2 to 0.5 OD.

Accuracy

- The method is validated against commercially available standards.

Stability

- The samples should be analyzed within 24 hours of preparation.

Figure A2.1: The COATEST® LMW Heparin/Heparin assay package insert.
A2.2 Healthy volunteer Information Sheet and Consent Form

Application Form for ethical consideration of research and teaching proposals involving human participants

[Reference Number as allocated upon approval by the Ethics Committee]
[Date]

Validation of an anti-activated Factor X (anti-Xa) assay used in the quantification of enoxaparin

INFORMATION SHEET FOR PARTICIPANTS

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the Aim of the Project?

What is enoxaparin?

Enoxaparin is an anti-clotting medicine used to treat abnormal or unwanted blood clotting, such as acute coronary syndromes, pulmonary embolism and deep vein thrombosis. It has its effect by increasing the action of a normal inhibitor in blood called antithrombin.

What are problems with enoxaparin?

Treatment with enoxaparin, like other anticoagulants, may result in bleeding if the dose is excessive, but clot formation if the dose is too low. This may result in heart attacks, strokes, or clots forming in the lungs. The amount of enoxaparin in the blood is determined using an assay called anti-Xa.

Aim of this study:

The aim of this study is to validate the anti-Xa assay used for measuring enoxaparin concentration.

What Type of Participants Are Being Sought?

We are seeking a healthy adult volunteer, aged 18-50 years who is well and has no bruising or bleeding symptoms. The participant will be excluded if he/she has had in the last month or currently:

- An inflammatory condition as a result of physical injury, immunological disorder (eg sprain, asthma, arthritis, etc.) or an infection
- Taking any prescription medicines that could suppress or treat an inflammatory response or that are known to affect clotting or bleeding time.
- A recent visit to the dentist: fillings or scaling – 7 days; extractions, gingival and other invasive procedures - 21 days.
Appendix 2: Additional material for Chapter 4

Application Form for ethical consideration of research and teaching proposals involving human participants

- Pregnant, breast feeding or taking hormonal treatments including oral contraceptives.

What Will The Participant Be Asked To Do?

Should you agree to take part in this project, you will be asked to give three tubes (2.6 mL each) of blood. This will be collected during a single session.

There are risks anticipated that are related to the collecting of blood samples. Though the blood will be collected by trained researchers/staff, bruising at the site of blood collection may occur.

The whole procedure will take less than 20 minutes.

You may choose not to take part in the project without any disadvantage to yourself of any kind.

Can The Participant Change his/her Mind and Withdraw from the Project?

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

What Data or Information will be Collected and What Use will be Made of it?

Your name, age, sex, contact details, blood volume, and date of blood collection will be recorded and stored as an electronic file in a password-protected computer in a locked office. Your details will be recorded to assess any linking between these characteristics and the results. A sample ID code will be used to identify these details. Contact information will be collected to contact you to make an appointment to collect blood samples. A log of date and volume of blood collected will be maintained.

Part of samples will be stored in a special freezer (-80°C) at the School of Pharmacy until the end of the study. After completion, all remaining samples will be destroyed.

No audio- or video-taping will be done.

The data collected will be used to assess the effect of haemoglobin (the protein that gives your blood its red colour) on the anti-Xa assay. This assay is currently the industry standard for measuring the amount of enoxaparin in a blood sample. Enoxaparin is a widely-used anti-clotting drug.

Mr Hesham Al-Sallami will be responsible for the safekeeping of data. Data will be accessible to study researchers only.

This project is partially funded by a grant from the New Zealand Pharmacy Education and Research Foundation (NZPERF). The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand).

A copy of the project results will be made available on request.
Appendix 2: Additional material for Chapter 4

Application Form for ethical consideration of research and teaching proposals involving human participants

The data collected will be securely stored in such a way that only those mentioned below will be able to gain access to it. At the end of the project any personal information will be destroyed immediately except that, as required by the University’s research policy, any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.

Reasonable precautions will be taken to protect and destroy data gathered by email. However, the security of electronically transmitted information cannot be guaranteed.

What if the Participant has any Questions?

If you have any questions about our project, either now or in the future, please feel free to contact:

Dr Natalie McIlwraith
School of Pharmacy
University Telephone Number: 479 5919

Mr Hesham Al-Sallami
School of Pharmacy
University Telephone Number: 479 7295

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (Ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
Application Form for ethical consideration of research and teaching proposals involving human participants

[Reference Number as allocated upon approval by the Ethics Committee]

[Date]

Validation of an anti-activated Factor X (anti-Xa) assay used in the quantification of enoxaparin

CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:-

1. My participation in the project is entirely voluntary;

2. I am free to withdraw from the project at any time without any disadvantage;

3. Personal identifying information (e.g. name, age, sex, contact details, date and volume of blood collected) will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for five years, after which they will be destroyed;

4. The risks anticipated are related to the collection of blood samples. Though the blood collection procedure will be carried out by trained personnel, minor bruising at the site of blood collection may occur;

5. No benefit will be gained from this study by participants apart from an altruistic sense of satisfaction from contributing to our knowledge of blood clotting kinetics and development of a clinically useful test;

6. The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand).

I agree to take part in this project.

................................................................. .................................................................
(Signature of participant) (Date)

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 08 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
Appendix 3: Additional material for Chapter 5
A3.1 Participant Information Sheet and Consent Form

Participant Information Sheet
Individualised versus conventional enoxaparin dosing study

You are being invited to take part in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what is involved. Please take time to read the following information carefully and discuss it with friends or relatives if you wish. You are entirely free to decide whether or not to take part in this trial, and if you choose not to take part, the care you are given by your own doctors will not be affected. If there is anything that is not clear, or if you would like more information, please do not hesitate to ask one of the investigators.

Introduction
Enoxaparin is a drug that is widely used throughout the world. It acts to make your blood thinner (less likely to clot). Studies have shown that this drug is helpful in people who have suffered angina, heart attacks, clots in the leg (deep vein thrombosis) or clots in the lungs (pulmonary emboli). You are being approached because the doctors caring for you have started you on enoxaparin. Enoxaparin can increase the risk of bruising and bleeding in some patients. This can happen in any part of the body.

We are currently conducting a study to investigate whether a dosing regimen designed for each individual patient (i.e. individualised) reduces the rate of unwanted bleeding when compared to standard therapy (i.e. conventional). Participants will be randomised to either group. In the conventional group, standard dosing of enoxaparin will be used which is based on body weight and kidney function. In the individualised group, doses will be calculated using a blood test, age, sex, height, weight, and kidney function. We would like to involve you in this study.

What the study hopes to answer
The main aim of this study is to investigate whether individualising the dose of enoxaparin will cause less bleeding complications than standard dosing.

What are the side-effects and risks of taking part?
Several blood samples will be withdrawn to measure the concentration of enoxaparin in your blood. Typically, participants can expect 1 to 3 samples taken over 2 to 4 days. This will be done by a trained investigator, Mr Hesham Al-Sallami, but bruises and mild discomfort at the site of needle insertion may occur.

You will not be at risk of any additional adverse effects as a result of being involved in the study as the treatment drug you’ll be receiving will not change.

Any questions or concerns about other drugs that you are taking should be discussed with the doctors or pharmacist looking after you.

In the unlikely event of a physical injury as a result of your participation in this study, you will be covered by the accident compensation legislation with its limitations. If you have any questions about ACC please feel free to ask the researcher for more information before you agree to take part in this trial.

Otago District Health Board
University of Otago

Contact: Hesham Al-Sallami – Investigator
Making address: School of Pharmacy PO Box 913 Dunedin
Tel (03) 4797295 Fax (03) 4797034 email: hesham.al-sallami@otago.ac.nz

Enoxaparin Participant Information Sheet, Oct 2010
Page 1 of 3 (version 5)
What the study involves
If you agree to take part, the investigators will arrange to see you and to ask some questions about your medical history. If you are eligible and wish to enter the study, you will be asked to sign a Consent Form and you will be given a copy to keep.

The investigator may then ask you further questions about your medical history and may look in your medical records if details need to be clarified. Only details which are relevant to this study will be looked at. Your medications and results of blood tests will be recorded.

In the conventional group, standard dosing of enoxaparin will be used which is based on body weight and kidney function.

In the individualised group, standard dosing of enoxaparin will be used for the initial dose; then Mr Hesham Al-Sallami will calculate subsequent doses and request your treating doctor to prescribe these doses. Doses will be calculated by using a blood test, age, sex, height, weight, and kidney function.

For every participant, a blood sample will be collected after the initial dose then every one or two days depending on the concentration of enoxaparin in your blood.

The investigators will visit each day while you are in hospital. This is to record any bruising or bleeding that you may experience. The study ends when you are discharged from hospital.

You may withdraw at any stage during the study, without needing to give a reason. This will not affect the medical care you can expect from your own doctors.

Throughout the study, your own doctors will remain fully responsible for all your medical care.

Other important information
In order to monitor your progress in the study, your details will be recorded on a computer database, and data will be stored on computer in the School of Pharmacy. This information will be kept securely and anonymously. If you consent to take part in the research, we may use your hospital and other health records for the purposes of analysing the results, and regulatory authorities may use them to check that the study is being carried out correctly. All such information will be used in confidence.

Should you have any questions at any point now or later on, please do not hesitate to talk to your doctor or the study investigators.

It is planned that the results of the study will be published in a medical journal. At no time would any information about you as an individual be released.

If you have any questions or concerns about your rights as a participant in this research study, you can contact an independent health and disability advocate. This is a free service provided under the Health and Disability Commissioner Act. Telephone: (local) 4792065, (NZ wide) 0800555050; Free Fax: (NZ wide) 08002787 (0800 2 SUPPORT), Email: advocacy@hdcc.org.nz. If there is a specific Maori issue/concern, please contact Linda Greeneall at 0800 977 766.

Ethics approval
This study has been reviewed by the Lower South Regional Ethics Committee. This is an independent research ethics committee, which includes people outside of the medical profession. You will retain the same rights as any other patient, whether or not you take part.

~ Thank you for your help ~
Contacts

Principal Investigator Employer-
Prof Stephen Duffull
Chair in Clinical Pharmacy, School of Pharmacy, University of Otago
Mailing address: School of Pharmacy, University of Otago, PO Box 913, Dunedin.
Telephone: 03-4795044 Fax 03-4797034
Email: stephen.duffull@stonebow.otago.ac.nz

Investigator-
Mr Hesham Al-Sallami
Postgraduate Student, School of Pharmacy, University of Otago
Mailing address: School of Pharmacy, University of Otago, PO Box 913, Dunedin
Telephone: 03-4797295; Fax 03-4797034 Email: hesham.al-sallami@stonebow.otago.ac.nz

Associated Investigators-
Dr Ruth Ferguson
Clinical Pharmacist, Department of Pharmacy, Dunedin Hospital
Mailing address: Department of Pharmacy, Dunedin Hospital, Private Bag 1921, Dunedin
Telephone: 03-4740999 (Tracer: 6373); Fax: 03-4747638
Email: ruth.ferguson@otagodhb.govt.nz

Dr John Schollum
Nephrologist, Department of Nephrology, Dunedin Hospital
Mailing address: Department of Nephrology, Dunedin Hospital, Private Bag 1921, Dunedin
Telephone: 03-4709242; Email: john.schollum@otagodhb.govt.nz

Dr Natalie Medlicott
Senior Lecturer, School of Pharmacy, University of Otago
Mailing address: School of Pharmacy, University of Otago, PO Box 913, Dunedin
Telephone: 03-4795919; Fax 03-4797034 Email: natalie.medlicott@stonebow.otago.ac.nz
Participant Consent Form

Individualised versus conventional enoxaparin dosing study

<table>
<thead>
<tr>
<th>Participant</th>
<th>PRINTED name (Given name and family name):</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Date of Birth</th>
<th>/</th>
<th>Study ID</th>
<th>Hospital ID</th>
</tr>
</thead>
</table>

REQUEST FOR INTERPRETER
(to be included on all consent forms)

<table>
<thead>
<tr>
<th>Language</th>
<th>Translation</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>I wish to have an interpreter.</td>
</tr>
<tr>
<td>Maori</td>
<td>E hia hia ana ahu ki tetahi kaiwhakamaori/kaiwhaka pakeha korero.</td>
</tr>
<tr>
<td>Samoan</td>
<td>Ou te mana’o ia i ai se fa’amatala upu.</td>
</tr>
<tr>
<td>Tongan</td>
<td>Oku ou fiema’u ha fakatonu ea.</td>
</tr>
<tr>
<td>Cook Island</td>
<td>Ka inangaro a i tetai tangata uri reo.</td>
</tr>
<tr>
<td>Niuean</td>
<td>Fia manako au ke faka aoga e taha tagata fakahokohoko kupa.</td>
</tr>
</tbody>
</table>

Other languages to be added following consultation with relevant communities.

Please tick (✓) to confirm you have read and understood the following

☐ I confirm that I have read and understood the Participant Information Sheet for the enoxaparin study.
☐ I have had the opportunity to ask questions and all of my questions have been answered to my satisfaction.
☐ I understand that my participation in the enoxaparin study is voluntary and that I am free to withdraw from the study at any time without my medical care or rights being affected.
☐ I understand that information about my progress in the enoxaparin study will be recorded on a computer database. I understand that this information will be kept securely and anonymously.

☐ I understand that my hospital and other health records may need to be looked at by authorised individuals from the enoxaparin study. I give permission for these individuals to have access to my records on the understanding that this will be done in confidence.

☐ I agree that samples of my blood may be analysed at the School of Pharmacy laboratory.

☐ I consent to any remaining samples being disposed of using standard disposal methods after the end of the study.

<table>
<thead>
<tr>
<th>PRINTED name of participant</th>
<th>Signature</th>
<th>Date</th>
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<tbody>
<tr>
<td>PRINTED name of study staff taking consent</td>
<td>Signature</td>
<td>Date</td>
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</table>

Once completed, please provide the participant a copy of this form.
A3.2 Participant Information Sheet and Consent Form

Clinical Report Form

<table>
<thead>
<tr>
<th>Patient Name:</th>
<th>Patient Number:</th>
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<tbody>
<tr>
<td>Height (m):</td>
<td>Weight (kg):</td>
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<tr>
<td>Age (y):</td>
<td>Sex (M/F):</td>
</tr>
<tr>
<td>Ethnicity:</td>
<td>Serum creatinine (mcmol/L):</td>
</tr>
<tr>
<td>Lean Body weight (kg):</td>
<td>BMI (mg/m²):</td>
</tr>
<tr>
<td>Calculated creatinine clearance (ml/min):</td>
<td></td>
</tr>
</tbody>
</table>

* a calculated using the equation by Jannmahasatian et al. Clin Pharmacokinet 2006;44:1051-65
* b calculated using the Cockcroft & Gault equation using lean (instead of total) body weight

Enoxaparin dosing details

<table>
<thead>
<tr>
<th>Date</th>
<th>Dose</th>
<th>Time</th>
<th>Serum creatinine (mcmol/L)</th>
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</table>
### Enoxaparin Conc (anti-Xa activity) details

<table>
<thead>
<tr>
<th>Date</th>
<th>Enoxaparin Dose</th>
<th>Sampling time</th>
<th>Anti-Xa activity IU/L</th>
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INR (if available) time, date, value: ____________________________

aPTT (if available) time, date, value: ____________________________

Other drugs that may affect risk of bleeding (e.g. warfarin, aspirin, penicillins, SSRIs)

<table>
<thead>
<tr>
<th>Start Date</th>
<th>Stop Date</th>
<th>Drug</th>
<th>Dose</th>
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</table>
**Bruising Chart (indicate on chart location, size & date of bruise)**

* Number each eligible bruise and describe changes over time

<table>
<thead>
<tr>
<th>Date</th>
<th>Site/bruise number</th>
<th>Mj/Mn</th>
<th>Objective evidence (e.g. Hb)</th>
</tr>
</thead>
<tbody>
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**Enoxaparin Clinical Report Form, Version 3 October 2019**

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Appendix 4: Additional material for Chapter 6
Figure A4.1 Histogram plots for patient covariates. Plot a shows an overlay of the covariate distributions of females (light shade) and males (dark shade). Plot b shows an overlay of the covariate distributions of Index dataset (light shade) and Test dataset (dark shade).
### A4.2 Fat-free mass model building

Table A4.1 Noteworthy steps in fat-free mass model development. AIC = Akaike information criterion (AIC); $\sigma_{\text{prop}}$ (CV%) = residual error variability expressed as coefficient of variation; BSV = between-subject variability.

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
<th>$\sigma_{\text{prop}}$ (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperbolic function with an intercept parameter and BSV on the intercept and the maturation half-time.</td>
<td>5827</td>
<td>28</td>
</tr>
<tr>
<td>Asymmetric hyperbolic model with a parameter on the maturation term to allow maturation to change once age reaches the maturation half-time.</td>
<td>5437</td>
<td>26</td>
</tr>
<tr>
<td>Weibull model with an intercept parameter and BSV on the shape and scale parameters.</td>
<td>5333</td>
<td>8</td>
</tr>
<tr>
<td>Exponential model with an intercept parameter and BSV on the intercept and the maturation rate constant.</td>
<td>5277</td>
<td>8</td>
</tr>
<tr>
<td>Sigmoid hyperbolic model with BSV on the sigmoidicity coefficient and the maturation half-time.</td>
<td>4778</td>
<td>28</td>
</tr>
<tr>
<td>Sigmoid hyperbolic model with upper and lower bounds of the sigmoid hyperbolic function and BSV on the upper bound of this function and a separate sigmoidicity coefficient allowed to be estimated when is above and below the maturation half-time</td>
<td>4526</td>
<td>6</td>
</tr>
</tbody>
</table>
A4.3 Fat-free mass model evaluation

Figure A4.2 Residual values versus predicted FFM using the three models. The empirical model is denoted M1, the maturation model M2, and the adult model M3. The line of identity is also shown.
A4.4 Covariate (BMI) visual predictive check plots

Figure A4.3 VPC plots for all models for females and males for the index dataset using BMI as a covariate. The upper row is M1, the middle row M2, and the lower row M3. The dotted lines are the percentiles for the simulated data and the solid lines are the percentiles for the observed data. Percentiles shown are 2.5th, 50th, and 97.5th.
Appendix 5: Additional material for Chapter 7
A5.1 NONMEM code for unfractionated heparin PKPD model estimation using Beal’s M3 method

```plaintext
024.2_hep.ctl  22/02/2015 12:23 a.m.

$PROBLEM PROJECT Paediatric Heparin model

$INPUT ID SEX AGE WT HT FFMA MAT FFM TIME AMT AMTX RATE RAX T DVID DVX

$DATA Paed_Hep_3.csv

;IGNORE (DVID.EQ.0)
;IGNORE (DVID.EQ.2)
;IGNORE (OLOQ.EQ.1)

$SUBROUTINE ADVAN1 TRANS1

$PK

TVCL=THETA(1)*FFM/15
TVV=THETA(2)*WT/20
TVD1=THETA(3)
TVE0=THETA(4)
TVSLP=THETA(5)
RUVCV=THETA(6)
RUVSD=THETA(7)

CL=TVCL*EXP(ETA(1))
V=TVV*EXP(ETA(2))
D1=TVD1
E0=TVEO*EXP(ETA(3))
SLP=TVSLP*EXP(ETA(4))

K=CL/V
S1=V

$ERROR

IF (DVID.EQ.1) THEN
  Y=CP*EXP(EPS(1))+EPS(2)
ENDIF

APTT=E0+SLP*CP
PROP=APTT*RUVCV
ADD=RUVSD
SD=SQRT(PROP*PROP+ADD+ADD)

DUM = (APTT-999)/SD

IF(DVID.EQ.2.AND.DV.LT.999) THEN
  F_FLAG=0
  Y=APTT+SD*EPS1
```
024.2 hep.ctl  22/02/2015 12:23 a.m.

53  ENDIF
54  IF(DVID.EQ.2.AND.DV.GT.999) THEN
55       F_FLAG=1
56       Y=PHI(DUM)
57  ENDIF
58
59  $THETA
60  0.603 FIX ;CL
61  0.751 FIX ;V
62  0.01 FIX ;D1
63  (35) ;E0
64  (1) ;SLP
65  (0,0.1,) ;RUVCV
66  (0,0.5,) ;RUVSD
67
68  $OMEGA
69  0.251 FIX ;PPVCL
70  0.161 FIX ;PPVV
71  0.1 ;PPVE0
72  0.1 ;PPVSLP
73
74  $SIGMA
75  0.0296 FIX ;ERRCV
76  8020 FIX ;ERRSD
77  1 FIX ;EPS1
78
79
80  $ESTIMATION METHOD=COND NSIG=6 INTER LAPLACIAN
81  MAXEVAL=9999 PRINT=1
82
83  $COVARIANCE
84
85  $TABLE ID SEX AGE WT HT TIME AMT RATE DVID Y
86  NOPRINT ONEHEADER FILE=hep.fit
A5.2 MATLAB code for unfractionated heparin PKPD model estimation using Beal’s M3 method

```matlab
%% virtual pop based on the dataset (as per Green et al, 2003)
clc
clear all
nrep=10000;

%% data
format short g
[NUM,TXT,RAW] = xlsread('FFM_MATLAB_2_modif'); % using bigger (FFM) dataset; PMA col del; wt ht swapped
% ID SEX AGE WT HT FM FPC DV BMC CA BMD TS
data = NUM;

%% covariates
useful_data=data(:,2:5);
emp_SEX=useful_data(:,1); emp_AGE=useful_data(:,2);
emp_WT=useful_data(:,3); emp_HT=useful_data(:,4);

females=useful_data(find(useful_data(:,1)==0),2:4);males=useful_data(find(useful_data(:,1)==1),2:4);
vc_f=cov(females);vc_m=cov(males);

cov_f = mvnrnd(mean(females),vc_f,nrep);cov_m = mvnrnd(mean(males),vc_m,nrep); %noneed for *3
upper = [18 100 180];lower = [3 10 60];lower=repmat(lower, nrep, 1);
upper=repmat(upper, nrep, 1); %noneed for *3

cov_f=cov_f.*(1.-(cov_f < lower));cov_f=cov_f.*(1.-(cov_f > upper));
cov_m=cov_m.*(1.-(cov_m < lower));cov_m=cov_m.*(1.-(cov_m > upper));

for ij=1:3
  a=find(cov_f(:,ij)==0);
  cov_f(a,:)=[];
  b=find(cov_m(:,ij)==0);
  cov_m(b,:)=[];
end

cov_f=cov_f(1:nrep/2,:);cov_m=cov_m(1:nrep/2,:);
flag=ones(nrep/2,1);
cov_m(:,4)=flag;cov_f(:,4)=(1-flag); % females are now denoted as 1-1=0

BMI = WT./(HT./100).^2;FFMA =((9270*WT./((6680+(216*BMI)))).*SEX+((9270*WT./((8780+(244*BMI)))).*(1-SEX));
MAT =((0.884+((1-0.884)./(1+(AGE/13.4).^(-12.7))).*SEX)+((1.11+((1-1.11)./(1+(AGE/7.05).^(-1.11))).*(1-SEX)));

emp_BMI=emp_WT./(emp_HT./100).^2; emp_FFMA
```

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Appendix 5: Additional material for Chapter 7

\[(9270 * \text{emp\_WT} / (6680 + (216 * \text{emp\_BMI}))) * \text{emp\_SEX} + (9270 * \text{emp\_WT} / (8780 + (244 * \text{emp\_BMI}))) * (1 - \text{emp\_SEX}); \]

\[
\text{emp\_MAT} = (((0.884 + ((1 - 0.884) / (1 + (\text{emp\_AGE} / 13.4)^{-12.7}))) * \text{emp\_SEX}) + ((1.11 + ((1 - 1.11) / (1 + (\text{emp\_AGE} / 7.05)^{-1.1}))) * (1 - \text{emp\_SEX}));
\]

\[
\text{emp\_FFM} = \text{emp\_FFMA} * \text{emp\_MAT};
\]

%% parameters

\[
\text{THETA} = [0.603 \ 0.751 \ 0.01 \ 37.4 \ 0.345]; \quad \text{[CL \ V \ D1 \ E0 \ SLP]}
\]

\[
\text{OMEGA1}=0.251; \quad \text{OMEGA2}=0.161; \quad \text{OMEGA3}=0.000001; \quad \text{OMEGA4}=0.376; \quad \text{[CL \ V \ E0 \ SLP]}
\]

\[
\text{SIGMA3}=(0.0357); \quad \text{SIGMA4}=(0.001); \quad \% \ \text{SIGMA1=sqrt(0.0296); SIGMA2=sqrt(8020)};
\]

\[
\text{MEANETA}=[0 \ 0 \ 0 \ 0 \ 0];
\]

\[
\text{OMEGA}=[0.251 \ 0.15 \ 0 \ 0 \ 0 \ 0.15 \ 0.161 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0.000001 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0.376];
\]

\[
\text{ETA} = \text{mvnrnd(MEANETA,OMEGA,nrep)};
\]

\[
\text{cl} = (\text{THETA}(1) * \text{FFM} / 15) * \exp(\text{ETA}(:,1));
\]

\[
\text{v} = (\text{THETA}(2) * \text{WT} / 20) * \exp(\text{ETA}(:,2));
\]

\[
\text{D} = \text{THETA}(3) * \exp(\text{ETA}(:,3));
\]

\[
\text{E0} = \text{THETA}(4) * \exp(\text{ETA}(:,4));
\]

\[
\text{SLP} = \text{THETA}(5) * \exp(\text{ETA}(:,5));
\]

%% bolus+infusion convolution model

\[
\text{a1} = \text{AGE} = 1; \quad \text{a2} = \text{AGE} > 1 \& \text{AGE} <= 12; \quad \text{a3} = \text{AGE} > 12;
\]

\[
\text{WT1} = \text{WT}(\text{a1}); \quad \text{WT2} = \text{WT}(\text{a2}); \quad \text{WT3} = \text{WT}(\text{a3});
\]

\[
\text{w1} = \text{mean(WT1)}; \quad \text{w2} = \text{mean(WT2)}; \quad \text{w3} = \text{mean(WT3)}; \quad \text{w} = \text{mean(WT)};
\]

\[
\text{FFM1} = \text{FFM}(\text{a1}); \quad \text{FFM2} = \text{FFM}(\text{a2}); \quad \text{FFM3} = \text{FFM}(\text{a3});
\]

\[
\text{r1} = 28; \quad \text{r2} = 20; \quad \text{r3} = 18;
\]

%% optimal dose FFM and success simulation

\[
\text{r\_FFM} = 1:0.1:3.5;
\]

for \( \text{ii} = 1: \text{length(r\_FFM)} \)
\[
\text{rate} = \text{r\_FFM}(\text{ii}) * \text{FFM};
\]

\[
\text{aPTT}(:,\text{ii}) = \text{E0} + \text{SLP} * (\text{rate} / \text{cl});
\]

\[
\text{avg} = \text{mean(aPTT)};
\]
end

\[
\text{P} = \text{polyfit(r\_FFM,avg,1)}; \quad \text{int} = (65 - \text{P}(:,2))/\text{P}(:,1); \quad \text{int} = \text{round} \left( \text{int} * 100 \right) / 100;
\]

\[
\text{A} = \left[ \text{int} \ 65 \right];
\]

figure(1); plot(r\_FFM,avg,'.r'); title('mean aPTT vs dose per FFM'); xlabel('dose (IU/kg) per FFM'); ylabel('mean aPTT (sec)');
hold on
plot(A(:,1),A(:,2),'k.');
str=num2str(A);

\[
\text{text(A(:,1)*1.1,A(:,2)*1,('(',' num2str(A(:,1)), ', ', num2str(A(:,2)), ', ')'))}
\]

\[
\text{aPTT\_FFM} = \text{E0} + \text{SLP} * (\text{int} * \text{FFM}) / \text{cl}; \quad \text{Success} = (\text{sum(aPTT\_FFM} >= 50 \& \text{aPTT\_FFM} <= 80)) / \text{nrep} * 100
\]

%% optimal dose WT and success simulation

\[
\text{r\_WT} = 1:0.1:3.5;
\]


for ii=1:length(r_WT)
    rate=r_WT(ii)*WT;
    aPTT(:,ii)=E0 + SLP.*(rate./cl);
    avg=mean(aPTT);
end

P=polyfit(r_WT,avg,1);  int=(65-P(:,2))/P(:,1); int=round(int*100)/100
A=[int 65];

figure(2); plot(r_WT,avg,'.r'); title('mean aPTT vs dose per WT');xlabel('dose (IU/kg) per WT');ylabel('mean aPTT (sec)');
hold on
plot(A(:,1),A(:,2),'k.');</ref>
str=num2str(A);
figure(2); plot(r_WT,avg,'.r'); title('mean aPTT vs dose per WT');xlabel('dose (IU/kg) per WT');ylabel('mean aPTT (sec)');
hold on
plot(A(:,1),A(:,2),'k.');</ref>
str=num2str(A);
figure(2); plot(r_WT,avg,'.r'); title('mean aPTT vs dose per WT');xlabel('dose (IU/kg) per WT');ylabel('mean aPTT (sec)');
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plot(A(:,1),A(:,2),'k.');</ref>
str=num2str(A);
figure(2); plot(r_WT,avg,'.r'); title('mean aPTT vs dose per WT');xlabel('dose (IU/kg) per WT');ylabel('mean aPTT (sec)');
hold on
plot(A(:,1),A(:,2),'k.');</ref>
str=num2str(A);
References
References

References


148. Peterson JL MK, Hasselblad V, et al. Efficacy and Bleeding Complications Among Patients Randomised to Enoxaparin or Unfractionated Heparin for


