Application of Nonlinear Mixed Effects Modelling in the Early Phases of Drug Development

by

Scott. F. Marshall B.Sc.(Hons.) M.R.Pharm.S.

A thesis submitted for the degree of Doctor of Philosophy in the Faculty of Medicine, University of Glasgow

Department of Medicine and Therapeutics

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SUMMARY

The origins of the "population approach" to pharmacokinetic (PK) and pharmacodynamic (PD) data analysis owes much to its use as a tool for understanding the variability in data collected during routine therapeutic drug monitoring. Subsequent promotion by leading regulatory scientists led the impetus that resulted in its regular and successful application in the analysis of the sparse data gathered during the later phases of drug development. Nevertheless, it is recognised that assessment of its full potential requires prospective application from the beginning of drug development process. This concept is embodied by the definition of "PK/PD as a general conceptual framework for drug development". However, early studies are inherently data rich and therefore lend themselves to noncompartmental analyses and conclusions based on standard statistical hypothesis testing. Furthermore, due to the high degree of experimental constraint and the homogeneity of the subjects under investigation, the benefits from the "population approach" in its traditional sense are not clearly evident. The potential advantages of applying the population approach, and, in particular, nonlinear mixed effects modelling (NONMEM), to data representative of those typically encountered in early drug development are explored in this thesis.

Normally, the safety of chronic drug treatment is assessed by measuring drug accumulation during multiple dosing to steady state. However, for drugs with long terminal half-lives dosing to steady state is not always practically possible, and model based predictions are often used. In the presented example, a previous standard two stage approach (STS) detected an equality between the absorption (Ka) and distribution (α) rate constants. However, using a nonlinear mixed effect model both rate constants were separately characterised. Subsequently, the model was shown to be useful in the prediction of

variability in steady state concentrations and in deducing that continuous treatment was unlikely to lead to chronic toxicity.

The standard bioequivalence study has one of the strictest experimental designs. However, due to drug toxicity or expected clinical bioinequivalence, there may be reason to establish bioequivalence in the target population. In these circumstances the number of samples that can be ethically or practically taken from each subject may be limited. A population pharmacokinetic approach to bioequivalence testing was compared to the standard noncompartmental approach using data from two routine studies. The point and 90% confidence interval estimate for relative difference in the area under the concentration time curve (AUC) and Ka was estimated directly from a two compartment model with first order absorption. After utilising the Wagner Nelson approximation the point and confidence interval for the relative difference in (the maximum concentration) Cmax was also directly calculated using a novel approach. The conclusion of bioequivalence in AUC and bioinequivalence in Cmax was consistent between the two approaches. After randomly reducing the dataset to 20% of the its original size, the point and confidence interval estimates for the relative difference were still similar to those originally estimated. This conclusion was influenced by the sample design and whether an additive or multiplicative bioequivalence model was utilised. It was also shown that advanced knowledge of the PK model was most likely to allow the bioinequivalence in Cmax to be identified.

It has been proposed that the use of cross-over or dose escalation designs for dose ranging studies in combination with more informative analysis could lead to a better characterisation of the dose response relationship. In the example presented, the dose response relationship for the 3-hydroxy-3-methylglutaryl Coenzyme A inhibitors (HMG COA) inhibitor, simvastatin, was estimated from a cross-over study which covered the current recommended dose range (10 to 40 mg). Analysis using nonlinear mixed effects modelling approach demonstrated that the selected doses only covered 20% (70 to 90%) of

the upper part of the estimated dose response relationship. It was concluded that a lower dose strength would be required to allow adjustment within the log-linear portion of the dose response relationship. The clinical implications of potential relationships between the pre-treatment cholesterol level and the model parameters were explored through prediction and simulation. On simulating the relationship between dose and the percentage of patients who would achieve reductions to below a recognised target concentration, it was found that a different set of dosages may better optimise clinical response.

Where strict experimental design is invalidated by study design or restricted recruitment, the resulting data can be unbalanced and not easily analysed by standard statistical methods. In the example presented, the number and size of doses of dofetilide used to test for PK/PD differences between patients with ischaemic heart disease (ISH) and healthy volunteers were different. A population PK/PD modelling approach was implemented, and no difference between the two groups could be detected. The Cmax and peak QTc ranges were predicted to be narrower following a fixed dose regimen in comparison to a dose per kilogram regimen. However, after incorporating the PK/PD variability, this was not predicted to manifest into an overall increase in the risk of Torsades de Pointes. Nevertheless, there was evidence to suggest that an upper total dose limit would be needed if a dose per kilogram regimen was to be adopted in future studies.

Although early drug development is, by nature, a piece-wise process, the application of more intuitive methods of analysis, such as nonlinear mixed effects modelling, was shown to provide a better understanding of the dose concentration response relationship and a useful tool with which to investigate study designs issues. On the basis of the analyses presented, the early prospective application of these techniques should have benefits in the optimisation of drug development.

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CHAPTER 1

INTRODUCTION

1.1 The phased approach to drug development

The term "drug development" covers the wide variety of activities required to take new chemical entities from discovery through to regulatory approval. The process is time-consuming, very costly and has a high failure rate. Only about one or two from every 10,000 compounds synthesised finally become licensed pharmaceuticals, so it is important that the process is optimally managed and controlled. The risk to healthy volunteers and patients taking part in clinical trials is minimised by reassessing the proposed therapeutic advantage of each chemical entity throughout the development process. A phased approach is most often applied to both streamline the development process and improve decision making. The commonly defined phases of drug development are briefly described below:

Preclinical

Identification of candidate drugs by comparing the activity and safety profiles via in vitro and animal models is the goal of the preclinical phase. Pharmacokinetic and pharmacodynamic data gathered during this phase are also used to guide human dosage regimen development and dose escalation strategies. The accuracy of predictions from in vitro and animal models can vary substantially, so expeditious progression to a point where human investigation can be initiated at minimum risk is the primary focus of the preclinical phase.

Phase I studies

The primary aim of Phase I is to determine safety and tolerability of a new drug in human volunteers. A secondary aim is to provide information on the absorption, distribution, metabolism and excretion and, in some cases, early proof of the therapeutic concept i.e. demonstration of the blood pressure reduction effects of a new antihypertensive.

Phase II studies

Progression from Phase I to Phase II represents the transition from healthy volunteers to patients with the disease state(s) of interest. Similar factors are explored in both settings, but, the focus of Phase II is to establish that the new drug is effective. A secondary aim is to establish a dose range which provides benefit while minimising the risk of adverse effects. This information is used in the design of the Phase III studies.

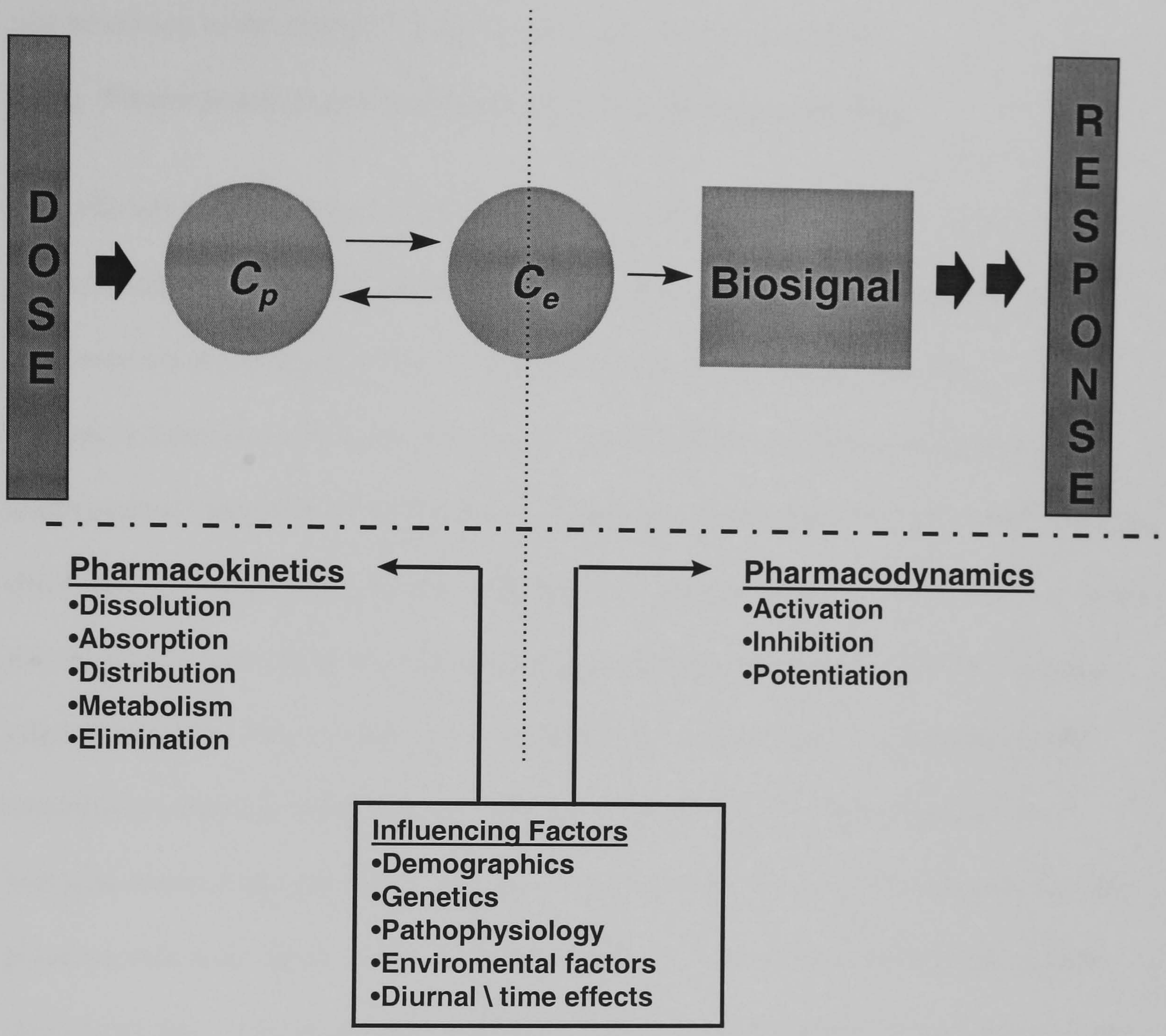
Phase III studies

These are comparative studies designed to assess the safety and effectiveness of the drug in conditions approximating those in which the drug would be used in clinical practice. The best dosage form and dose or range of doses from Phase II are administered to a larger and more varied patient population. One of the additional aims is the detection of groups of patients who may be at increased risk of adverse effects or who require a higher dose to achieve an adequate therapeutic response.

1.2 Role of pharmacokinetic and pharmacodynamic modelling in drug development

The overall understanding of a complex system can be considered to be the sum of the knowledge of its component parts. The components underlying the relationship between dose and response (Figure 1.1) are more fully described below.

Figure 1.1 Relationship between dose and response



1.2.1 Pharmacokinetics and pharmacokinetic modelling

The term Pharmacokinetics was defined by WHO as "the study of the absorption, distribution metabolism and elimination of drugs" (World Health Organisation, 1970). An apt definition by Wagner describes it as "the study of the time courses of drug and metabolite concentrations and amounts in biological fluids, tissues and excreta,....and the construction of suitable models to interpret the data " (Wagner, 1968). The latter highlights the need to model or reduce the data to a set of meaningful parameters which can be used to make predictions for future experiments. The functional form of the pharmacokinetic model is dependent upon both the processes to be modelled and the future

predictions required, but usually involves relating dose, dose frequency, and route of administration to the change in plasma concentration (Cp) over time.

1.2.2 Pharmacodynamics and pharmacodynamic modelling

The principles surrounding the establishment of an appropriate dosage regimen rest heavily on the assumption that a functional relationship, albeit complex, exists between the concentration at the site of action (Ce) and the response ultimately produced. Pharmacodynamics is the study of this relationship and PD modelling involves the mathematical expression of the inhibition, activation or potentiation of the biological signal (biosignal) which underlies the drug response. Therefore, drug potency and tissue or organ sensitivity can be usefully summarised by using models linking concentration to clinical effect (Holford & Sheiner, 1982a, b). While the benefits of a new drug cannot be fully established until large scale efficacy trials are conducted, the relationships between surrogate markers of response to dose or concentration can be used to indicate therapeutic potential and guide future dosing. The measurement of drug effect and its interpretation through the principles of pharmacodynamics is now considered to be a very important part of early drug development.

1.2.3 The linking of pharmacokinetics and pharmacodynamics: Past and present approaches

The full potential of PK/PD modelling was not fully realised until the effect compartment or link model was popularised in the late 1970's early 1980's (Kelman & Whiting, 1979; Sheiner et al., 1979; Whiting et al., 1980; Holford & Sheiner, 1982b) which was some years after the seminal paper by Segre (Segre, 1968). It has now become the standard methodology in modelling the temporal displacement between concentration and effect (hysteresis), a complexity which can often arise in non-steady state PK/PD studies (Hudson

et al., 1983; Meredith et al., 1983; Guy et al., 1984; Fisher et al., 1985; Hinderling et al., 1985; Kelman et al., 1986).

Elaboration of the basic models has also led to the development of semi-parametric approaches, where the PD and/or PK relationship is characterised non-parametrically, but a parametric link model is used to account for the hysteresis (Unadkat et al., 1986; Verotta & Sheiner, 1988; Fuseau & Sheiner, 1989). Moreover, convolution techniques which allow the temporal delay to be characterised by poly-exponential and non-parametric spline functions have also been proposed (Verotta & Sheiner, 1991; Gumbleton et al., 1994).

Other physiological approaches to describing the relationships between concentration and effect have more recently been proposed. Protein binding (Pedraz et al., 1992) , formation of competitive or non-competitive agonists and antagonists (Gupta et al., 1993) and arterio-venous drug concentration differences (Gumbleton et al., 1994) have been proposed as alternative pharmacokinetic mechanisms by which both displacement of effect from concentration (counterclockwise -hysteresis loop) or displacement of concentration from effect (clockwise -proteresis loop) could occur.

A physiological modelling approach to PK/PD was used some time ago to describe the effect of warfarin on prothrombin complex activity (Nagashima et al., 1969; Sheiner, 1969) and further described by Holford and Sheiner (1982b). More recently it has been applied to study the pharmacodynamics of corticosteriods (Kong et al., 1989; Wald et al., 1991; Wald & Jusko, 1992; Lew et al., 1993) and prolactin suppression (Francheteau et al., 1991). By way of these and other examples, Jusko and co-workers characterised a family of indirect response models for stimulation or inhibition of the production or loss of endogenous substances or mediators (Dayneka et al., 1993) and proposed their utility in characterisation of the lag-time between concentration and effect for other therapies (Jusko & Hui, 1994). They also demonstrated that mis-specification of an effect compartment model in place of

an indirect model would result in erroneous conclusions i.e. that Ce_{50} and Emax were dose dependent (Dayneka et al., 1993; Jusko & Hui, 1994).

The establishment of appropriate methods for undertaking a PK/PD modelling approach has subsequently led to its extensive application in many therapeutic areas i.e. (Swerdlow & Holley, 1987; Dingemanse et al., 1988; Donnely et al., 1989; Reid & Meredith, 1990; Mandema & Danhof, 1992) and has been the focus of specialist symposia i.e. (Danhof & Peck, 1994). More recently the importance of these approaches have gained favour within the drug industry (Steimer et al., 1993; Van Peer et al., 1993) and the drug regulatory agencies (Peck & Collins, 1990; Peck, 1992a, 1993). A more detailed discussion of the advantages of PK/PD modelling in the area of antiarrhythmic drug therapy is shown below, and serves as the background to PK/PD analysis described in Chapter 7.

Hysteresis in the concentration versus ECG time intervals has been commonly shown for many antiarrhythmic drugs after intravenous administration i.e.amiodarone (Rodden, 1993), ajmaline (Padrini et al., 1993) diltiazem (Schwartz & Abernethy, 1987), flecainide (Wang et al., 1988), procainamide (Galeazzi et al., 1976), and verapamil(Abernethy et al., 1986). Consequently, one of the initial applications of the effect compartment theory was in the PK/PD modeling of antiarrhythmics i.e quinidine (Holford et al., 1981) digoxin (Kelman & Whiting, 1979) and disopyramide (Whiting et al., 1980).

As an alternative, the slopes of the concentration ECG interval relationship can be obtained from the post infusion data (Echizen et al., 1985; Abernethy et al., 1986; Schwartz & Abernethy, 1987). However, Schwartz et.al. (1989) demonstrated that this technique resulted in biased slope estimates, which over predict the steady state QTc. Furthermore, since the descending limb of the hysteresis loop (data post end of infusion) can often approximate a sigmoid relationship, dose dependent Emax and CE_{50} parameter estimates have in some cases been wrongly reported (Echizen et al., 1985; Schwartz & Abernethy, 1987).

1.3 Pharmacokinetic and pharmacodynamic variability

The factors which are commonly found to influence the pharmacokinetics and pharmacodynamics and, therefore, help explain the variability in the PK/PD model are also shown in Figure 1.1. In the following two sections the components of the overall variability are discussed with reference to these factors:

Interindividual variability

The interindividual variability in drug response can result from either pharmacokinetic or pharmacodynamic differences between subjects. Differences in diet and disease state can affect the rate and extent of drug absorption. Similarly, differences in body size, body weight, tissue composition and tissue binding can account for interindividual differences in drug distribution. The variability in elimination often depends on both genetic and environmental factors. Metabolic rates can be either or both genotypically or phenotypically different. Differences in overall elimination can be further altered by the effect of ageing or disease. Both pathophysiological and genetic differences are major factors influencing the interindividual variability in pharmacodynamics.

Intraindividual Variability

Residual or intraindividual variability is related to several sources. "True" intraindividual variability is most often obscured by the variability inherent in the measurements of concentration or response i.e. assay error or the error in the recordings of dosing and sampling times. "True" intraindividual variability results from moment to moment or occasion to occasion changes in physiology, in some cases these have a rhythm and can be modelled i.e. diurnal variation in blood pressure.

1.3.1 Population approach

The aim of the population approach is to assess the central tendency of the pharmacokinetic and pharmacodynamic response and to quantify the variability around it. Although, the concept of measuring and accounting for variability in pharmacokinetics is not new, the development of novel statistical methods has allowed PK/PD modelling to implemented across all phases of drug development. An outline of the available statistical methodologies and the development of the population approach are described below.

1.3.2 Statistical methodologies used in population pharmacokinetics

The standard two stage approach (STS) is often used to obtain estimates of the average pharmacokinetic parameters and their associated variability from rich experimental data. With this approach the estimation of interindividual variability and the investigation of covariate relationships are undertaken in a separate step to the nonlinear regression used to estimate the individual model parameters. Provided that the study design is reasonably balanced, the average parameter estimates should theoretically agree with the "True" values. However, it would be expected that the interindividual variabilities would be upwardly biased since they include the uncertainty in the estimation of the individual parameter estimates. Furthermore, particular problems arise with the STS approach when the data per individual is sparse, since obtaining parameter estimates for every individual may become difficult. In such circumstances naive pooling of data (NPD) can be used to obtain average parameter estimates. However, this approach ignores the concept of the individual, and estimates of interindividual variability are not available.

Statistically, a non-linear mixed effect model, allowing for repeated measurements , is necessary to allow estimation of parameters in a single stage. "Mixed effects" refers to the combination of both fixed effects (parameters and covariate relationships) and random

effects (intra- and inter- individual variability). A parametric approach to the estimation problem was developed by Sheiner and Beal and first implemented as the first order (FO) method within the program NONMEM (Beal,SL & Sheiner, 1980). The term "parametric" refers to the assumption that the variability in both parameter estimates and measurements follows a Gaussian distribution. The first order method utilises the first term of the Taylor series expansion of the nested (intraindividual within interindividual) random effects. The resultant linearisation simplifies the problem to one which is more easily estimated. Despite this approximation, the method has been shown to be superior to the NPD method in estimating the population mean (structural) parameter estimates and superior to the STS in estimating interindividual variability of mean parameters when the data is simulated as either sparse clinical (Sheiner & Beal, 1980; Sheiner, 1984; Steimer et al., 1984; Hashimoto et al., 1994) or rich experimental (Sheiner & Beal, 1981, 1983; Beal, 1984) from a linear (Sheiner & Beal, 1981, 1983; Sheiner, 1984; Steimer et al., 1984) or nonlinear (Sheiner & Beal, 1980; Hashimoto et al., 1994) PK (PD) model. Nevertheless, the algorithm is prone to inaccuracies when interindividual variation is large (White et al., 1990) or when the parameter estimates are highly correlated (Steimer et al., 1984).

Other approaches have also been developed. Mallet (1986) introduced the non-parametric maximum likelihood method which differs from NONMEM in that it is not dependent on any prior assumptions about the distribution of the parameter estimates within the population. A Bayesian method has also be suggested (Racine-Poon & Smith, 1990) and developed using Gibbs Sampling into several exportable packages.

An EM (expectation maximisation) -algorithm developed to analyse linear models (Dempster et al., 1977; Laird & Ware, 1982) has been further developed for estimation of non-linear models by utilising different linearisation methods (Amisaki & Tatsuhara, 1988; Aarons, 1993). A Bayesian approach implementing the EM-algorithm has also been proposed (Racine-Poon, 1985).

More recent versions of NONMEM have developed along similar lines and now include a first order conditional (FOCE) algorithm which is similar to the Lindstrom and Bates method (Lindstrom & Bates, 1990). Details of statistical aspects of the NONMEM software are discussed in chapter 3.

1.3.3 The population approach -Past and present developments

It was the FDA who first raised concerns that the pharmacokinetics of new drugs were not being sufficiently investigated during the drug development process. In particular, to aid in the selection of appropriate doses, they outlined the need for the pharmacokinetics of new drugs to be studied in the elderly population (Temple, 1983, 1985) . In these documents they introduced the concept of the pharmacokinetic screen, where trends between patient demographics and steady state trough plasma concentrations were investigated. This simplistic approach was first adopted for practical reasons, since extensive pharmacokinetic sampling in elderly patients was not ethically permissible. The data was often sparse and biased as the number and frequency of samples could vary between patients and visits. A formalised approach adopting more sophisticated methodology was required to deal with these data. Sheiner et al. had earlier alluded to methodology which could be used in these situations and highlighted the clear rationale for their development as an aid to both drug development and clinical evaluation (Sheiner et al., 1972, 1977).

Despite this obvious need, the application of the population approach was for many years quite limited. Underlying this was a degree of healthy scepticism within the drug industry (Darrow, 1985; Colburn, 1989). The main reasons for this was complexity of the methodology, the lack of general acceptance of the statistical techniques, and the explorative nature of the analyses. However, at that time therapeutic drug monitoring was at its peak, and retrospective analysis using the population approach was used to

investigate factors which influenced the pharmacokinetics of drugs with a narrow therapeutic index (Whiting et al., 1986). These analyses provided information for both dose initiation and subsequent adjustment via Bayesian feedback (Kelman et al., 1982; Sheiner & Beal, 1982).

As a result of the continued academic interest, the approach slowly gained favour within the drug industry. During the 1990's there has been an explosion of interest, as reflected by the large increase in publications and the formation of specific user groups (i.e. PAGE). The support through the COST B1 initiative has been pivotal in both the further development and wider application of the approach within the drug industry (Bechtel & Alvan, 1998). As well as two conferences, specialist meetings on PK/PD software (Aarons et al., 1994) and performing population PK/PD studies (Aarons et al., 1996) have added greatly to the developments in this areas. The first conference entitled "New strategies in drug development and clinical evaluation; the population approach" highlighted the short-comings to be overcome in order for the approach to become more acceptable. Jochemsen (1992) revealed that the European pharmaceutical industry was beginning to employ the population approach and incorporate it into study design. However there had only been a limited amount of prospective application and it was felt that a conceptual shift within the industry was required if the approach was to become established (Olson, 1992). While individuals within FDA continued to champion the approach (Peck, 1992a), the European guidelines did not reflect the growing interest (Gundert-Remy, 1992).

The second COST B1 "Conference on the population approach: measuring and managing variability in response, concentration and dose" in Geneva in 1997 demonstrated that many advances had been made. The five intervening years had seen an appreciable development addressing theoretical issues around estimation methods, statistical models and design. A staggering growth in both awareness and interest in the approach was exemplified by the three fold increase in the number of scientific publications (Vozeh, 1997). The present day

value of the approach was highlighted by the growth in prospectively planned population PK/PD analyses (Bruno, 1997; Fuseau et al., 1997; Jorga et al., 1997; Mandema, 1997), and the number of subsequent submissions to the FDA which had included the approach (25%) (Ette, 1997). Furthermore, the FDA and MPA regulators presented a number of cases where population PK was being used as evidence for labelling statements (Ette, 1997; Wade, 1997).

Now, the potential of population pharmacokinetic and pharmacodynamic modelling and its application to the drug development process receives similar attention from academic (Aarons, 1992; Sale & Blaschke, 1992; Sheiner & Ludden, 1992; Grasela & Antal, 1993; Rosenbaum et al., 1995) industry (Samara & Granneman, 1997) and regulatory agencies (Peck et al., 1992b).

1.3.4 Application of PK/PD modelling in the early phases of drug development

The application of PK/PD modelling to data obtained from the early phases (I/II) of drug development and its the subsequent use in the design of the Phase III studies has been discussed in several papers (Steimer et al., 1993; Van Peer et al., 1993; Peck, 1997). In particular, Sheiner, who previously questioned the adequacy of the current practices used in drug development (Sheiner, 1991), has proposed an alternative strategy with PK/PD modelling as the cornerstone of the optimised process. By partitioning Phases I/II and III into cycles of "learning" and "confirming", respectively, he highlighted that early development should serve to fully evaluate the "therapeutic response surface" (Sheiner, 1997); a mandate that requires a greater flexibility both in the design and analysis of the early studies (Sheiner & Rubin, 1995). Although some examples showing the advantages of using PK/PD modelling with rich experimental data have been highlighted (Sambol,

1991; Karlsson et al., 1995; Schoemaker & Cohen, 1996), further work exploring the advantages and disadvantages of utilising these methodologies in the early phases of drug development is required to support conceptual change proposed by Sheiner and others.

CHAPTER 2

OUTLINE AND GENERAL AIMS

The general aim of this thesis is to explore how nonlinear mixed effects modelling can be used in the analysis of data taken from the early stages of drug development. The advantages and disadvantages of applying these techniques are examined through a series of examples-

Drug safety analysis - **Chapter 4**

Bioequivalence testing- **Chapter 5**

Dose response analysis - **Chapter 6**

Dose concentration response analysis - **Chapter 7**

The order or presentation represents the transition from the application of pharmacokinetic modelling (Chapters 4 and 5) to pharmacodynamic modelling (Chapter 6) and finally to an example of integrated PK/PD modelling (Chapter 7). The common pharmacokinetic and statistical methods are outlined in Chapter 3. Each subsequent chapter is an integral piece of work; with aims, methods, results, discussion and conclusions. While Chapters 4, 6 and 7 are focused towards the drug under investigation, all Chapters assess the potential advantages of applying a mixed effect modelling approach to standard early drug development problems.

Conclusions from the four separate analysis are presented in **Chapter 8**.

CHAPTER 3

GENERAL METHODS

This chapter describes the pharmacokinetic and statistical methods which are common to the analyses presented in the later Chapters. Analysis specific methods are presented within each subsequent Chapter.

3.1 The nonlinear mixed effects model

As previously discussed, a non-linear mixed effects model can be used to describe the fixed and random effects associated with a model based approach to a PK/PD problem (Chapter 1). In the sections below, the intraindividual and interindividual submodels are described along with the methods used to estimate both simultaneously.

3.1.1 Intraindividual submodel

In PK/PD analyses, it is usually assumed that observations can be described by the following model

$$y_{ij} = f(P_i, X_{ij}, \varepsilon_{ij}) \qquad \text{Eq (3.1)}$$

Where y_{ij} is the jth observation (concentration and /or response) from the ith individual, $f()$ is a general function of all arguments listed which includes a structural model that relates the independent variables, X_{ij} (e.g. time and dosage history), to the observations given the ith individuals vector of model parameters P_i (such as CL,V, D_{50} and Emax). The term ε_{ij} accounts for the error between the observations y_{ij} and model predictions $\hat{y}_{ij}$, and is normally assumed to be independently symmetrically distributed with an expected mean value of zero and variance $\nu\sigma^2$. The parameter ν represents the dependence between model predictions $\hat{y}_{ij}$ and the intraindividual error variance. Changes to ν alter how the intraindividual variability model is implemented. If there is no dependence i.e. the variance remains constant irrespective of the magnitude of the predictions, the error model

is said to homoscedastic or additive. In this case, ν (described above) equals 1 and σ is the standard deviation (SD). However, in many PK/PD cases the error model is found to be heteroscedastic i.e. the error variance changes with the magnitude of the prediction. A limited heteroscedastic case is where the error variance is proportional to the magnitude of $\hat{y}_{ij}$. In this case, ν equals $\hat{y}_{ij}^2$, the variance of ε_{ij} becomes $\sigma^2 \hat{y}_{ij}^2$ and σ is the coefficient of variation (%CV).

In NONMEM additive and proportional intraindividual error models are defined as follows

$$Y = F + EPS(1) \qquad \text{Eq 3.1}$$

$$Y = F *(1+EPS(1)) \qquad \text{Eq 3.2}$$

where Y is y_{ij}, F is $\hat{y}_{ij}$ and EPS(1) is ε_{ij}. An exponential expression, as shown below can also be used

$$Y = F *EXP (EPS(1)) \qquad \text{Eq 3.3}$$

However, with the FO approximation (see section 3.2) this is operationally identical to the proportional error model (Eq 3.2).

More complex intraindividual error models can also be defined. In the general heteroscedastic case the error variance is equal to $\sigma^2 \hat{y}_{ij}^{\zeta_i}$, where ζ_i is an estimated variance parameter. The advantage of this is model is that it can smoothly interpolate between a homoscedastic model (ζ_i=0) and the proportional model (ζ_i=2).

Unfortunately, observations at or around zero cause estimation problems. Often the combined exponential (or proportional) and additive model is used. In this case two independent errors, ε_{ij1} and ε_{ij2}, and therefore two independent variances $\hat{y}_{ij}^2 \sigma_{EXP}^2$ and σ_{ADD}^2 are estimated. The σ_{EXP} is expressed as a (%CV) and σ_{ADD} is expressed

as a standard deviation (SD). In NONMEM, the variances and covariances for the ε_{ij}'s are estimated as a block matrix (Σ).

In NONMEM the combined exponential and additive intraindividual error model is written as follows

$$Y = F * EXP(1) + EPS(2) \qquad \text{Eq 3.4}$$

This was the most complex model tested in this thesis and has the advantage that it can be easily reduced to an additive or exponential error model.

3.1.2 Interindividual model

The individual structural parameter estimates P_i's are distributed around their typical values Θ. The following describes the general relationship

$$P_{ik} = g_k(\theta_k, Z_i, \eta_{ki}) \qquad \text{Eq (3.5)}$$

Where P_{iK} is the kth parameter in the vector of individual parameters P_i, θ_k is a parameter of Θ which singly (or in combination with other parameters from Θ) describes the population average or typical value P_k. The term Z_i represents the vector of covariates for the ith individual. e.g. the ith's individuals demographics, biochemistry or concomitant medication. The function $g_k()$ is a general function of all arguments listed, and relates P_k to Z_i through θ_k, while estimating the difference (η_{ki}) between P_k and P_{ik}. The η_{ki}'s are assumed to be independently, multivariately distributed, with mean expected values of zero and variances of ω_κ^2. The variances and covariances for the η_{ki}'s are estimated in NONMEM as a block matrix (Ω). In comparison to the additive model, the exponential error model prevents individual parameter estimates (see section 3.2.2) from becoming negative.

Examples of additive, proportional and exponential interindividual error models for clearance (CL) are described as follows

$$CL = TVCL + ETA(1) \quad \text{Eq 3.6}$$

$$CL = TVCL *(1+ ETA(1)) \quad \text{Eq 3.7}$$

$$CL = TVCL *EXP (ETA(1)) \quad \text{Eq 3.8}$$

where TVCL is the typical value (P_K) of CL and ETA(1) is η_{ki} .

3.1.3 General nonlinear mixed effects model

On suppressing $g_k()$, the nesting of the interindividual and the intraindividual models can be expressed by the general model:

$$y_{ij} = S(X_{ij}, Z_i, \Theta, \eta_i, \varepsilon_{ij}) \quad \text{Eq 3.9}$$

where $S(\quad)$ becomes a general function incorporating the terms of both submodels.

3.2 Estimating the nonlinear mixed effects model

As discussed in section 1.3.2, estimation of the nonlinear mixed effects has been implemented in a number of software packages (Aarons et al., 1994). However, since only NONMEM was used in this thesis only the methods related to this package are presented.

3.2.1 First Order estimation method (FO)

NONMEM minimises the extended least squares objective function (Eq 3.10)

$$O_{els} = \sum_i \left[\log\left|\mathrm{var}(y_i)\right| + \left(y_i - E(y_i)\right)\mathrm{var}(y_i)^{-1}\left(y_i - E(y_i)\right)^t \right] \quad \text{Eq 3.10}$$

where O_{els} is the extended least squares objective function, $\mathrm{var}(y_i)$ is the variance-covariance of the ith individual's vector of observations, $E(y_i)$ is the expectation of y_i i.e. $\hat{y}_i$ and the subscript t denotes the transpose of the matrix. The log term included in the

O_{els} can be viewed as a penalty to avoid continuous increases in the variance which would decrease the objective function without a decrease in residuals. Under Gaussian conditions O_{els} is only different to -2log-likelihood of the fit (-2LL) by a constant. This attribute is utilised in model development and the testing of covariate relationships (3.4.1).

Closed form solutions of $E(y_i)$ and $\text{var}(y_i)$ can only be obtained in the special case when the general model (Eq 3.9) is linear in its random effects. However, a linearisation can be used as an approximate solution when this is not the case. This approximation uses a first order Taylor series expansion about the expected values of η_{ki}'s and ε_{ij}'s i.e. zero.

If the general function (3.9) is simplified to the vector $M_i(\Theta, \eta_i, \varepsilon_i)$, such that

$$y_{ij} = M_i(\Theta, \eta_i, \varepsilon_i) = \left(M_{i1}(\Theta, \eta_i, \varepsilon_{i1}), M_{i2}(\Theta, \eta_i, \varepsilon_{i2}), M_{i3}(\Theta, \eta_i, \varepsilon_{i3}), \ldots, M_{ini}(\Theta, \eta_i, \varepsilon_{ini}) \right) \quad \text{Eq 3.11}$$

where the ith individual's dosing history, covariates and j individual observations are suppressed, and the η_i is the ith individual's vectors of η -values and ε_{ij} is the ith individual's vectors of ε 's for observation j. The expectation of the partial derivatives of $M_i(\Theta, \eta_i, \varepsilon_i)$ with respect to η_i and ε_i can be denoted by matrices G_i and H_i, respectively i.e.

$$G_i = \frac{d M_i}{d \eta_i}(\Theta, 0, 0) \quad \text{Eq 3.12}$$

$$H_i = \frac{d M_i}{d \varepsilon_i}(\Theta, 0, 0) \quad \text{Eq 3.13}$$

As a result, $E(y_i)$ is approximately given by

$$E(y_i) \approx M_i(\Theta, 0, 0) + G_i \eta_i + H_i \varepsilon_i \quad \text{Eq 3.14}$$

and if G_i^t and H_i^t correspond to the transpose of G_i and H_i, respectively, var$\left(y_i\right)$is approximately given by

$$S_i^2(\Theta,\Omega,\Sigma) \approx G_i \Omega G_i^t + DiagH_i \Sigma H_i^t \qquad \text{Eq 3.15}$$

where Ω is the variance covariance matrix of the η_{ki} 's, and Σ is the variance covariance of the ε_{ij} 's as discussed previously. Diag is the matrix diagonal

The use of the linear approximation to estimate $E\left(y_i\right)$ and var$\left(y_i\right)$ in the extended least squares (ELS) objective function is known as the **first order (FO) estimation method** and was the first method implemented with NONMEM. The O_{els} for the population model under the FO approximation becomes:

$$\sum_i \left[\log\left|S_i^2(\Theta,\Omega,\Sigma)\right| + \left(y_i - M_i(\Theta,0,0)\right)\left(S_i^2(\Theta,\Omega,\Sigma)\right)^{-1}\left(y_i - M_i(\Theta,0,0)\right)^t \right] \qquad \text{Eq 3.16}$$

The resultant objective function value (OFV) can be used to compare models (see section 3.3.1).

3.2.2 Individual parameter estimates

With the FO estimation method, individual posterior Bayes parameter estimates can be obtained after the ELS problem has been minimised. The objective function which is minimised with respect to the η_i -values is as follows

$$\sum_i \left[\log\left|S_i^2(\Theta,\Omega,\Sigma)\right| + \left(y_i - M_i\left(\Theta,\eta_i,0\right)\right)\left(S_i^2(\Theta,\Omega,\Sigma)\right)^{-1}\left(y_i - M_i\left(\Theta,\eta_i,0\right)\right)^t \right] \qquad \text{Eq 3.17}$$

$$+ \eta_i^t \Omega^{-1} \eta_i$$

The population parameters (Θ,Ω,Σ) are fixed to that obtained from the fit so only the second (or Bayesian) term is estimated. Since the individual parameter estimates are

obtained after the objective function has been minimised the term "Posthoc" is used to describe them.

3.2.3 First order conditional estimation FOCE

When the model is highly non-linear in η_i the FO method may under certain circumstances produce biased estimates. Bias can occur with non-linear PK or PD models and may be exacerbated by multiple dose models. Additional estimation methods are available to reduce the bias with the FO method. The conditional estimation methods produce both estimates of population parameters and estimates of random interindividual effects, simultaneously. The FOCE methods are two stage processes, at each iteration conditional estimates of η_i ($\hat{\eta}_i$) are obtained using the current estimates of Θ, Ω, Σ. The estimates of Ω, Σ are then updated based on $\hat{\eta}_i$. The following objective function is minimised

$$\sum_i \left[\log\left|S_i^2(\Theta,\Omega,\Sigma)\right| + \left(y_i - M_i(\Theta,\hat{\eta}_i,0)\right)\left(S_i^2(\Theta,\Omega,\Sigma)\right)^{-1}\left(y_i - M_i(\Theta,\hat{\eta}_i,0)\right)^{t'} \right]$$

Eq 3.18

Two FOCE methods exist, by default the "no interaction" method is specified. In this case Σ is estimated based on the mean parameter model. When "interaction" is specified the prediction of Σ is based on the conditional estimates of $\hat{\eta}_i$

When the intraindividual error is independent of the predicted value i.e. additive error, there can be no interaction between the $\hat{\eta}_i$'s and ε_i's. Therefore, specifying an interaction can only give different parameter estimates when a heteroscedastic error model is utilised. There is a dramatic increase in computation time with the FOCE methods, so the FO method is usually used by default.

3.3 Model building

The process of model building involves balancing the need to obtain the best description of a set of observations against the convenience and utility of the model. While the individual observations are themselves the most accurate representation of the data, they are not very easy to use. The arithmetic mean, on the other hand, is convenient to use but is not a good description of the majority of the data. The "best" model lies somewhere between these two extremes. The stepwise process for determining the most appropriate structural, variance and covariate models is outlined below.

3.3.1 Structural model identification

The structural models employed for the characterisation of the pharmacokinetic and pharmacokinetic/pharmacodynamic relationships may or may not utilise knowledge from previous analyses. Refinement of previous models by fixing or deleting parameters from the model may be necessary where the data is insufficient to support the full model.

Difficulties in obtaining satisfactory minimisation or poor parameter precision (i.e. large SE) may be used to identify when models are overly complex i.e. not supported by the data. If the underlying model is not known or found to be insufficient, knowledge from the basic pharmacology and pharmacokinetics can be used to propose suitable alternative models.

Selection of the final structural model is based on a combination of the following criteria :

1) Significant decrease in objective function value (OBV). This is determined using either the likelihood ratio test (LRT), if competing models form a full/reduced pair (see section 4.1), or the Akaike information criterion (AIC) (Yamaoka et al., 1978)

i.e. for two models a and b

$\Delta AIC = OFVa - OFVb + 2(Pa - Pb)$

where P is the number of free parameters

If $\Delta AIC > 0$ model b is choosen over a

2) Improvement in the goodness of fit (GOF) plots i.e. observed (DV) vs predicted (PRED) plots; residual (RES) and weighted residual (WRES) versus time after dose plots. Subjectively assessed by looking for patterns and bias in the residuals.

3) Improvement in the goodness of fit (GOF) plots for the individual data i.e. observed (DV) vs individual predicted (IPRED) plots; individual residual (IRES) and individual weighted residual (IWRES) versus time after dose plots.

4) Parameter precision calculated based on the SE of the parameter estimates which are obtained using a quadratic approximation. As stated above, a model may be considered too complex if one or more of the parameter estimates is not statistically significantly different from zero i.e. 95 % CI includes zero. This is approximately equivalent to the SE (%) (SE as a percentage of the population parameter estimate) being greater than 50%.

3.3.2 Variability model identification

The combined additive and exponential error model (section 3.1.1) was the most complex intraindividual variability model used. The significance of each component was tested using the LRT. Either a proportional or an exponential interindividual error model was used.

3.3.3 Covariate identification and selection

Graphical analysis was used to detect potential relationships between covariates and Posthoc or FOCE derived individual parameter estimates. The trends in the plots were used to determine whether the relationship was likely to be linear or nonlinear. The significance of each relationship was formally tested by comparing the objective function obtained with and without the additional parameter(s) used to describe the relationship. The likelihood ratio test was used (LRT) to compare the difference in the objective

function values between the two models (known as the "full" and the "reduced" model pair). The difference is approximately χ^2 distribution with degrees of freedom (df) equal to the difference in the number of free parameters. When one parameter is fixed in the reduced model a decrease in objective function value 3.84 is significant at $p<0.05$.

After testing all covariates univariately a step-wise procedure was used to incorporate all the significant covariates. In the analyses presented there were only a small number of covariates of interest, so all combinations of covariates from the univariate analyses were tested multivariately. After the full models were built, the final models were obtained by removing each of the covariates in turn and performing a LRT. Since the aim of these analyses was to generate hypotheses for testing in subsequent studies, the same p-value ($p<0.05$) was used to include and exclude covariates.

CHAPTER 4

APPLICATION OF NONLINEAR MIXED EFFECTS MODELLING IN THE DRUG SAFETY ANALYSIS OF RANITIDINE BISMUTH SUBCITRATE (RBS)

In this chapter a nonlinear mixed effects analysis of the distribution / elimination pharmacokinetics of bismuth following multiple dosing with ranitidine bismuth subcitrate (RBS) is described. The results are compared to a previous analysis where individual fitting was carried out using models which assume that the absorption rate Ka is equal to initial distribution rate constant α. Predictions of steady state bismuth accumulation are performed and the safety of extended courses of RBS treatment is assessed.

4.1 Introduction

4.1.1 Ranitidine bismuth subcitrate (RBS)

RBS is a novel salt of ranitidine formed from ranitidine hydrochloride and bismuth citrate complex. It therefore possesses both the antisecretory actions of ranitidine (Prewitt et al., 1991), and the mucosal protective (Hudson et al., 1993) and Anti-helicobacter pylori (Fraser et al., 1993; Webb et al., 1995) properties of bismuth. RBS was developed for treatment of relapse of benign gastric ulcer, duodenal ulcer and eradication of *Helicobacter pylori.*

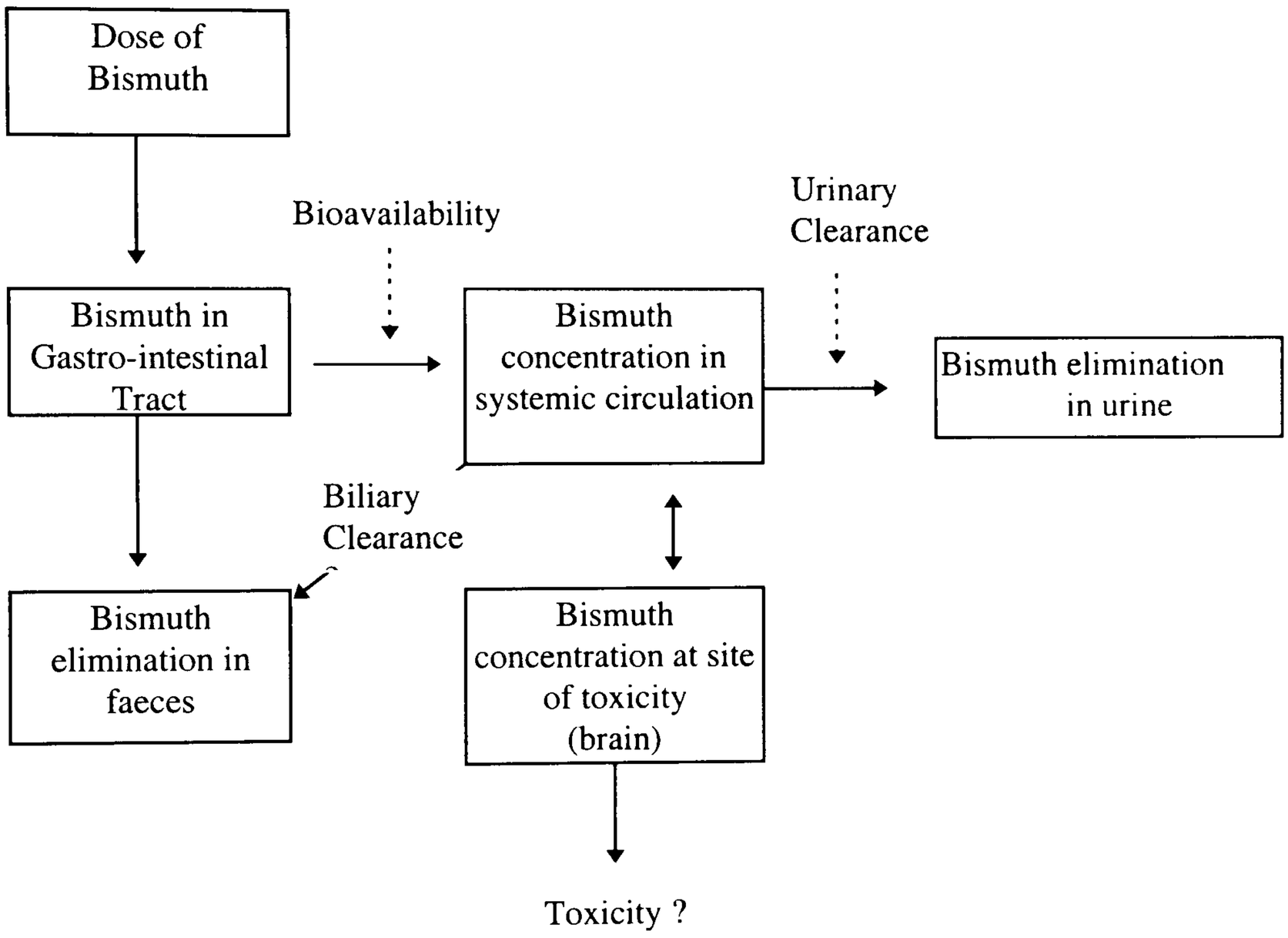
4.1.2 Bismuth pharmacokinetics

The absorption of bismuth depends on the solubility of the salt in which it is administered. The most commonly used are the salicylate (Peptobismol ®) or subcitrate salt (tripotassium dicitrato bismuthate) (De-nol ®). While only trace amounts are absorbed from the relatively insoluble subnitrate and salicylate salts (Nwokolo et al., 1990a, b), absorption from the more soluble subcitrate salt has been demonstrated (Wagstaff et al., 1988; Froomes et al., 1989; Gavey et al., 1989; Nwokolo et al., 1989; Benet, 1991; Madaus et al., 1991) and a bioavailability of ~ 0.28% has been estimated based upon urinary measurements (Benet, 1991).

A pictorial representation of the processes governing the pharmacokinetics of absorbed

bismuth after oral bismuth subcitrate (BS) administration is shown in Figure 4.1. The primary route of elimination is by urinary excretion. While biliary clearance may be as much as 90% of renal clearance, hepato-biliary re-circulation may reduce the net biliary loss (Mclean et al., 1989).

Figure 4.1 Schematic diagram of bismuth pharmacokinetics and pharmacodynamic characteristics (adapted from Benet 1991)



Previously, the multiple distribution phase pharmacokinetics of bismuth have been shown to require at least a tri-eponential model for the post peak decline in plasma concentration (Benet, 1991). Bismuth accumulation following regular dosing has been well documented with apparent steady state levels being achieved in 30-40 days (Froomes et al., 1989; Gavey et al., 1989; Nwokolo et al., 1989) . However, a general elimination half-life of 21 days (Froomes et al., 1989) and terminal elimination half-life of 21-72 days (Benet, 1991) have been estimated, so accumulation may continue for up to 4-12 months.

The presence of food can reduce the percentage absorbed (Nwokolo et al., 1989); and co-

administration of H_2-antagonists can increase the absorption of bismuth from certain formulations by decreasing gastric pH (Nwokolo et al., 1991). Since it is primarily eliminated via the renal route, caution is advised in the treatment of patients with renal impairment. In particular, a meta-analysis of several bismuth studies demonstrated a negative correlation between trough concentration and creatinine clearance (Lacey, 1994). The small increase in trough levels was not deemed to be clinically significant. Most of the intra- and inter-individual variability in bismuth levels remains largely unexplained and considered to be the result of variable absorption and low intrinsic bioavailability (Benet, 1991).

4.1.3 Bismuth toxicity

While gastro-intestinal side-effects i.e. blackening of the stools are the most commonly reported side-effect with bismuth, more severe CNS toxicity has also been reported. During the 1970's, oral administration of bismuth salts resulted in over a thousand reported cases of neurological disorders which were characterised by myoclonic jerks, severe confusion, hallucination and epileptic seizures (Martin-Bouyer et al., 1981). Hillemand and co-workers proposed average steady state blood concentrations greater than 50ng.ml^{-1} and 100ng.ml^{-1} (equivalent to plasma levels of 77.5 and 155, respectively) to be "safety" and "alarm" levels for bismuth toxicity, respectively (Hillemand et al., 1977). However, these have more recently been shown to be over cautious (Benet, 1991). Furthermore, previous episodes of CNS toxicity have mainly been associated with salts other than subcitrate and treatment duration extending beyond that required clinically (Martin-Bouyer et al., 1981).

Nevertheless, the delay from the onset of therapy until the appearance of toxicity may be due to the slow accumulation into the CNS. A full understanding of the pharmacokinetics

of bismuth following treatment with RBS is required to help understand the potential for toxicity upon prolonged treatment.

4.1.4 Drug and therapeutic safety evaluation

The primary focus of the initial healthy volunteer studies is to determine the relationship between dose/concentration and adverse events. For a new product containing a combination of drugs, these relationships may have previously been established. Nevertheless, when the combination has the potential to interact, these relationships will have to be reassessed. In multiple dose safety and tolerability studies it is desirable that dosing continue until steady state levels have been achieved. However, with drugs which have a long terminal half-life this is not always practical.

4.2 Data and previous results

4.2.1 Study design & data

A double blind randomised multiple dose study in healthy volunteers, was undertaken to investigate the pharmacokinetics of bismuth and ranitidine following administration of the RBS formulation. The randomisation assigned one third of subjects to placebo, and the remainder to 800mg of RBS twice daily for 28 days. After the morning doses on days 1, 14, and 28, plasma samples were taken pre-dose and at 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0 hours post dose. During the intervening weeks, trough samples were collected on days 4, 7, 10, 14, 18, 21 and 25. After day 28, the subjects in the active group entered into an open phase where further samples were measured daily for one week, twice weekly for the following three weeks and then weekly until either plasma levels fell below the assay's limit of quantification or 28 weeks had elapsed from the final dose.

Subjects had a mean age and weight of 32.3 (SD 8.8) years and 77.7 (SD 6.7) kg, respectively.

One subject was lost to follow up before the last full profile study day, the other 17 subjects completed the initial study phase. Two subjects (5 & 7) were lost to follow up after one week post final dose and had plasma bismuth levels of 1.2 and 2.31 $ng.ml^{-1}$ at this time. The rest of the subjects were monitored for an average of 58 (SD 24.2) days post dosing, at which point the bismuth concentration was 0.24 (SD 0.04) $ng.ml^{-1}$. In total the eighteen patients provided 977 bismuth concentrations.

4.2.2 Drug assay

Plasma bismuth concentration measurements were determined using a validated inductively coupled plasma mass - spectroscopy method which had a lower limit of quantification of 0.2 $ng.ml^{-1}$ and precision of 13.2% (Tye et al., 1992).

4.2.3 Previous results using a Standard Two Stage (STS) approach

Koch et al have previously modelled the data individually using PC-NONLIN version 3 (Koch et al., 1996). In this analysis, the most appropriate distribution model was initially determined from the following models using the AIC (Chapter 3) to select between models.

2 Compartment

$$C_t = Ae^{-\alpha t}\cdot\frac{1-e^{-N\alpha\tau}}{1-e^{-\alpha\tau}} + Be^{-\beta t}\cdot\frac{1-e^{-N\beta\tau}}{1-e^{-\beta\tau}} - \left(A+B\right)e^{-Kat}\cdot\frac{1-e^{-NKa\tau}}{1-e^{-Ka\tau}} \quad \text{Eq 4.1}$$

3-Compartment

$$C_t = Ae^{-\alpha t}\cdot\frac{1-e^{-N\alpha\tau}}{1-e^{-\alpha\tau}} + Be^{-\beta t}\cdot\frac{1-e^{-N\beta\tau}}{1-e^{-\beta\tau}} + Ce^{-\gamma t}\cdot\frac{1-e^{-N\gamma\tau}}{1-e^{-\gamma\tau}} - \left(A+B+C\right)e^{-Kat}\cdot\frac{1-e^{-NKa\tau}}{1-e^{-Ka\tau}} \quad \text{Eq 4.2}$$

4-Compartment

$$C_t = Ae^{-\alpha t}\cdot\frac{1-e^{-N\alpha\tau}}{1-e^{-\alpha\tau}} + Be^{-\beta t}\cdot\frac{1-e^{-N\beta\tau}}{1-e^{-\beta\tau}} + Ce^{-\gamma t}\cdot\frac{1-e^{-N\gamma\tau}}{1-e^{-\gamma\tau}} + De^{-\delta t}\cdot\frac{1-e^{-N\delta\tau}}{1-e^{-\delta\tau}}$$
$$-\left(A+B+C+D\right)e^{-Kat}\cdot\frac{1-e^{-NKa\tau}}{1-e^{-Ka\tau}} \qquad \text{Eq 4.3}$$

The equations describe plasma concentration (C_t) as a function of time (t), coefficient constants (A, B, C and D), absorption/ distribution/ elimination rate constants (Ka, α, β, γ, δ,) and the standard multiple dose function $(1-e^{-Nx\tau})/(1-e^{-xt})$; where x represents the respective rate constant (Ka,α, β, χ, δ,), N is the number of equal doses administered by time t, and τ is the dosing interval.

However, it was reported that on fitting these models, the standard errors for Ka and α were found to be very large i.e. >100%. Inaccurate and imprecise estimates can occur when the absorption rate constant value is similar to that of a distribution or elimination rate constant (this is considered further in the discussion-section 4.6.2). To overcome this problem, models for the special case where the absorption rate is equal to a distribution or elimination rate have been derived for the one (Dost, 1968) and two compartment models (Wijnand, 1988). These were extended by Koch et al to allow the fit of up to 4 compartments after multiple doses i.e.

2-Compartment

$$C_t = Ate^{-kt}\cdot\frac{1-e^{-Nk\tau}}{1-e^{-k\tau}} + A\tau e^{-kt}\cdot\frac{1}{1-e^{-k\tau}}\cdot\left[\frac{e^{-k\tau}-e^{-Nk\tau}}{1-e^{-k\tau}} - N\,e^{-Nk\tau} + e^{-Nk\tau}\right]$$
$$+Be^{-\beta t}\cdot\frac{1-e^{-N\beta\tau}}{1-e^{-\beta\tau}} - Be^{-kt}\cdot\frac{1-e^{-Nk\tau}}{1-e^{-k\tau}} \qquad \text{Eq 4.4}$$

3-Compartment

$$C_t = Ate^{-kt} \cdot \frac{1-e^{-Nk\tau}}{1-e^{-k\tau}} + A\tau e^{-kt} \cdot \frac{1}{1-e^{-k\tau}} \cdot \left[\frac{e^{-k\tau} - e^{-Nk\tau}}{1-e^{-k\tau}} - N e^{-Nk\tau} + e^{-Nk\tau} \right]$$

$$+ Be^{-\beta t} \cdot \frac{1-e^{-N\beta\tau}}{1-e^{-\beta\tau}} + Ce^{-\gamma t} \cdot \frac{1-e^{-N\gamma\tau}}{1-e^{-\gamma\tau}} - \left(B+C\right)e^{-kt} \cdot \frac{1-e^{-Nk\tau}}{1-e^{-k\tau}} \qquad \text{Eq 4.5}$$

4-Compartment

$$C_t = Ate^{-kt} \cdot \frac{1-e^{-Nk\tau}}{1-e^{-k\tau}} + A\tau e^{-kt} \cdot \frac{1}{1-e^{-k\tau}} \cdot \left[\frac{e^{-k\tau} - e^{-Nk\tau}}{1-e^{-k\tau}} - N e^{-Nk\tau} + e^{-Nk\tau} \right]$$

$$+ Be^{-\beta t} \cdot \frac{1-e^{-N\beta\tau}}{1-e^{-\beta\tau}} + Ce^{-\gamma t} \cdot \frac{1-e^{-N\gamma\tau}}{1-e^{-\gamma\tau}} + De^{-\delta t} \cdot \frac{1-e^{-N\delta\tau}}{1-e^{-\delta\tau}}$$

$$-\left(B+C+D\right)e^{-kt} \cdot \frac{1-e^{-Nk\tau}}{1-e^{-k\tau}} \qquad \text{Eq 4.6}$$

where k is the combined absorption and distribution rate constant. The individual parameter estimates for the best fit model using this approach are shown in Table 4.1.

Table 4.1 Individual parameter estimates for the best fit models obtained using PC-NONLIN and the adapted Wijnand equations

Three compartment model (Eq 4.5)

ID	A $ng.ml^{-1}.hr^{-1}$	SE (A) %	B $ng.ml^{-1}$	SE (B) %	C $ng.ml^{-1}$	SE (C) %	k hr^{-1}	SE (k) %	β hr^{-1}	SE (β) %	γ hr^{-1}	SE (γ) %
2	3.5	18	0.50	24	0.028	13	1.2	14	0.075	27	0.0015	12
3	3.6	27	0.58	18	0.021	23	1.4	23	0.033	21	0.0012	22
4	9.4	13	0.33	12	0.082	7	1.6	8	0.026	22	0.0010	5
10	1.40	31	0.35	8	0.020	10	3.3	43	0.041	11	0.0011	11
11	4.6	28	0.68	26	0.033	18	1.3	22	0.064	29	0.0015	18
13	13	20	0.72	15	0.067	6	2.7	15	0.11	19	0.0018	5
15	7.5	41	1.2	23	0.032	13	2.4	41	0.11	24	0.0012	14
17	14	25	0.78	16	0.012	25	2.5	18	0.058	16	0.0011	36
21	3.1	14	0.26	11	0.064	9	1.4	11	0.020	19	0.0012	7
24	14	25	0.25	61	0.28	5	2.3	16	0.10	91	0.0020	4
25	0.65	52	0.29	16	0.077	18	1.8	68	0.019	32	0.0014	11
27	8.6	9	0.69	11	0.10	6	1.6	7	0.065	17	0.0026	3

Four compartment model (Eq 4.6)

ID	A $ng.\ ml^{-1}.hr^{-1}$	SE (A) %	B $ng.ml^{-1}$	SE (B) %	C $ng.ml^{-1}$	SE (C) %	D $ng.ml^{-1}$	SE (D) %	k hr^{-1}	SE (k) %	β hr^{-1}	SE (β) %	γ hr^{-1}	SE (γ) %	δ hr^{-1}	SE (δ) %
8	88	45	3.4	56	0.37	23	0.028	25	4.99	32	0.53	41	0.018	27	0.0011	21
14	10	41	1.6	62	0.44	59	0.042	12	2.83	51	0.32	75	0.033	47	0.0012	10
19	7.5	19	1.0	52	0.16	23	0.026	31	2.64	22	0.43	38	0.016	34	0.0019	23

4.3 Aims

The aim of this analysis was to:

1) Determine the disposition characteristics of bismuth following multiple oral dosing in healthy volunteers using nonlinear mixed effect modelling

2) Compare the population modelling approach to the standard two stage approach with regard to estimation of α and Ka

3) Predict the steady state levels and therefore assess the potential for toxicity from prolonged treatment with RBS

4.4 Methods

4.4.1 Noncompartmental analysis

The area under the plasma time curve for the first 12 hours post dose (AUC_{0-12}) was calculated for each full profile day using the linear trapezoidal rule. The maximum concentration post dose (Cmax) and minimum concentration after 12 hours (Cmin) were determined directly from the data. Ratios of the individual parameters were used to assess the accumulation from day 1 to day 14 and from day 14 to day 28.

4.4.2 Population pharmacokinetic analysis using micro or macro rate constant models

Micro rate constant model

An initial population analysis was undertaken utilising the ADVAN5 subroutine from the PREDPP library of pharmacokinetic models available within NONMEM. Two and three compartment models with first order input were fitted using the FO and FOCE methods (Chapter 3).

Macro rate constant model

Due to problems in obtaining appropriate model fits (see results and discussion), a second population analysis was undertaken using the coefficient and macro rate constant models Eq 4.1 to 4.3. For comparison, the adapted Wijnand compartment models were also fitted Eq 4.4 to 4.6. Interindividual variability was estimated on all parameters using an exponential error model. Intraindividual variability was estimated using a combined exponential and additive error model (Chapter 3). The NMTRAN user supplied PRED records for implementation of equations 4.2 and 4.5 are shown in Appendix 1.1.

Model dependent estimation of $AUC_{0\text{-}12}$, Cmax and Cmin

The population mean and individual model dependent estimates of Cmax and Cmin on days 1, 14 and 28 were obtained by setting N to 1, 27 or 55 in Eq.4.1 to 4.6 and substituting in the corresponding population and individual pharmacokinetic parameter estimates. While Cmin was obtained by directly substituting t=12, Cmax was obtained by varying t until a maximum was reached. This process was automated using the solver routine in EXCEL (version 5). $AUC_{0\text{-}12}$ on days 1, 14 and 28 were obtained by integrating the equations over the 12 hour dosing interval. For the standard three compartment model (Eq 4.2) the following equation is obtained

$$AUC_{0-\tau} = \frac{A}{\alpha}\cdot\left(1-e^{-N\alpha\tau}\right)+\frac{B}{\beta}\cdot\left(1-e^{-N\beta\tau}\right)+\frac{C}{\gamma}\cdot\left(1-e^{-N\gamma\tau}\right)-\frac{A+B+C}{Ka}\cdot\left(1-e^{-NKa\tau}\right)$$

Eq 4.7

The values of Cmin and Cmax at steady state were obtained from the equivalent steady state equations i.e. for the three compartment model

$$C_{tss}=Ae^{-\alpha t}.\frac{1}{1-e^{-\alpha\tau}}+Be^{-\beta t}.\frac{1}{1-e^{-\beta\tau}}+Ce^{-\gamma t}.\frac{1}{1-e^{-\gamma\tau}}$$
$$-\left(A+B+C\right)e^{-Kat}.\frac{1}{1-e^{-Ka\tau}} \qquad \text{Eq 4.8}$$

$AUC_{0\text{-}12}$ at steady state was determined by taking the integral of these equations over the 12 hour dosing interval i.e. for the three compartment model

$$AUC_{ss}=\frac{A}{\alpha}.+\frac{B}{\beta}+\frac{C}{\gamma}-\frac{A+B+C}{Ka}$$

The prediction intervals for AUC, Cmin and Cmax from day 1 up to steady state dosing (i.e. (median and 25-75th and 5-95th percentiles) were obtained by simulating for 1000 patients using the final population mean and variability estimates.

4.4.3 Assessment and comparison of the noncompartmental and population model dependent estimates of accumulation.

Model based predictions of accumulation in $AUC_{0\text{-}12}$, Cmin and Cmax were compared to the noncompartmental estimates. The noncompartment estimates were log-normally distributed, so the geometric mean was compared to the average population estimates obtained using the exponential interindividual variability model.

4.5 Results

4.5.1 Noncompartmental analysis

The individual and geometric mean, $AUC_{0\text{-}12,}$ Cmin and Cmax for days 1, 14 and 28 are shown in Table 4.2. The average measures indicated that noncompartmental parameters increased during the course of the study. The standard deviations were large across all parameters and days. All subjects except individuals 3, 7, 8 and 11 demonstrated a progressive increase in both $AUC_{0\text{-}12}$ and Cmin during treatment.

Table 4.2 Noncompartmental estimates of AUC_{0-12}, Cmin and Cmax on days 1, 14 and 28

Day	1	14	28	1	14	28	1	14	28
	AUC_{0-12}	AUC_{0-12}	AUC_{0-12}	Cmin	Cmin	Cmin	Cmax	Cmax	Cmax
	$ng.ml^{-1}.hr$	$ng.ml^{-1}.hr$	$ng.ml^{-1}.hr$	$mg.ml^{-1}$	$mg.ml^{-1}$	$mg.ml^{-1}$	$mg.ml^{-1}$	$mg.ml^{-1}$	$mg.ml^{-1}$
ID									
2	7.23	16.02	19.33	0.20	0.89	1.27	2.35	2.90	2.45
3	7.61	35.87	42.57	0.28	1.81	1.69	1.18	10	10.33
4	7.66	50.47	52.95	0.29	2.56	3.53	2.57	10.75	6.39
5	9.93	30.25	40.03	0.34	2.22	2.62	4.13	3.34	6.33
7	*	67.84	35.49	*	2.53	1.92	*	15.79	6.62
8	17.16	52.26	39.39	0.30	2.41	1.91	7.70	13.84	18.86
10	3.63	14.73	16.08	0.20	0.95	1.13	0.40	2.02	1.61
11	8.32	48.91	24.78	0.34	1.55	1.74	1.84	15.26	2.80
13	7.76	24.37	33.56	0.27	1.44	2.58	2.92	4.39	3.51
14	13.70	33.40	35.02	0.35	2.18	2.51	6.07	5.18	3.92
15	14.11	17.80	28.58	0.40	0.84	1.64	5.38	2.21	5.75
17	12.48	18.70	19.45	0.47	0.94	1.17	4.44	3.58	3.73
19	*	19.24	23.68	*	1.10	1.41	*	4.12	3.84
21	5.05	30.83	52.86	0.22	2.19	3.11	0.93	3.54	12.26
23	13.18	37.05	*	0.38	2.59	*	4.55	3.97	*
24	7.83	62.29	154.29	0.30	4.70	9.31	2.95	6.11	17.37
25	4.75	33.06	67.34	0.20	2.24	4.40	0.85	4.68	12.42
27	9.77	38.29	41.90	0.38	2.23	2.88	2.18	5.83	5.24
Geometric mean	**8.65**	**31.71**	**36.40**	**0.30**	**1.77**	**2.24**	**2.45**	**5.33**	**5.78**

* Indicates where the data was insufficient for parameter estimation

The relationship between Cmax and duration of treatment was highly variable for all individuals. This was exemplified by the largest measured concentration being sampled on day 14 in 9 individuals. The accumulation of bismuth during the treatment course is shown in terms of the ratios of the parameters on day 14 to day 1 and on day 28 to day 14 (Table 4.3). As expected, the geometric mean accumulation in Cmax, Cmin and AUC_{0-12} during the first two weeks (2.07, 6.00 and 3.61, Table 4.3) was much greater than that during the second two weeks (1.07, 1.29 and 1.16, Table 4.3). Although further significant increases would not be expected, predictions of the final steady state estimates require the application of modelling techniques.

4.5.2 Population analysis

Micro rate constant model

The summary of runs made using the micro rate constant models is shown in Table 4.4. Using FO estimation and an exponential error model, the objective function for the three compartment model (Run2) was significantly smaller than that for a two compartment model (Run1). The combined additive plus exponential intraindividual variability model (Run3) was significantly better than when the exponential error model alone was used (Run2). Figure 4.2 shows plots of population predicted concentration versus the observed concentration for a three compartment model using the FO and FOCE estimation methods (Run 3 and Run 5). The fit with the FOCE method was less biased than with the FO method. However, the run terminated unsuccessfully due to rounding errors, even after rerunning with new starting estimates. A successful termination and lower objective function was obtained by reducing the number of ω terms in the model (Run 6). This suggested that the model with the full interindividual variability structure had terminated at a local minimum (Run 5).

Table 4.3 Ratios of the noncompartmental estimates of AUC_{0-12}, Cmax and Cmin for days 1, 14 and 28

Ratio	AUC_{0-12}	AUC_{0-12}	Cmin	Cmin	Cmax	Cmax
Day : Day	14:1	28:14	14:1	28:14	14:1	28:14
ID						
2	2.22	1.21	4.45	1.43	1.23	0.84
3	4.72	1.19	6.46	0.93	8.47	1.03
4	6.59	1.05	8.83	1.38	4.18	0.59
5	3.05	1.32	6.53	1.18	0.81	1.90
7	*	0.52	*	0.76	*	0.42
8	3.04	0.75	8.03	0.79	1.80	1.36
10	4.06	1.09	4.75	1.19	5.05	0.80
11	5.88	0.51	4.56	1.12	8.29	0.18
13	3.14	1.38	5.33	1.79	1.50	0.80
14	2.44	1.05	6.23	1.15	0.85	0.76
15	1.26	1.61	2.10	1.95	0.41	2.60
17	1.50	1.04	2.00	1.24	0.81	1.04
19	*	1.23	*	1.28	*	0.93
21	6.10	1.71	9.95	1.42	3.81	3.46
23	2.81	*	6.82	*	0.87	*
24	7.96	2.48	15.67	1.98	2.07	2.84
25	6.96	2.04	11.20	1.96	5.51	2.65
27	3.92	1.09	5.87	1.29	2.67	0.90
Geometric mean	**3.61**	**1.16**	**6.00**	**1.29**	**2.07**	**1.07**

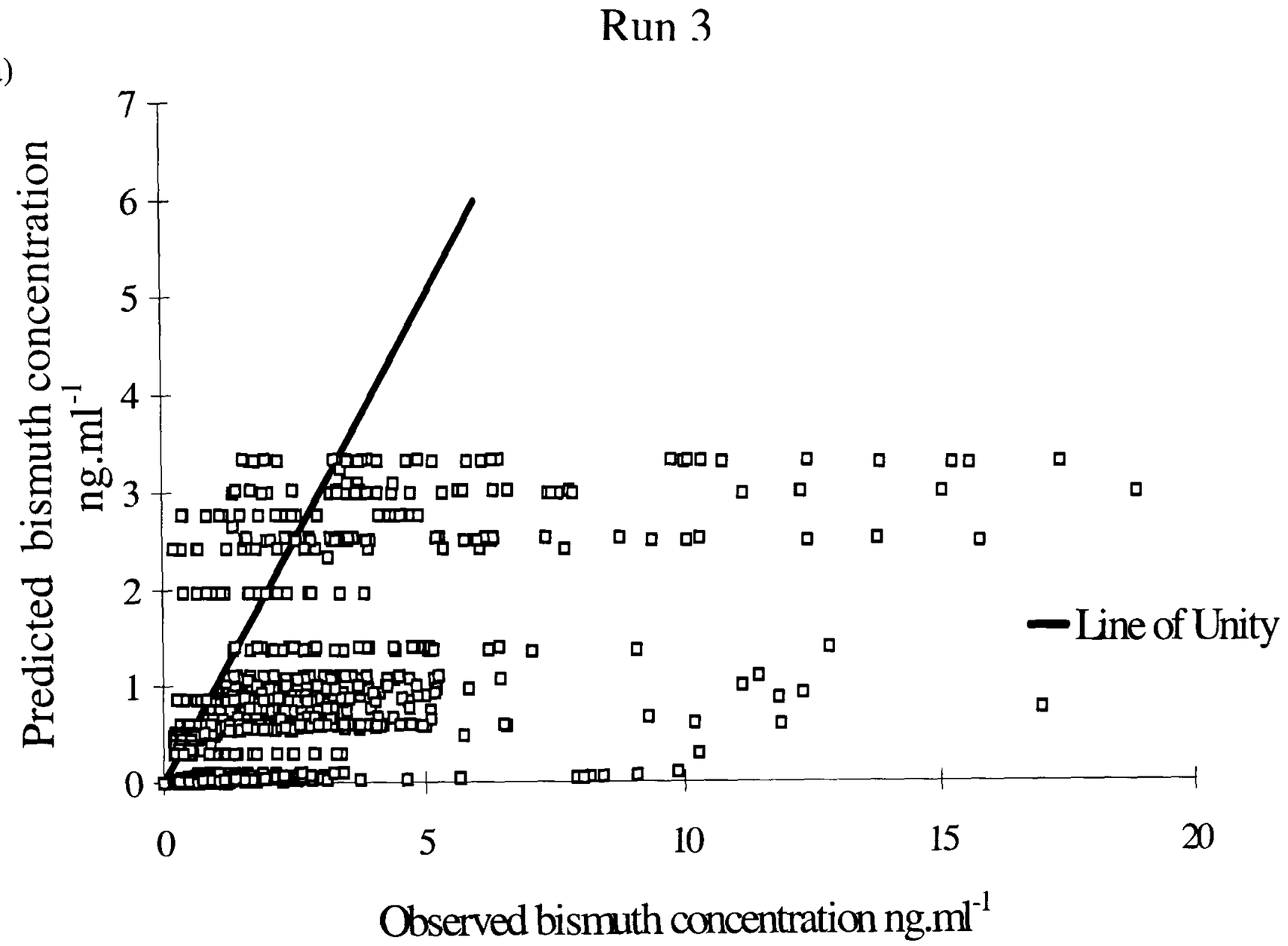
* Indicates where the data was insufficient for parameter estimation

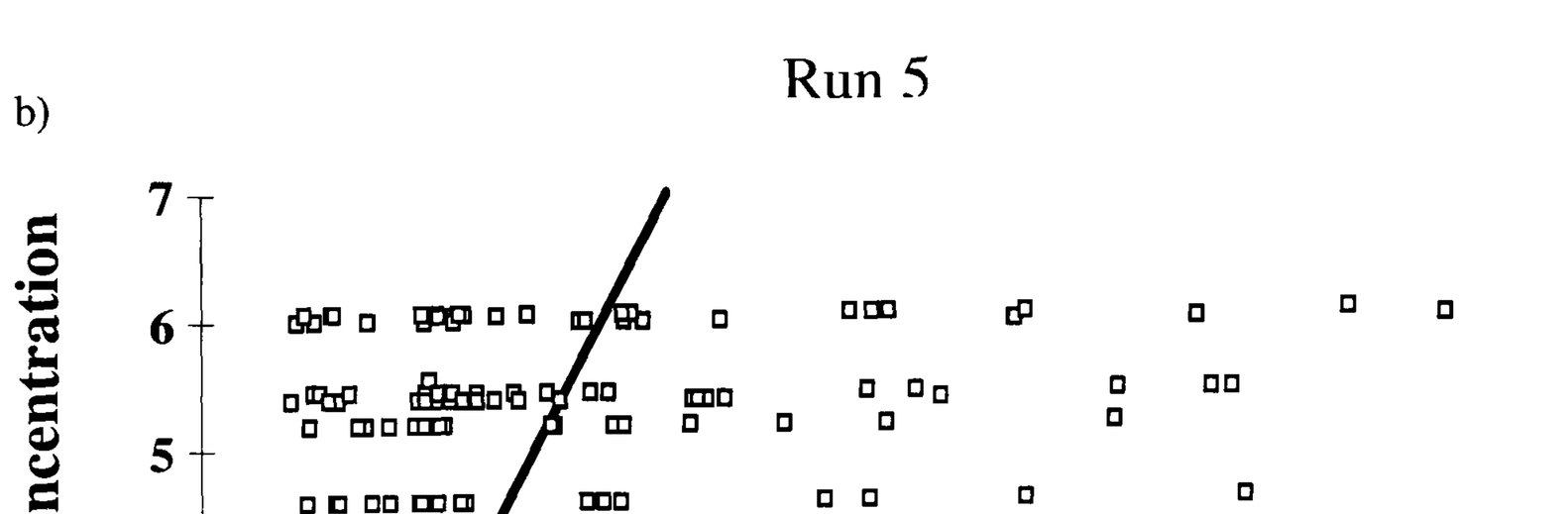
Table 4.4 Model development for the micro rate constant models

Run no	Estimation method	No compartments	Objective function	No θ Parameters	No ω parameters	No σ parameters	Successful minimisation	No significant figures	Standard errors estimated
1	FO	2	1191.3	5	5	1	yes	3.3	ne
2	FO	3	405.1	7	7	1	yes	3.1	ne
3	FO	3	313.0	7	7	2	yes	3.1	ne
4	FOCE interaction	2	830.8	5	5	1	yes	3.5	ne
5	FOCE interaction	3	188.0	7	7	2	No	-0.5	ne
6	FOCE interaction	3	35.7	7	4	2	yes	3.3	yes

ne indicates where the standard errors were not estimable

Figure 4.2 Predicted concentration versus observed concentration plots for a) Run 3 (3 compartment model using the FO estimation) and b) Run 5 (3 compartment model using the FOCE estimation)





Macro rate constant model

Model development of the macro rate constant population models is shown in Table 4.5 and the corresponding population parameter estimates are shown on Table 4.6. Run times were much shorter with this parameterisation (12 to 24 hours) and the objective functions for equivalent models were substantially lower. There was a large decrease in objective function upon fitting a 3 compartment model in comparison to a 2 compartment model (Run 8 vs Run 7). The decrease in objective function upon fitting a 4 compartment model was smaller but still statistically significant ($P<0.005$), (Run 9 vs Run 8). However, the covariance step was not successful and therefore standard errors could not be estimated. Figure 4.3 shows the weighted residuals versus time plots for the 2, 3 and 4 compartment models. While the bias in the weighted residuals indicates that there was mis-specification of the washout phase (times > 672 hours) when the two compartment model was fitted (Figure 4.3a), there was no evidence of a bias with the three and four compartment models (Figure 4.3 b and c). Plots of the individual predictions are shown on Figure 4.4. While there was a large difference between the individual predictions from the two and three compartment models, the individual predictions for the three and four compartment model were very similar i.e. only 26 of 977 individual predictions using the four compartment model were >+25% or <-25% of corresponding three compartment model individual predictions. Thirteen of these predictions were for three individuals where the individual fitting demonstrated that a four compartment model was better. Therefore, while for some individuals the concentration time profile was better characterised by a four compartment model, a three compartment model was most appropriate across the majority of the subjects. Precise population average estimates of Ka and α were obtained (Table 4.6). In general, interindividual variability estimates were large and imprecisely estimated (Table 4.6).

Table 4.5 Model development of the macro rate constant models

Run no	Estimation method	No compartments & model type	Objective function	No θ Parameters	No η parameters	No ε parameters	Successful minimisation	No significant figures	Standard errors estimated
7	FOCE interaction	2 standard	293.7	5	5	2	yes	4.2	yes
8	FOCE interaction	3 standard	15.6	7	7	2	yes	4.1	yes
9	FOCE interaction	4 standard	-1.58	9	9	2	yes	4.5	no
10	FOCE interaction	2 Wijnand	554.2	4	4	2	yes	4.4	yes
11	FOCE interaction	3 Wijnand	25.9	6	6	2	yes	4.0	yes
12	FOCE interaction	4 Wijnand	2.7	8	8	2	yes	4.7	no

Table 4.6 Parameter estimates for the macro rate constant models

Run no	A $ng.ml^{-1}$ SE %	α hr^{-1} SE %	B $ng.ml^{-1}$ SE %	β hr^{-1} SE %	C $ng.ml^{-1}$ SE %	γ hr^{-1} SE %	D $ng.ml^{-1}$ SE %	δ hr^{-1} SE %	Ka hr^{-1} SE %	ω_A SE %	ω_α SE %	ω_B SE %	ω_β SE %	ω_c SE %	ω_γ SE %	ω_D SE %	ω_δ SE %	ω_{ka} SE %	[1]σ_{Add} SE %	σ_{Exp} SE %
7	2.8	0.30	0.088	0.0028					8.2	80%	83%	78%	74%					59%	0.2	33%
	20%	25%	22%	21%					14%	42%	55%	47%	80%					47%		18%
8	7.0	1.20	0.57	0.046	0.044	0.0014			4.4	40%	41%	61%	40%	69%	44%			39%	0.2	29%
	22%	12%	18%	15%	19%	16%			19%	44%	70%	63%	41%	49%	141%			85%		21%
9	5.3	0.96	0.54	0.036	0.011	0.0011	0.001	0.00023	5.8	45%	51%	27%	25%	106%	17%	340%		75%	0.2	29%

Run no	A $ng.ml^{-1}.hr^{-1}$ SE %	k hr^{-1} SE %	B $ng.ml^{-1}$ SE %	β hr^{-1} SE %	C $ng.ml^{-1}$ SE %	γ hr^{-1} SE %	D $ng.ml^{-1}$ SE %	δ hr^{-1} SE %	Ka hr^{-1} SE %	ω_A SE %	ω_α SE %	ω_B SE %	ω_β SE %	ω_c SE %	ω_γ SE %	ω_D SE %	ω_δ SE %	ω_{ka} SE %	σ_{Add} SE %	σ_{Exp} SE %
10	10	1.3	0.13	0.0034						67%	19%	57%	56%						0.2	36%
	19%	10%	15%	14%						63%	65%	30%	35%							21%
11	18	2.3	0.64	0.050	0.045	0.0014				64%	30%	59%	38%	69%	44%				0.2	29%
	17%	9%	18%	16%	18%	16%				63%	40%	57%	40%	45%	153%					20%
12	18	2.4	0.41	0.21	0.37	0.033	0.042	0.0013		62%	29%	147%	ne	27%	23%	71%	41%		0.2	29%

1) σ_{Add} was fixed to 0.2 $ng.ml^{-1}$ the LOQ (i.e. the variance σ^2_{Add} was fixed to 0.04)

Figure 4.3 Population weighted residuals versus time plots for a) Run 7 (2 compartment model using the FOCE estimation), b) Run 8 (3 compartment model using the FOCE estimation) and b) Run 9 (4 compartment model using the FOCE estimation).

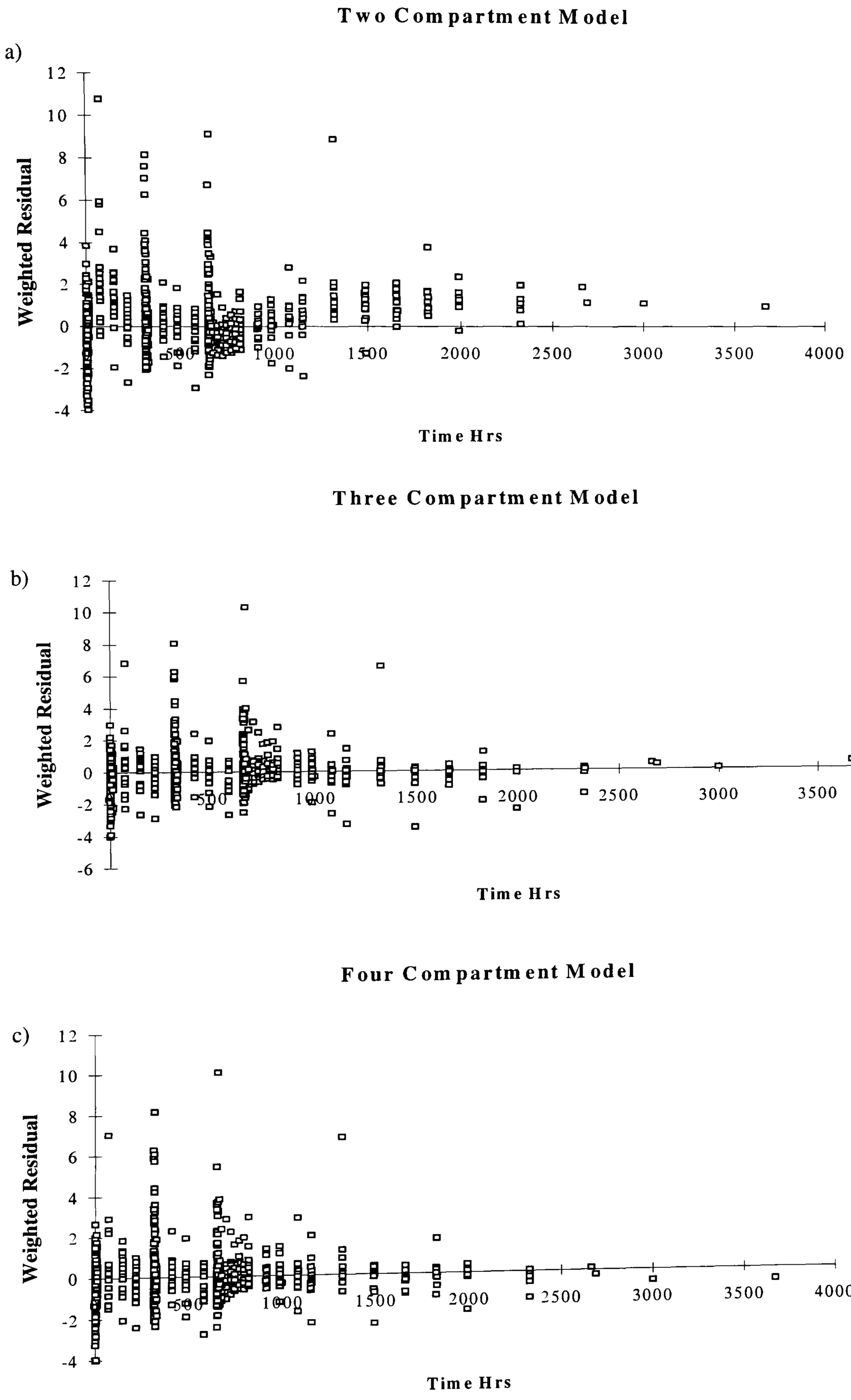
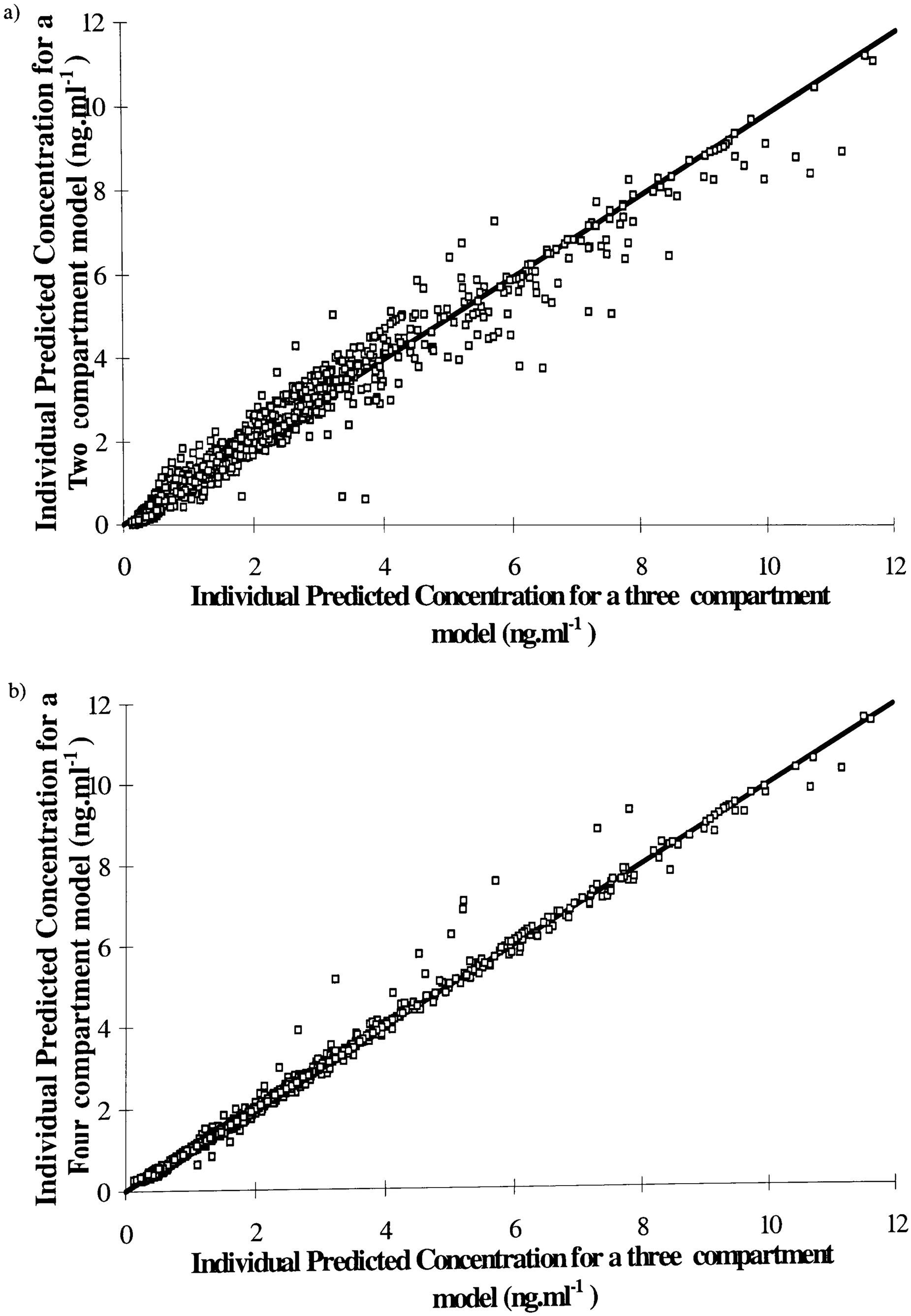


Figure 4.4 Comparison of individual predictions from the population macro rate constant models: a) Two (Run7) versus three compartment model (Run8) and b) Four (Run9) versus three compartment model (Run8)



Individual patient parameters for the standard macro rate constant three compartment model are shown in Table 4.7. A selection of individual patient fits using the standard macro rate constant model is shown in Figure 4.5.

The model development and parameter estimates using the Wijnand macro rate constant models are also shown in Tables 4.5 and 4.6, respectively. On comparing equivalent models, the objective functions were larger than for the standard macro rate constant models. For the two compartment (Run 2 vs Run 10) and three compartment (Run 8 vs Run11) models the differences in objective function were statistically significant ($P<0.001$ and $P< 0.01$, respectively). A comparison of the individual predictions for the three compartment models is shown in Figure 4.6. Only 4 of the 977 Wijnand model predictions were >+25% or <-25% of the standard model predictions.

For the three compartment model, the estimates of B, β,C, γ estimates were similar for the standard and Wijnand models (Run 8 and Run 11, Table 4.6). The combined absorption/distribution rate constant, k, was estimated to be 2.3 hr^{-1} (Run 11), and therefore, in between the values of 1.2 and 4.4hr^{-1}, estimated for α and Ka, respectively. The estimate of the exponential component of intraindividual variability was the same for Runs 8 and 11.

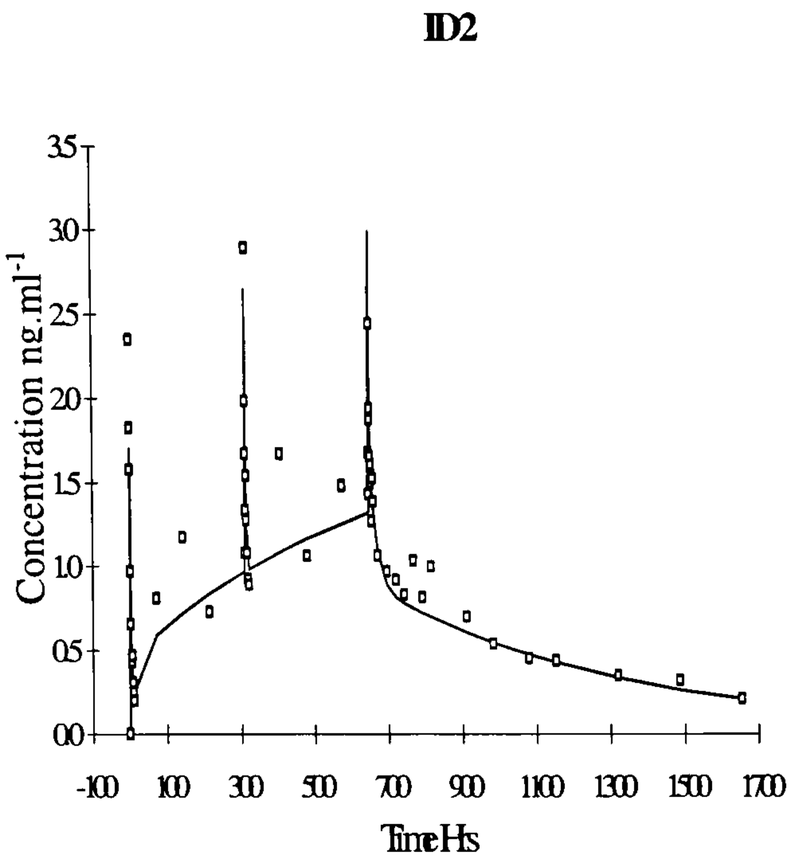
The individual patient parameters for the Wijnand macro rate constant three compartment model are also shown in Table 4.7. The individual estimates of B, β,C, and γ estimates were similar for the standard and Wijnand macro rate constant models. Therefore, while using the Wijnand macro rate constant model increased the objective functions, it did not affect the estimate of the intermediate and terminal rate constants. The magnitude of k in comparison to α and Ka, was consistent with that shown with population parameters. Since separate estimates of α and Ka were discernible there was no requirement to further utilise the Wijnand macro rate constant model.

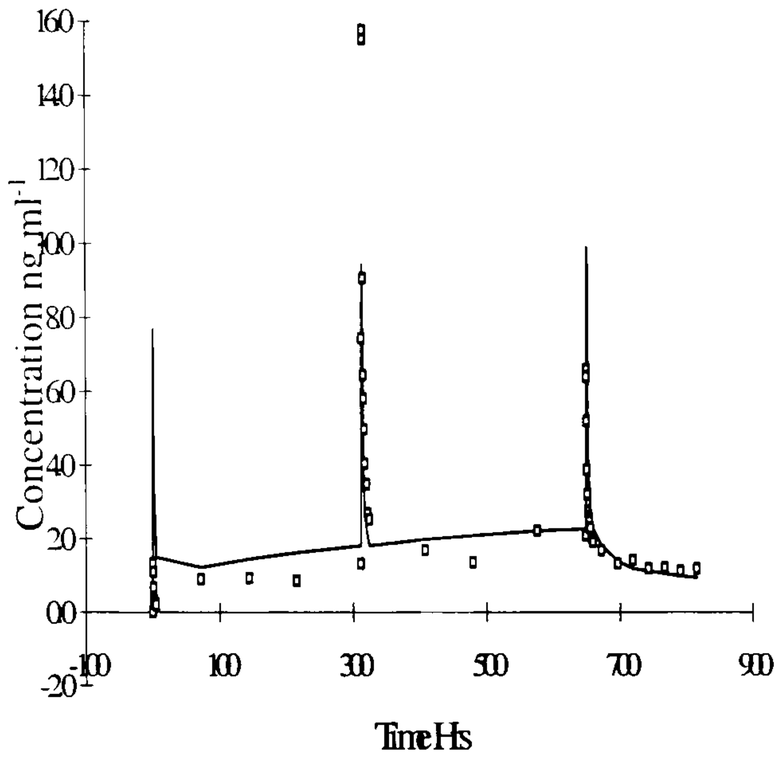
Table 4.7 Individual parameter estimates and calculated half-lifes for the macro rate constant models

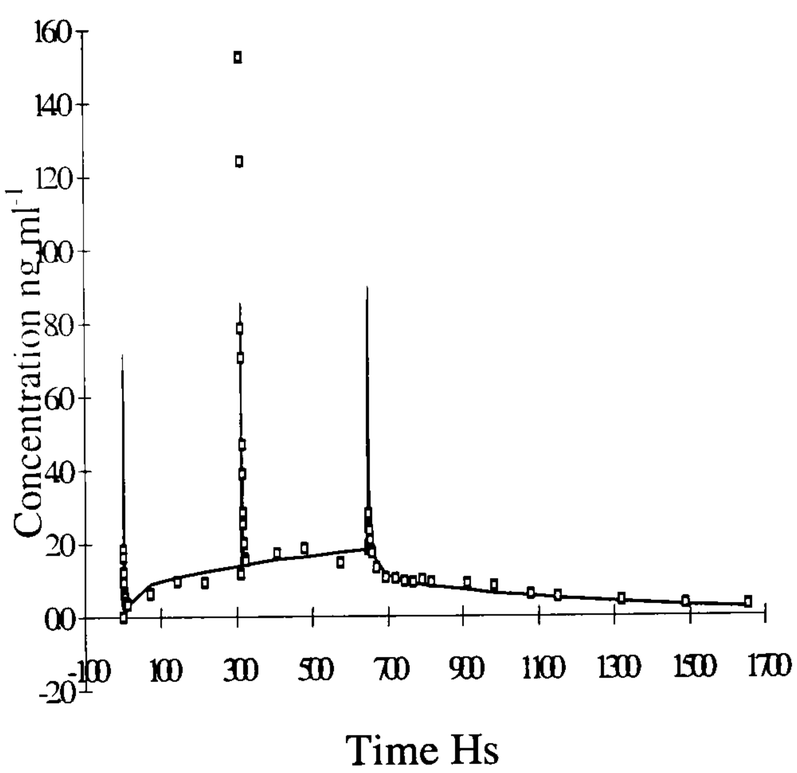
Standard (Run8)											
ID	A	α	B	β	C	γ	Ka	$T_{1/2}$ α	$T_{1/2}$ β	$T_{1/2}$ γ	$T_{1/2}$ Ka
	$ng.ml^{-1}$	hr^{-1}	$ng.ml^{-1}$	hr^{-1}	$ng.ml^{-1}$	hr^{-1}	hr^{-1}	mins	hrs	days	mins
2	5.6	1.2	0.45	0.057	0.025	0.0014	2.4	35	12	20	17
3	11	1.0	0.76	0.045	0.024	0.0015	4.6	40	15	19	9.1
4	7.9	1.1	0.43	0.035	0.082	0.0011	3.8	36	20	27	11
5	6.8	1.5	0.63	0.032	0.046	0.0010	4.7	28	22	29	8.8
7	12	0.52	0.75	0.046	0.040	0.0018	3.5	80	15	16	12
8	11	1.7	3.4	0.097	0.034	0.0013	7.5	25	7	22	5.6
10	6.6	2.4	0.33	0.041	0.021	0.0013	2.6	17	17	22	16
11	13	0.76	0.51	0.043	0.031	0.0015	3.9	55	16	19	11
13	5.9	1.6	0.47	0.056	0.053	0.0014	4.3	26	12	21	9.7
14	6.9	1.1	0.75	0.046	0.040	0.0011	5.3	36	15	26	7.9
15	6.8	1.2	0.84	0.072	0.029	0.0012	5.2	34	10	24	8.0
17	6.7	1.4	0.78	0.065	0.016	0.00046	3.7	29	11	63	11
19	5.7	1.6	0.36	0.040	0.030	0.0020	3.6	27	18	14	11
21	7.3	1.7	0.38	0.037	0.071	0.0013	6.0	24	19	22	6.9
23	6.9	1.0	0.66	0.034	0.056	0.0013	3.6	42	20	22	12
24	6.4	1.6	0.26	0.058	0.29	0.0019	4.4	26	12	15	9.5
25	8.3	1.8	0.46	0.028	0.078	0.0015	5.8	23	24	20	7.1
27	5.8	1.1	0.59	0.045	0.083	0.0023	2.9	37	15	12	14
Population (NONMEM)	**7.0**	**1.2**	**0.57**	**0.046**	**0.044**	**0.0014**	**4.4**	**35**	**15**	**21**	**9.6**

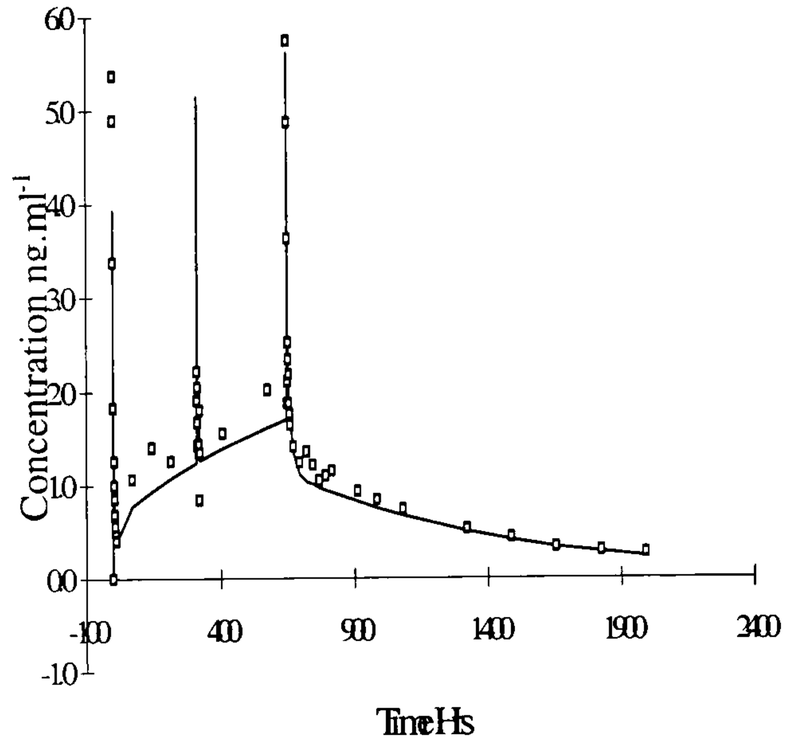
Wijnand (Run11)									
ID	A	k	B	β	C	γ	$T_{1/2}$ k	$T_{1/2}$ β	$T_{1/2}$ γ
	$ng.ml^{-1}.hr^{-1}$	hr^{-1}	$ng.ml^{-1}$	hr^{-1}	$ng.ml^{-1}$	hr^{-1}	mins	hrs	days
2	6.9	1.7	0.48	0.061	0.025	0.0015	24	11	20
3	31	2.0	0.85	0.048	0.025	0.0015	20	14	19
4	31	2.0	0.48	0.039	0.083	0.0011	21	18	27
5	31	2.5	0.66	0.033	0.046	0.0010	16	21	29
7	31	1.3	1.0	0.049	0.042	0.0019	32	14	15
8	31	3.2	3.4	0.096	0.034	0.0013	13	7.2	22
10	4.0	3.3	0.32	0.042	0.022	0.0013	12	16	22
11	29	1.5	0.63	0.048	0.032	0.0016	28	14	18
13	15	2.6	0.51	0.060	0.054	0.0014	16	11	21
14	24	2.4	0.88	0.053	0.040	0.0011	18	13	25
15	23	2.4	0.97	0.080	0.029	0.0012	17	8.6	24
17	15	2.3	0.81	0.067	0.016	0.00045	18	10	64
19	11	2.4	0.39	0.043	0.031	0.0021	17	16	14
21	26	3.0	0.42	0.041	0.073	0.0013	14	17	21
23	16	1.8	0.73	0.038	0.058	0.0013	23	18	22
24	17	2.6	0.29	0.064	0.29	0.0019	16	11	15
25	29	3.1	0.48	0.030	0.079	0.0015	14	23	20
27	10	1.8	0.65	0.050	0.085	0.0023	23	14	12
Population (NONMEM)	**18**	**2.3**	**0.64**	**0.050**	**0.045**	**0.0014**	**18**	**14**	**21**

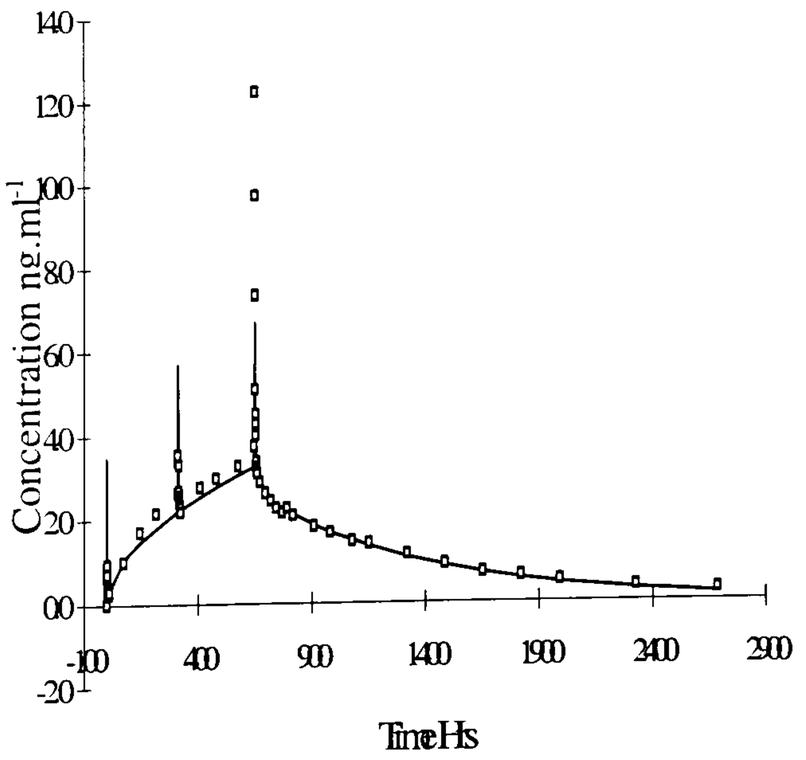
Figure 4.5 Individual predictions from the standard population macro rate constant model (Run 8) versus time











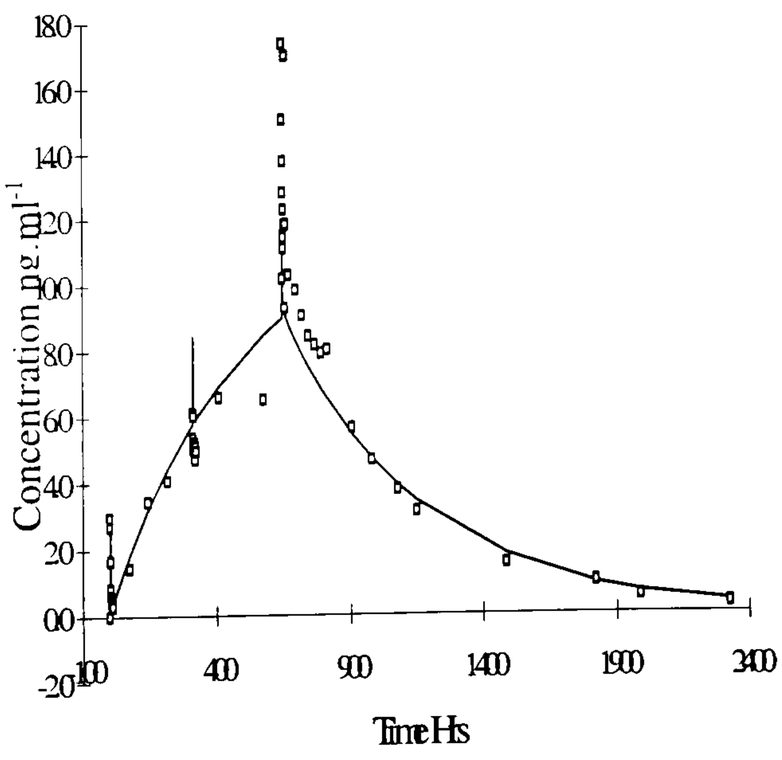
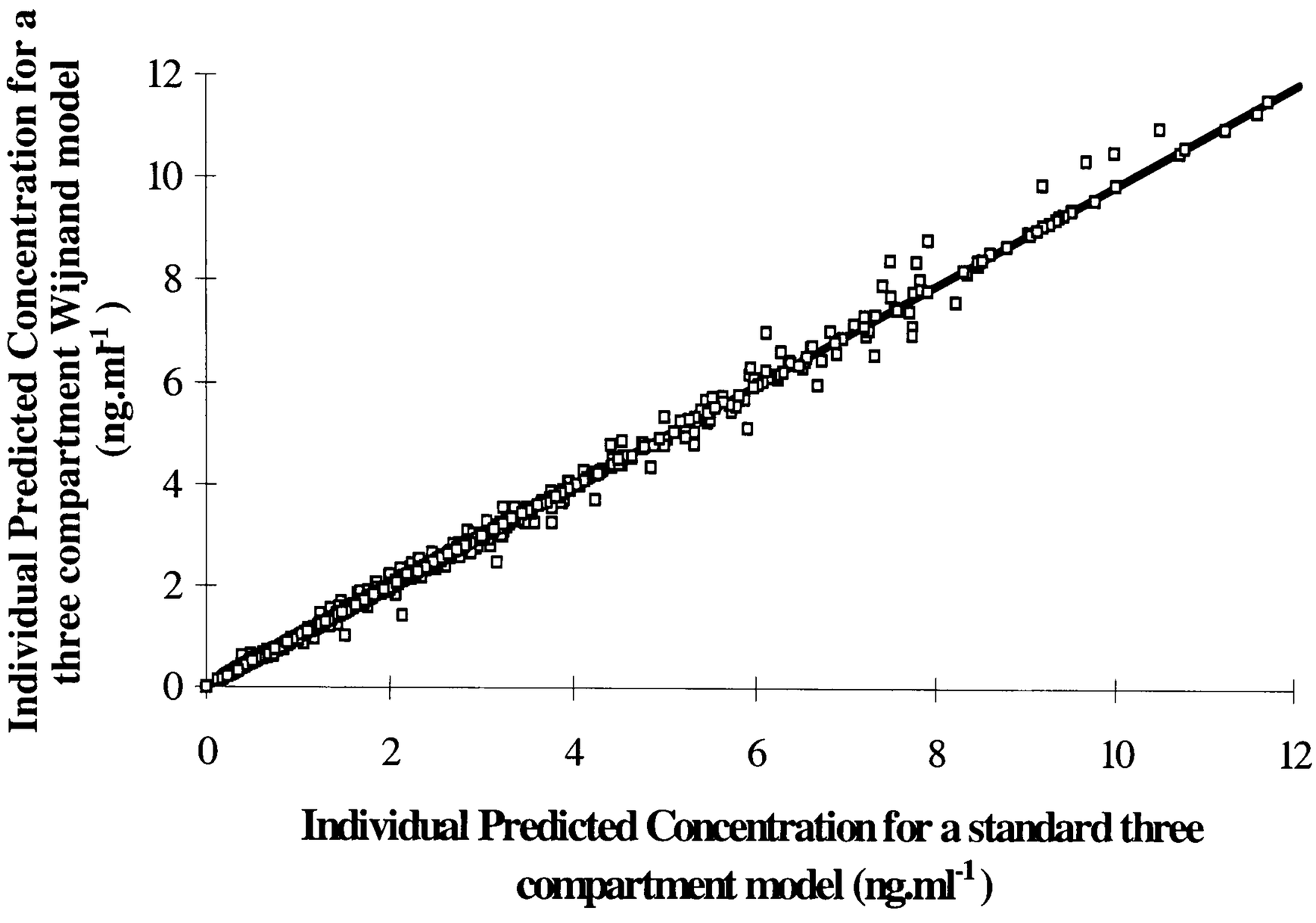


Figure 4.6 Comparison of individual predictions from the population macro rate constant models: Three compartment Wijnand model versus standard three compartment model



Model dependent estimates of AUC_{0-12}, Cmax and Cmin and comparison with the noncompartmental estimates of accumulation

The predicted estimates of AUC_{0-12}, Cmax and Cmin for days 1, 14 and 28 and at steady state, using the population and individual estimates from Run 8 are shown in Table 4.8. The ratios of day 14 to day 1 estimates, and day 28 to day 14 estimates and SS to day 28 estimates are shown in Table 4.9. Figure 4.7 shows a comparison of the geometric mean estimates of AUC_{0-12}, Cmax and Cmin on days 1, 14 and 28 from the noncompartmental analysis and the corresponding population typical estimates from the population analysis . The ratios of the AUC_{0-12} indicate that the model based estimate of accumulation was smaller from day 1 to day 14 (2.94 Table 4.9 vs 3.61 Table 4.3) and greater from day 14 to day 28 (1.28 Table 4.9 vs 1.16 Table 4.3). However, the resultant estimates of AUC_{0-12} on day 28 were very similar (36.1 $ng.ml^{-1}$.hr Table 4.8 vs 36.4$ng.ml^{-1}$.hr Table 4.2).

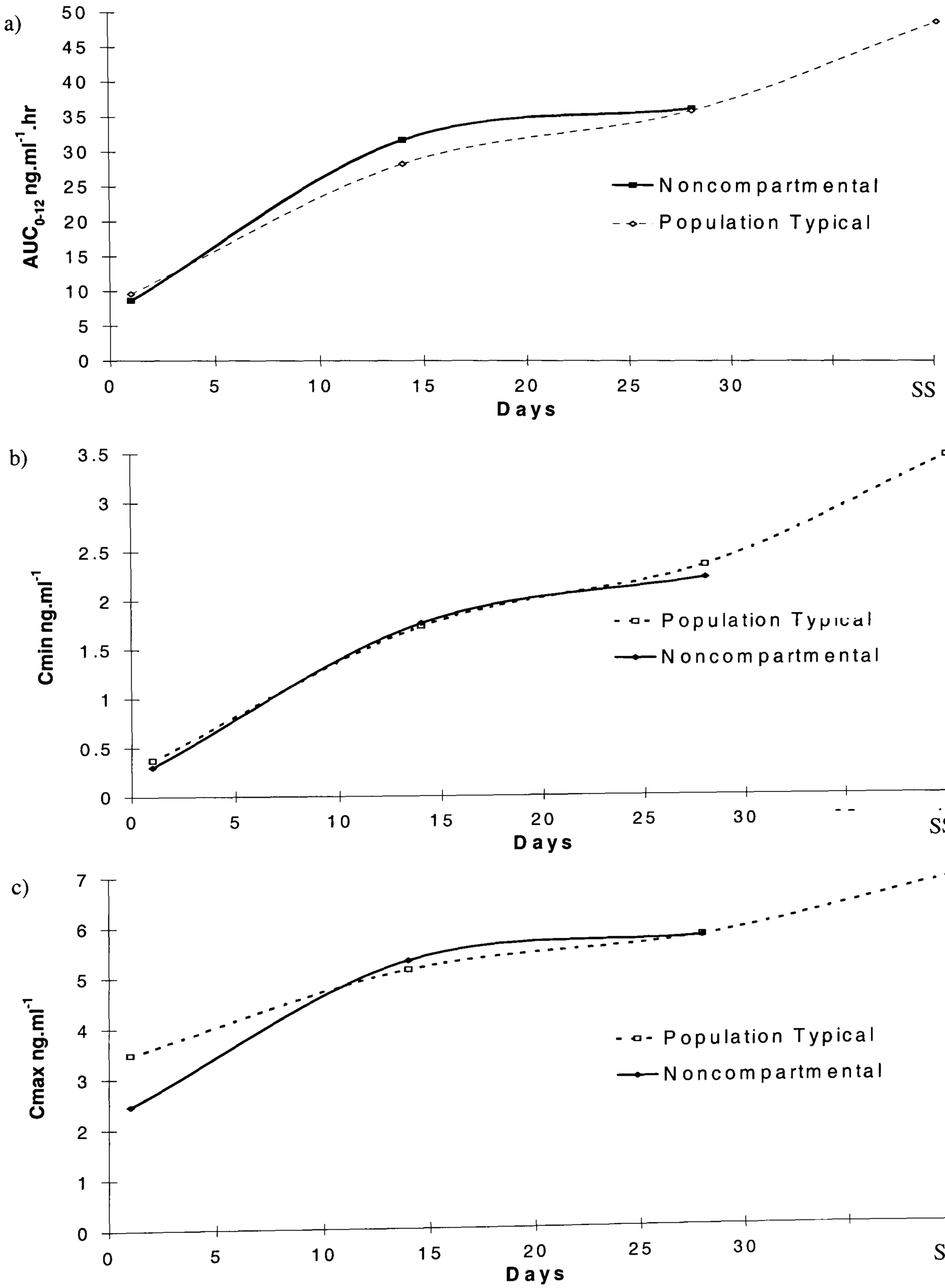
Tables 4.8 Model predicted estimates of AUC_{0-12}, Cmax and Cmin for days 1, 14 and 28, and at steady state based on the individual parameter estimates from Run 8

Day	1	14	28	SS	1	14	28	SS	1	14	28	SS
	AUC_{0-12}	AUC_{0-12}	AUC_{0-12}	AUC0-12	Cmin	Cmin	Cmin	Cmin	Cmax	Cmax	Cmax	Cmax
	$ng.ml.hr^{-1}$	$ng.ml.hr^{-1}$	$ng.ml.hr^{-1}$	$ng.ml.hr^{-1}$	$ng.ml^{-1}$	$ng.ml^{-1}$	$ng.ml^{-1}$	$ng.ml^{-1}$	$ng.ml^{-1}$	$ng.ml^{-1}$	$ng.ml^{-1}$	$ng.ml^{-1}$
ID												
2	6.31	16.4	20.4	27.2	0.25	0.99	1.33	1.88	1.70	2.65	3.00	3.56
3	15.1	31.1	34.9	40.9	0.47	1.59	1.91	2.40	5.92	7.47	7.80	8.30
4	10.0	39.5	55.3	93.6	0.37	2.68	4.04	7.17	3.75	6.36	7.73	10.9
5	9.80	35.5	44.7	69.2	0.48	2.41	3.20	5.20	3.20	5.55	6.35	8.37
7	26.4	45.2	50.7	57.7	0.49	1.85	2.32	2.88	5.99	9.48	9.96	10.5
8	29.3	48.7	54.5	65.3	1.11	2.28	2.77	3.66	8.45	10.7	11.2	12.1
10	3.52	13.8	17.5	24.7	0.22	0.98	1.30	1.88	0.48	1.44	1.76	2.35
11	18.2	33.0	37.8	45.3	0.34	1.43	1.83	2.44	7.18	8.57	8.99	9.60
13	6.90	24.4	33.2	48.6	0.29	1.63	2.38	3.64	2.41	3.99	4.76	6.03
14	11.9	31.6	39.1	56.1	0.47	1.91	2.55	3.93	4.11	5.97	6.61	8.01
15	11.2	23.6	28.9	40.4	0.38	1.26	1.72	2.66	3.94	5.16	5.62	6.57
17	9.33	19.3	23.4	48.8	0.38	1.05	1.40	3.51	2.87	3.88	4.23	6.36
19	5.73	18.1	21.8	25.7	0.25	1.17	1.48	1.80	1.73	3.14	3.46	3.78
21	7.50	32.0	44.2	66.9	0.32	2.24	3.27	5.11	3.47	5.69	6.71	8.58
23	12.0	39.1	48.8	66.8	0.50	2.53	3.36	4.83	3.66	6.13	6.31	8.46
24	8.05	76.0	113	156	0.41	5.95	9.09	12.5	2.65	8.44	11.6	15.2
25	8.64	39.5	52.0	72.6	0.41	2.81	3.87	5.54	3.72	6.46	7.54	9.25
27	9.41	35.0	44.0	52.1	0.43	2.38	3.14	3.79	2.44	4.76	5.26	6.21
Population	**9.63**	**28.3**	**36.1**	**49.1**	**0.37**	**1.74**	**2.37**	**3.49**	**3.47**	**5.15**	**5.80**	**6.94**

Tables 4.9 Ratios of the model predicted estimates (Run 8) of AUC_{0-12}, Cmax and Cmin for days 1, 14 and 28, and at steady state

Ratio	AUC_{0-12}	AUC_{0-12}	AUC_{0-12}	Cmin	Cmin	Cmin	Cmax	Cmax	Cmax
Day : Day	14:1	28:14	SS:28	14:1	28:14	SS:28	14:1	28:14	SS:28
ID									
2	2.59	1.25	1.33	3.95	1.35	1.42	1.56	1.13	1.19
3	2.05	1.12	1.17	3.38	1.20	1.26	1.26	1.04	1.06
4	3.97	1.40	1.69	7.33	1.50	1.78	1.70	1.22	1.41
5	3.62	1.26	1.55	5.07	1.33	1.63	1.74	1.14	1.32
7	1.71	1.12	1.14	3.77	1.25	1.24	1.58	1.05	1.06
8	1.66	1.12	1.20	2.06	1.22	1.32	1.26	1.05	1.08
10	3.91	1.27	1.41	4.39	1.32	1.45	2.99	1.22	1.34
11	1.81	1.15	1.20	4.19	1.28	1.33	1.19	1.05	1.07
13	3.53	1.36	1.46	5.62	1.46	1.53	1.66	1.19	1.27
14	2.65	1.24	1.43	4.05	1.33	1.54	1.45	1.11	1.21
15	2.11	1.23	1.40	3.30	1.36	1.55	1.31	1.09	1.17
17	2.07	1.21	2.09	2.81	1.33	2.51	1.35	1.09	1.50
19	3.15	1.20	1.18	4.68	1.26	1.21	1.82	1.10	1.09
21	4.27	1.38	1.51	7.06	1.46	1.56	1.64	1.18	1.28
23	3.07	1.24	1.36	5.12	1.32	1.44	1.68	1.03	1.34
24	9.45	1.49	1.38	14.61	1.53	1.38	3.18	1.38	1.30
25	4.57	1.32	1.40	6.88	1.38	1.43	1.74	1.17	1.23
27	3.72	1.26	1.18	5.59	1.32	1.21	1.95	1.10	1.18
Population	**2.94**	**1.28**	**1.36**	**4.65**	**1.37**	**1.47**	**1.49**	**1.13**	**1.20**

Figure 4.7 Comparison of population model dependent estimates (Run 8) of a) AUC_{0-12}, b) Cmax and c) Cmin with the equivalent geometric mean estimates from the noncompartmental analysis on days 1, 14 and 28, and at steady state (SS)



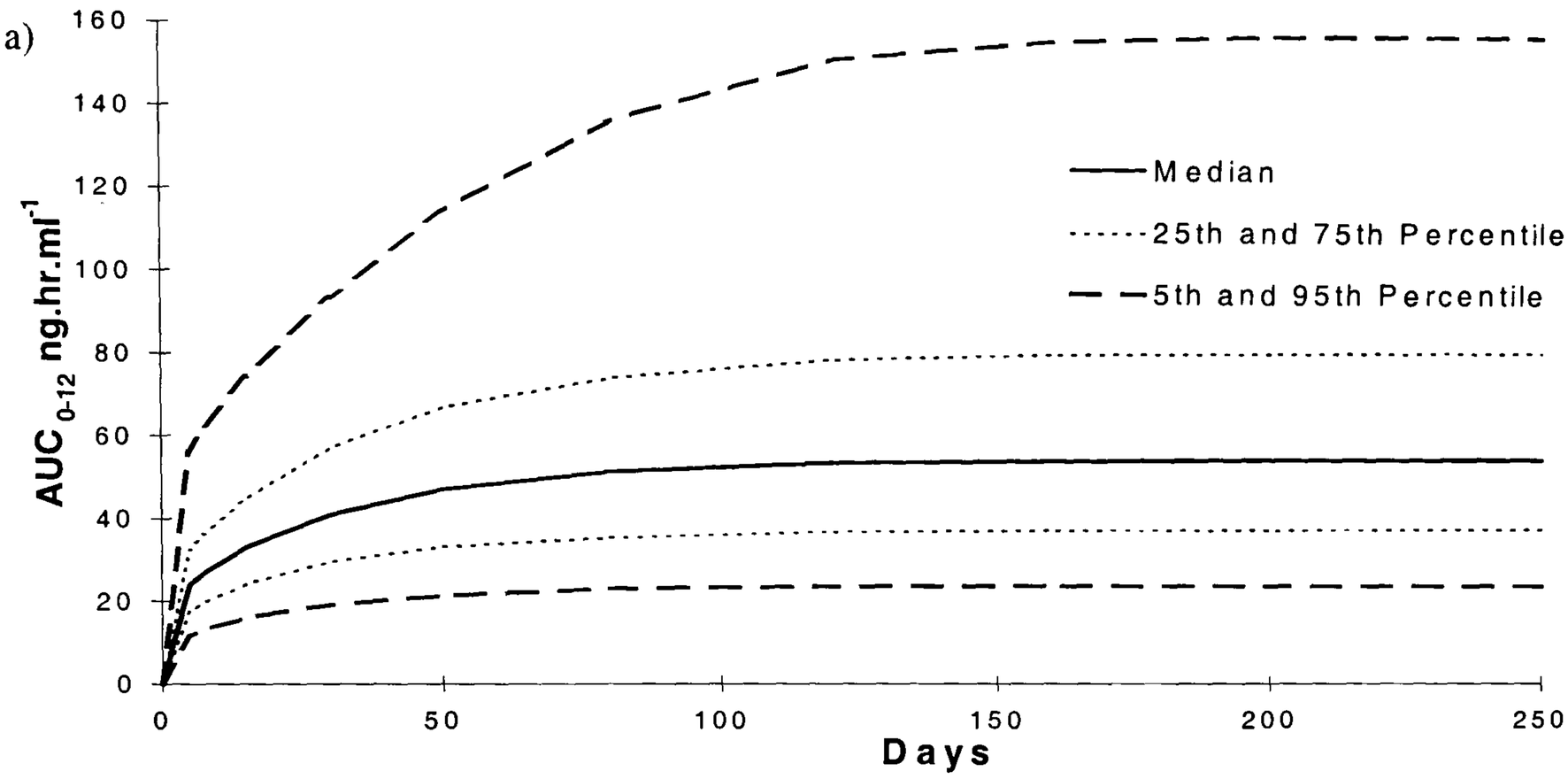
The model dependent estimates of Cmin on days 1, 14 and 28 (0.37, 1.74 and 2.37 $ng.ml^{-1}$ Table 4.8, respectively) were very similar to the observed values (0.30, 1.77 and 2.24 $ng.ml^{-1}$ Table 4.2, respectively). Although the model dependent estimate of Cmax was substantially greater on day 1 (3.47 , Table 4.8 vs 2.45 Table 4.2), the model dependent estimates of accumulation in Cmax over the first 14 days was smaller, so the actual estimates on day 14 (5.15 Table 4.8 vs 5.33 Table 4.2) and subsequently day 28 (5.80 Table 4.8 vs 5.78 Table 4.2) were similar for the noncompartmental and compartmental approaches.

It was predicted that the accumulation from day 28 to the steady state levels would on average result in a further 36%, 47% and 20% increase in AUC_{0-12}, Cmin and Cmax, respectively. The predicted estimates of AUC_{0-12}, Cmin and Cmax at steady state were 49.1 $ng.ml^{-1}.hr$, 3.49 $ng.ml^{-1}$ and 6.94 $ng.ml^{-1}$, respectively (Table 4.8).

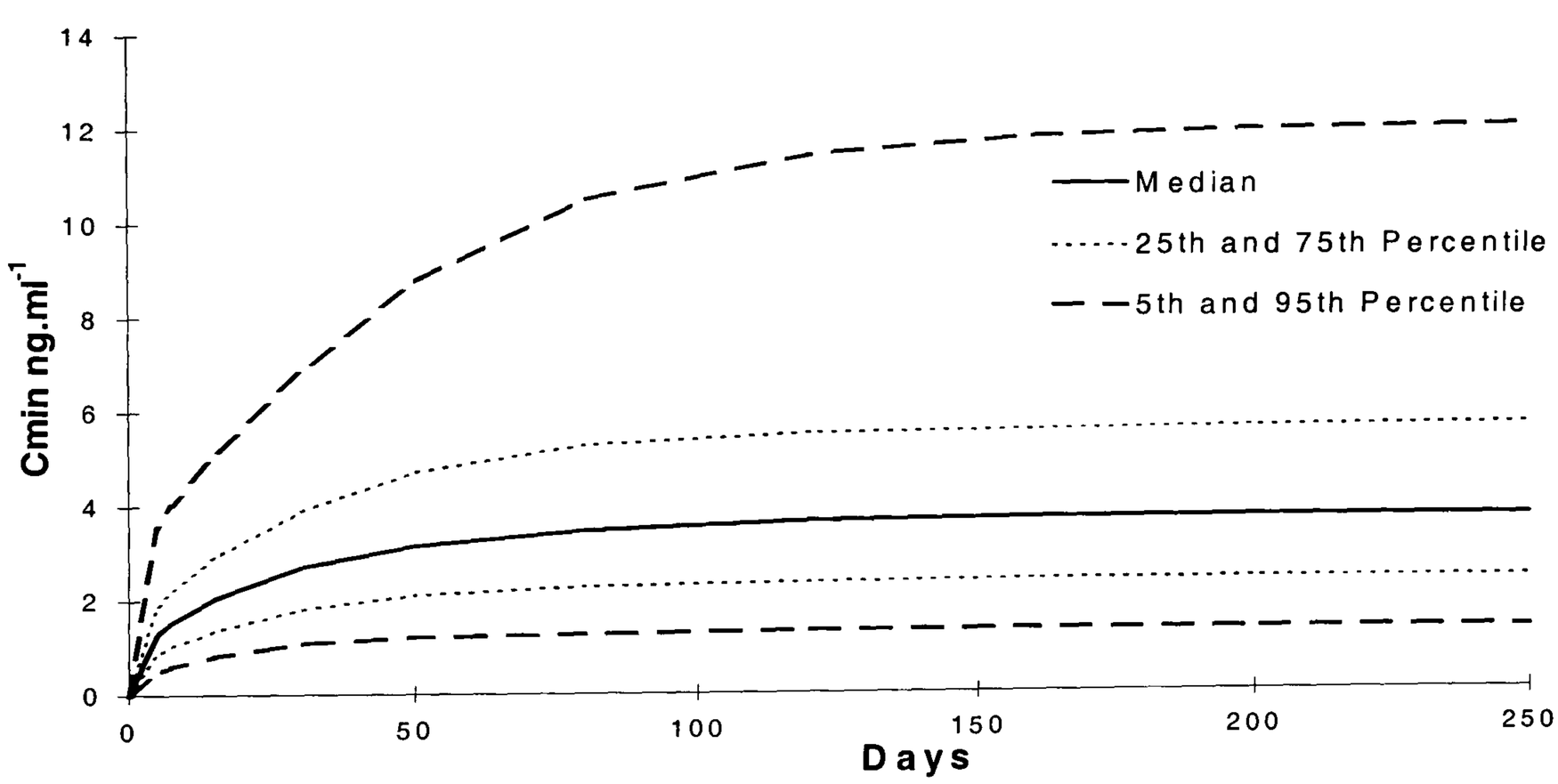
The prediction intervals (median and 25-75th and 5-95th percentiles) for AUC, Cmin and Cmax from day 1 up to steady state are shown in Figure 4.8. At steady state the upper limit for Cmax was predicted to be well below the previously proposed "safety" limits (50 $ng.ml^{-1}$, section 4.1.3).

Figure 4.8 Predicted estimates of a) AUC_{0-12}, b) Cmax and c) Cmin following a 255 day treatment course of RBS.

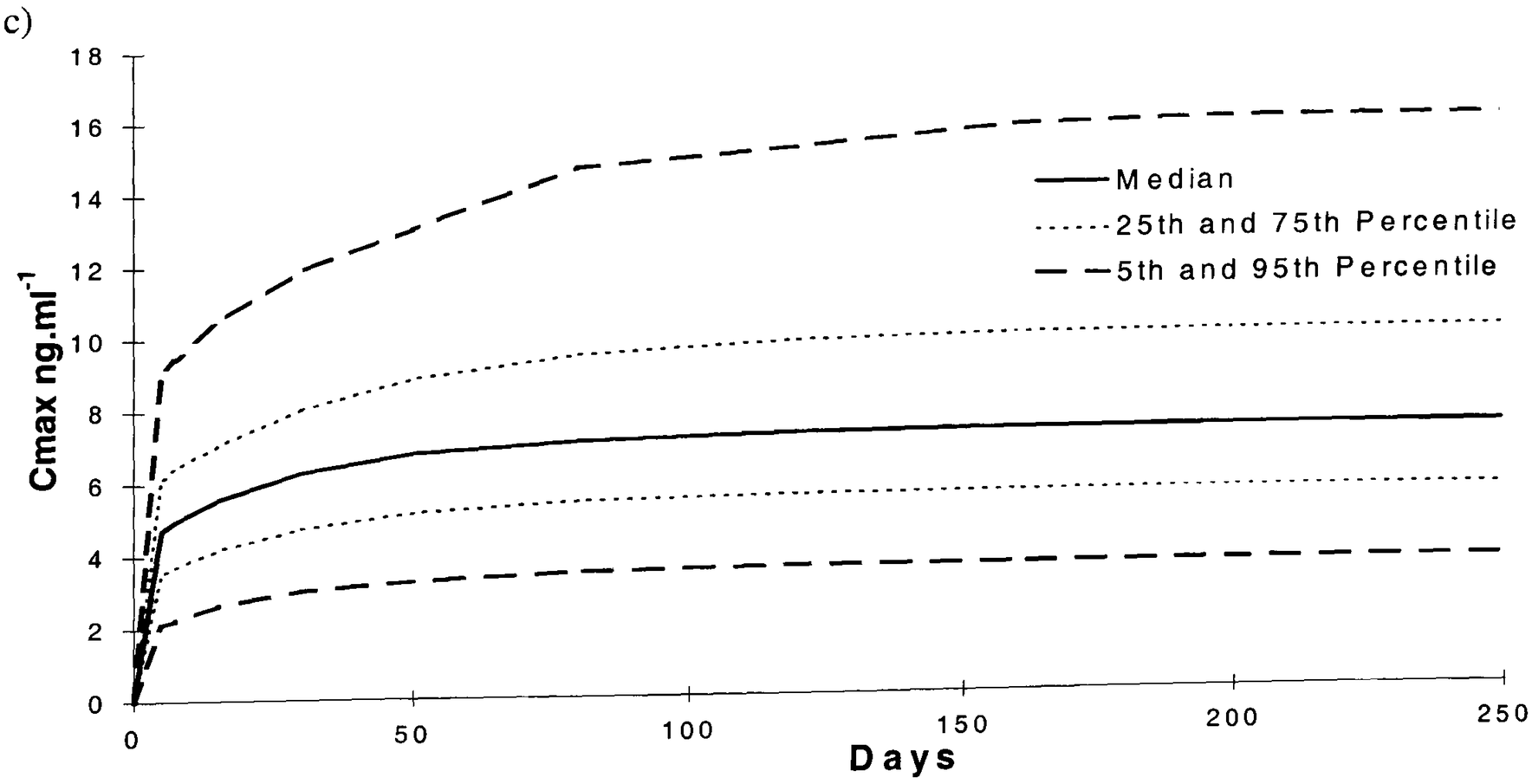
a)



b)



c)



4.6 Discussion

4.6.1 Safety assessment of RBS treatment

As previously highlighted (4.1.3), most incidences of toxicity have been due to treatment periods which extended beyond those currently used in routine management of relapsed gastric or duodenal ulcer. It is therefore unlikely that episodes of CNS toxicity would occur with any bismuth formulation given in accordance with current clinical practice. Nevertheless, the drug regulatory agencies require that the safety of an individual drug product be demonstrated. Furthermore, since alteration of intra-gastric acidity has been shown to increase the absorption of bismuth from RBS (Nwokolo et al., 1991), plasma levels could theoretically increase upon co-administration with ranitidine. The noncompartmental geometric mean estimates of AUC_{0-12}, Cmax and Cmin on day 14 (31.7 $ng.ml.hr^{-1}$, 5.3 $ng.ml^{-1}$ and 1.8 $ng.ml^{-1}$, respectively) were comparable with levels previously reported on day 10 of a 500 mg twice daily RBS study (34$ng.ml.hr^{-1}$, 5 $ng.ml^{-1}$ and 2 $ng.ml^{-1}$, respectively) (Lacey et al., 1994). However , since the equivalent elemental bismuth doses were 240.8 mg and 150.5 mg, respectively, it would be expected that in the present study the average AUC_{0-12}, Cmax and Cmin would be significantly higher. The differences may be an artefact of the small sample sizes in each study and the large interindividual variability in general (Table 4.2). The three 12 hour profiles allowed the estimation of bismuth accumulation via noncompartmental techniques (Table 4.3). Since accumulation ratios were a lot smaller over the second two weeks of dosing it would be expected that further accumulation upon dosing to steady state would be limited. In fact, it was predicted that the accumulation from day 28 to the steady state levels would on average result in a further increase in AUC_{0-12}, Cmin and Cmax of 36%, 47% and 20%, respectively.

All plasma concentrations measured over the 28 day dosing period were lower than 19$ng.ml^{-1}$, a value which is approximately one quarter of the proposed 77.5 $ng.ml^{-1}$

safety limit. Similarly, the 5th to 95th percentile range for the predictions of Cmax at steady state were shown to be much lower than this limit. Furthermore, there has been no clinical evidence that the transitory nature of the Cmax values relate to toxicity, and the limits as previously discussed have themselves been considered to be overcautious (Benet, 1991).

4.6.2 Model dependent methods

Due to the necessity for at least a three compartment model to describe the typical healthy volunteers pharmacokinetics, ADVAN 5 (for general linear models) from the PREDPP library was utilised. However, the complexity of the models, the use of ADVAN 5 and the requirement for FOCE increased computation time considerably (3-7 days per run). In addition, neither successful minimisation or standard error estimation could be obtained using the full off-diagonal interindividual error structure. In comparison, the coefficient and macro rate constant parameterisation, implemented using a PRED subroutine, was found to decrease computation time and provide successful termination. Avoiding ADVAN 5 was probably the main reason for the improvement in run-times.

As previously stated, the combined exponential and additive models allowed the variance to be calculated as separate homogenous and heterogenous components (Chapter 3). Often, both the heterogenous and homogenous intraindividual variability are estimated. In this analysis, the additive variance was fixed to 0.04 $(LOQ)^2$ due to the protracted nature of the terminal phase at or around the LOQ and the inability to obtain a precise estimate.

The population approach determined that a three compartment model would best describe the pharmacokinetics of bismuth. This confirms the results of the individual fitting and is consistent with a Bayesian analysis of this data which has recently been

reported (Bennett et al., 1997). The population typical parameter estimates for both population methods i.e. A, B, C, α, β, γ and Ka were (Bayesian / NONMEM, Run 8 Table 4.6) 4.5/ 7.0 $ng.ml^{-1}$, 0.59 / 0.57 $ng.ml^{-1}$, 0.051 / 0.044 $ng.ml^{-1}$, 0.87 / 1.20 hr^{-1}, 0.035 / 0.046hr^{-1} and 0.0013 / 0.0014 hr^{-1} and 4.76 / 4.4 hr^{-1}, respectively. The small differences may be due to the random effects being assumed to be normal and log normal for this analysis and the Bayesian analysis, respectively. The estimated half-lives from this analysis are similar to those from the Bayesian analysis i.e. (Bayesian / NONMEM) $T_{1/2}\alpha$, $T_{1/2}$ β $T_{1/2}$ γ and $T_{1/2}$ Ka were 48 / 35 mins, 20 / 15 hours and 22.2/21 days, respectively. However, they do not coincide with the range in the half-lives previously reported by Benet (1991) i.e. 1 to 4 hours, 5 to 11 days and 21 to 72 days. Nevertheless, the washout curve available with this dataset should allow the distribution and elimination phases to be appropriately characterise.

As previously discussed , the inflated standard errors when the data was individually modelled using a two, three and four compartment model with PC-NONLIN was perceived to be due to an equality between absorption and initial distribution parameter estimates α (Koch et al., 1996). Previous investigations of this phenomena have focused on the problems of estimating an equality between Ka and Ke parameters of a one compartment model with first order absorption. Application of regular residual methods (Gibaldi & Perrier, 1982b) , curve stripping programmes i.e. (R-Strip & Minsq., 1987) and the Wagner Nelson method have been shown to give inaccurate parameter estimates when Ka <3 Ke or 3 Ka <Ke (Chan & Miller, 1982; Gibaldi & Perrier, 1982a; Garrett, 1993). The failure of these methods is due to overlap with the absorption phase preventing the determination of a "true" terminal elimination phase. In comparison, nonlinear regression has previously been shown to provide accurate estimates of Ka and Ke when there is at least a small difference between the values (Chan & Miller, 1982; Patel, 1984). However, the closer the parameter estimates the

greater the potential for computational problems. Depending on the parameter estimation algorithm utilised, both slow and failed convergence can occur, and standard errors can become greatly inflated when the correlation between Ke and Ka approaches 1 (Purves, 1993). In the cases where parameters can be considered to be identical, the models derived by Dost (1968) and Wijnand (1988) can be applied. Several noncompartmental techniques have been proposed to aid in the identification of whether this assumption can be made, however, all are limited to the one compartment single dose case (Bialer, 1980; Macheras, 1985; Garrett, 1993). In comparison, the application of nonlinear regression allows both identification of an equality in the rate constants and subsequent estimation of the combined parameter regardless of the underlying data or compartmental model. While there was evidence in the individual fitting approach that the large standard errors on the parameter estimates arose from a similarity between the α and Ka, the population typical rate constants were determined to be 3.7 fold different and precisely estimated (Table 4.6). The combined absorption and initial distribution rate constant k (from the Wijnand model) was equivalent to the average of the separate estimates of Ka and α (from the standard model). This result is consistent with previous comparisons of these models (Patel, 1984).

Ka and α equality has previously been induced by adding small amounts of random error to data simulated using distinct values for Ka and α (Purves, 1993). As part of this investigation, it was shown that the WLS (Weighted Least Squares) method used by Koch et al (1996) erroneously concluded equality of rate constants more often than the more complex IRLS (Iteratively Reweighted Least Squares) method. To this regard, the application of the ELS (Extended Least Squares) method with individual data requires further investigation.

There may be other reasons why the SE of α and Ka were found to be inflated upon individual fitting. The highly variable absorption which is inherent with drugs of low bioavailability (Hellriegel et al., 1996) may have prevented accurate estimation of Ka and α. Since different and precise estimates of Ka and α were also obtained using the Bayesian approach (Bennett et al., 1997), the application of population methods has been shown to be beneficial in characterising the underlying model which was otherwise difficult when using an individual fitting approach.

Despite the significant reduction in the objective function upon estimating separate α and Ka parameters, the comparison of the standard three compartment model with the corresponding Wijnand model showed that assuming equality did not greatly change the predictions (Figure 4.6). This is consistent with the previous work which showed the predictions from the two types of model could be very similar (Wijnand, 1988) and that mis-specification of the absorption phase does not affect the estimation of other population model parameters (Wade et al., 1993).

For the three compartment model in this study the initial, intermediate and terminal phases accounted for 7%, 27% and 66% of steady state AUC_{0-12}, respectively. Therefore, inaccurate estimation of the initial phase would not greatly affect the estimation of exposure. In a simulation analysis Bennett et al (1997) showed that on fitting a simple one compartment model to data simulated using the three compartment model, there was bias in the estimate of CL/F. However, it was less than the bias in the estimate of V/F. This result is consistent with the majority of AUC and hence CL/F being associated with a single elimination phase but Cmax and hence V/F being dependent on characterising the full absorption and distribution profile for bismuth.

4.7 Conclusions

While the noncompartmental approach allowed appropriate estimates for accumulation during the 28 day treatment course, estimation of expected steady state levels requires the application of modelling techniques. While individual patient accumulation is of ultimate clinical importance, the typical healthy volunteer accumulation and variability is important in the extrapolation to the later phases of drug development. The application of the population approach has been shown in this chapter to be useful in this respect.

The initial STS approach suggested that distinct Ka and α rate constants could not be determined. In resolving the problem, the dual absorption and elimination rate constant was estimated using adaptations of derived models for the special case where Ka equals α. However, precise population estimates of both Ka and α could be obtained using the FOCE method implementing user supplied PRED codes.

The problems associated with the population modelling of complex distribution functions and extensive multiple non steady state dosing were exemplified. For these complex cases the benefits of the FOCE over the FO estimation method was demonstrated. The resulting extraordinary CPU times were largely overcome by avoiding PREDPP, and by employing simpler user supplied PRED routines.

The results indicate that the population approach provided an appropriate description of healthy volunteer data and a suitable base model for future patient investigations. It was shown that accumulation to toxic levels was unlikely with the present formulation and dosage regimen.

CHAPTER 5

APPLICATION OF NONLINEAR MIXED EFFECTS MODELLING TO BIOEQUIVALENCE TESTING

In this chapter, the application of the mixed effects modelling to bioequivalence testing is investigated. A compartmental modelling approach is compared to the standard non-compartmental analysis. The robustness of the mixed effect modelling approach is assessed by testing the hypotheses of bioequivalence before and after the datasets have been reduced by 80%. Both additive and multiplicative bioequivalence models are utilised in the analysis. Finally, the potential role of the population pharmacokinetic approach in the assessment of bioequivalence is discussed.

5.1 Introduction

5.1.1 The concept of bioavailability and bioequivalence

Bioavailability, an abbreviated term for **biological availability**, has been defined by the FDA as "the rate and extent to which the active ingredient, or therapeutic moiety, is absorbed and becomes available at the site of action". In practical terms, the proportion of a drug which becomes systemically available is known as the **absolute bioavailability** (**F**), and all intravascular doses are, by convention, 100% bioavailable. The **relative bioavailability** of an extra-vascular drug product is estimated by comparing the concentration time profiles after intra-vascular and extra-vascular administration. This value is dependent on both the physiochemical properties of the drug and the release characteristics of the formulation.

In **comparative bioavailability** studies, the concentration time profiles of different formulations of the same drug, or different drug products, are compared. In **bioequivalence studies** the average concentration time profiles from different formulations are compared to ensure that the products are interchangeable. Bioequivalence is concluded when the two formulations are shown to be equivalent in terms of both the **"rate"** and **"extent"** of absorption.

5.1.2 Parameters used to determine bioequivalence

Many different pharmacokinetic parameters can be used to describe and assess the drug concentration time profiles, e.g. $T_{1/2}$,Ke, Mean residence time (MRT) etc. The preferred parameters are; $AUC_{0-\infty}$ to assess the total amount of drug which becomes bioavailable (i.e. the "extent" of absorption); Cmax, the maximum observed concentration; and Tmax, the time at which the maximum concentration is observed. Together Cmax and Tmax are used to assess the rate at which the drug becomes bioavailable.

5.1.3 Background and history of bioequivalence studies

After the innovating company's patent has expired, a drug can be manufactured and marketed under its "approved" (generic) name. Generic prescribing, which can potentially reduce treatment cost, has led to an increase in the number of generic manufacturers and the marketing of generic drug products. A method to ensure that efficacy and safety is maintained across formulations is therefore required.

The concept of bioavailability/ bioequivalence became a major public issue in the late 1960's, when the therapeutic responses between proprietary and generic products for several drugs were shown to be different. By 1970, the FDA required evidence of "biological availability" in applications for the approval of certain drugs. Subsequently, this requirement has developed into the regulations currently used in bioavailability and bioequivalence studies. (FDA, 1992) The statistical methodology employed for bioavailability / bioequivalence studies has evolved over the years to cope with alterations to the regulatory requirements. Recently, the difference between population bioequivalence (where the safety of prescribing a drug for the first time is ensured) and individual bioequivalence (where the safety of switching from one product to another is ensured) has been identified (Anderson & Hauck, 1990; Schall & Luus, 1993; Hauck &

Anderson, 1994; Schall & William, 1996). Draft guidelines have been prepared which identify separate criteria for each of these issues (FDA, 1997).

5.1.4 Development of the statistical concepts in bioequivalence testing

In bioequivalence testing, the rate and extent of absorption from a test formulation (T) is compared to a reference formulation (R). If the average population values for the test formulation (μ_T) and the reference formulation (μ_R) are equal, then the formulations are concluded to be bioequivalent. However, μ_T and μ_R may differ statistically without the difference being clinically important.

Following the inaugural meeting of the bioavailability committee in 1971, the proposed statistical hypothesis for the assessment of bioequivalence was the subject of much debate. During the following decade there were many important contributions to the statistical methodology used in bioequivalence testing. Amongst the most prominent were the power approach, (Schurimann, 1981), the confidence interval approach (Westlake, 1972, 1976, 1979; Metzler, 1974), the reformulated hypothesis approach (Schurimann, 1981; Anderson & Hauck, 1983) and the Bayesian approach (Rodda & Davis, 1980; Mandallaz & Mau, 1981). The methods were initially compared and contrasted by Metzler and Huang (1983). More recently the new approaches of population and individual bioequivalence have been discussed (FDA, 1997). However, the two one sided test approach (Schurimann, 1987, 1989) which is operationally identical to the confidence interval approach (Pidgen, 1992) used in this chapter, is the current regulatory standard.

5.1.5 Confidence interval approach

If the population mean parameter estimates for the test and reference formulations are given by μ_T and μ_R, a hypothesis test based on the confidence interval of the mean difference can be postulated. The concept is to show bioequivalence by rejecting the Null Hypothesis of bioinequivalence. The Null Hypothesis is given by

Ho: $\mu_T - \mu_R \leq a_1$ or $\mu_T - \mu_R \geq a_2$

where a_1 and a_2 are the limits for the region of acceptance, where $a_1 < a_2$. The alternative hypothesis of bioequivalence is therefore given by

H_1: $a_1 < \mu_T - \mu_R < a_2$

The (1-α) x 100% confidence interval for the sample mean difference is given by

$$\mu'_T - \mu'_R \pm \left(t1-\alpha/2,n-1\right) \cdot \sqrt{\frac{2S^2}{n}}$$

where:

μ'_R is the sample mean for the reference formulation

μ'_T is the sample mean for the test formulation

S^2 is the mean square error from the analysis of variance between formulations, subjects and periods.

n is the number of subjects

$t1-\alpha/2,n-1$ is the appropriate two tailed t-value with α usually set at 0.1 or 0.05 for 90% or 95% confidence, respectively.

The intervals in their absolute form are difficult to interpret, so confidence intervals for the relative difference are normally calculated

$$\theta_{RD} = \frac{\mu'_T - \mu'_R}{\mu'_R} \qquad \text{Eq 5.1}$$

The FDA have adopted a policy of accepting bioequivalence when the 90% confidence intervals are within 20% (i.e. $-a_1=a_2=0.2$) of the reference formulation. (Westlake, 1972, 1981; Pidgen, 1992; Chow & Liu, 1992a).

5.1.6 Normality vs ln-normality (additive vs multiplicative model)

The individual Cmax or $AUC_{0-\infty}$ parameters (Y), for a comparison of two formulations using a standard randomised 2x2 cross-over study, can be described by the effects model-

$Y=\mu+I+P+F+E$

where μ is the overall mean; I is the random intersubject effect; P is the period effect; F is the formulation effect and E is the random intrasubject error in observing Y. Both I and E are independent and normally distributed with a mean of zero. Since the parameters Cmax and $AUC_{0-\infty}$, are also considered to be normally distributed, the bioequivalence model is additive in both fixed and random effects.

However, assumptions of normality for Cmax and $AUC_{0-\infty}$ are often invalid, since the underlying distributions can be positively skewed or truncated at zero. In addition, it has been shown that intrasubject and intersubject variances often lack homogeneity (Chow & Liu, 1992b). Ln- transformation can correct for this skew. In using this model it is assumed that the effects model is described by the following multiplicative relationship

$Y=\mu \times I \times P \times F \times E$

Upon ln-transformation the effects can again be described by an additive model

$\ln(Y)=\ln(\mu) + \ln(I) + \ln(P) + \ln(F) + \ln(E)$

This concept has direct application to the pharmacokinetic relationships which underly the estimation of the parameters used in the bioequivalence test i.e.

$$AUC_{0-\infty} = \frac{F.DOSE}{CL} \qquad \text{Eq 5.3}$$

Upon ln-transformation this is converted to the additive model

$$\ln(AUC_{0-\infty}) = \ln F - \ln CL + \ln DOSE \qquad \text{Eq 5.4}$$

such that ln $AUC_{0-\infty}$ is expressed as a function of the sum of formulation effect (lnF) and subject effect (lnCL). Therefore, the ln-transformation could reduce skew, normalise variance and ensure that effects are additive (Steinijans & Hauschke, 1990).

An additional advantage is that the ln-transformed (multiplicative) model provides a confidence interval for the ratio of two means (θ_{lnRD}) without the need for further manipulation (Mandallaz & Mau, 1981)-

$$\theta_{\ln RD} = \ln\mu_T^{'} - \ln\mu_R^{'} = \ln\left(\frac{\mu_T^{'}}{\mu_R^{'}}\right) \qquad \text{Eq 5.5}$$

The Null Hypothesis of bioinequivalence for the ratio of the two formulation means becomes-

$$\text{Ho: } \ln\left(\frac{\mu_T^{'}}{\mu_R^{'}}\right) \leq \ln a_1 \text{ or } \ln\left(\frac{\mu_T^{'}}{\mu_R^{'}}\right) \geq \ln a_2$$

while the corresponding alternative hypothesis of bioequivalence becomes-

$$H_1\text{: } \ln a_1 < \ln\left(\frac{\mu_T^{'}}{\mu_R^{'}}\right) < \ln a_2$$

Following the 20% rule the limits for a_1 and a_2 would be 0.8 & 1.2, respectively. However on the logarithmic scale these are not symmetrical around unity. A symmetrical decision scheme using the limits 0.8 & 1.25 has been proposed (Pabst & Jaegar, 1990) ,and is now considered to be a regulatory standard (Chow & Liu, 1994).

Usually, both normal and log-normal models are applied and the assumptions of normality and ln-normality are checked in each case.

5.1.7 Application of the population approach to bioequivalence data

Previously, compartmental approaches have been used to directly compare the absorption rates, as an addition to standard noncompartmental hypothesis testing (Graves & Chang, 1989; Piotrovskij et al., 1995). It has also been shown to be useful in the comparison of immediate and controlled release formulations when the difference in dosing frequencies would not allow bioequivalence to be established noncompartmentally (Miller & Ludden, 1993). The utilisation of mixed effect modelling in the general context of bioequivalence testing was first proposed by Kaniwa et al. (1990), and more recently by Li et al. (1994) and Pentikis et al. (1996). Kaniwa et.al. directly compared compartmental and noncompartmental methods for estimating bioequivalence in terms of $AUC_{0-\infty}$ and Cmax. For six different drugs, the point and 95% CI for θ_{RD} in $AUC_{0-\infty}$ and Cmax were similarly estimated, by the two approaches. The same number of Null Hypotheses, was rejected by both approaches (6 in total). They also showed that five out of six Null Hypothesis were still rejected when data was reduced by 80%.

5.2 Aims

The aim in this chapter was to confirm and extend the work of Kaniwa et al. (1990), and discuss where the population pharmacokinetic approach to bioequivalence testing would be useful in drug development and the control of generic equivalents.

1) Comparison of noncompartmental and compartmental approaches to bioequivalence testing.

The previous comparisons of the compartmental and the noncompartmental approaches to bioequivalence testing utilised datasets which were either obviously bioequivalent or bioinequivalent in both $AUC_{0-\infty}$ and Cmax. In this analysis, a dataset which is bioequivalent in $AUC_{0-\infty}$ but bioinequivalent in Cmax is investigated. The effect of mis-specifying the compartmental model and the NONMEM estimation method used in the computation of the model parameters are also assessed.

2) The Pharmacokinetic model and Cmax derivation

The regulatory bodies require that Cmax is used to assess the rate of absorption. Kaniwa et al. (1990) showed that the θ_{RD} in Cmax can be estimated using the mean population parameters. However, the confidence intervals for the θ_{RD} were approximated using the SE estimates for the absorption rate of the test formulation. A compartmental method for determining the point and CI estimate for the θ_{RD} in Cmax is therefore required. In this analysis, an approach for estimating the point and CI estimates for the θ_{RD} in Cmax, directly from a one compartment model, is proposed. A surrogate metric for the direct estimation of Cmax from a two compartment model is also proposed.

3) Additive and Multiplicative bioequivalence models

The regulatory agencies require that both additive and multiplicative bioequivalence models be tested. The compartmental methods for implementing a multiplicative bioequivalence model are described and used to estimate the point and CI estimates for the θ_{lnRD} in $AUC_{0-\infty}$ and Cmax.

4) Power of the population approach in assessing bioequivalence from reduced datasets

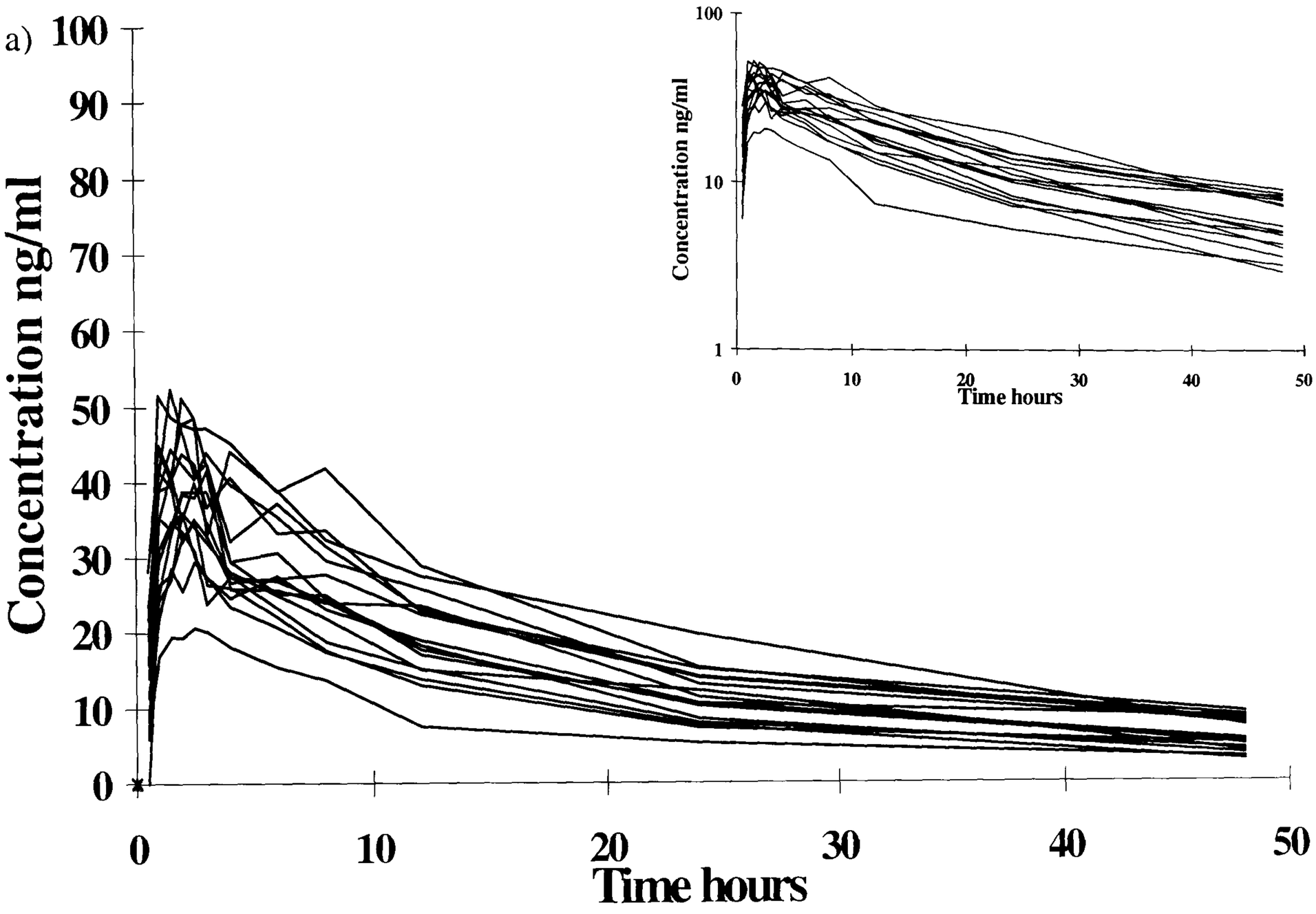
Kaniwa et al (1990) previously demonstrated that the results of the hypothesis tests were maintained even when the data sets were reduced by 80%. In this analysis, the dataset was reduced by assuming three different sampling schemes, representing three different methods for the collection of sparse bioequivalence data .

5.3 Bioequivalence Data

Data from two bioequivalence studies, comparing the reference Natrilix formulation and a test indapamide formulation (Harris Pharmaceuticals), were available and used in the assessment of the population compartmental approach to bioequivalence testing . Both had a two period randomised cross-over design. In the first sudy, 16 young male healthy volunteers received one 2.5mg tablet of each formulation, and in the second study 14 young male healthy volunteers received two 2.5 mg tablets of each formulation. Plasma concentrations were measured at 12 time points: 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48 hours after each dose.

The individual plasma concentration versus time profiles for the 2.5mg and 5.0mg studies are shown, split by formulation, in Figures 5.1 and 5.2, respectively. In each case the peak concentrations were highest for the test formulation.

Figure 5.1 Individual concentration and log concentration time profiles for a) The reference and b) The test formulations after a 2.5mg dose



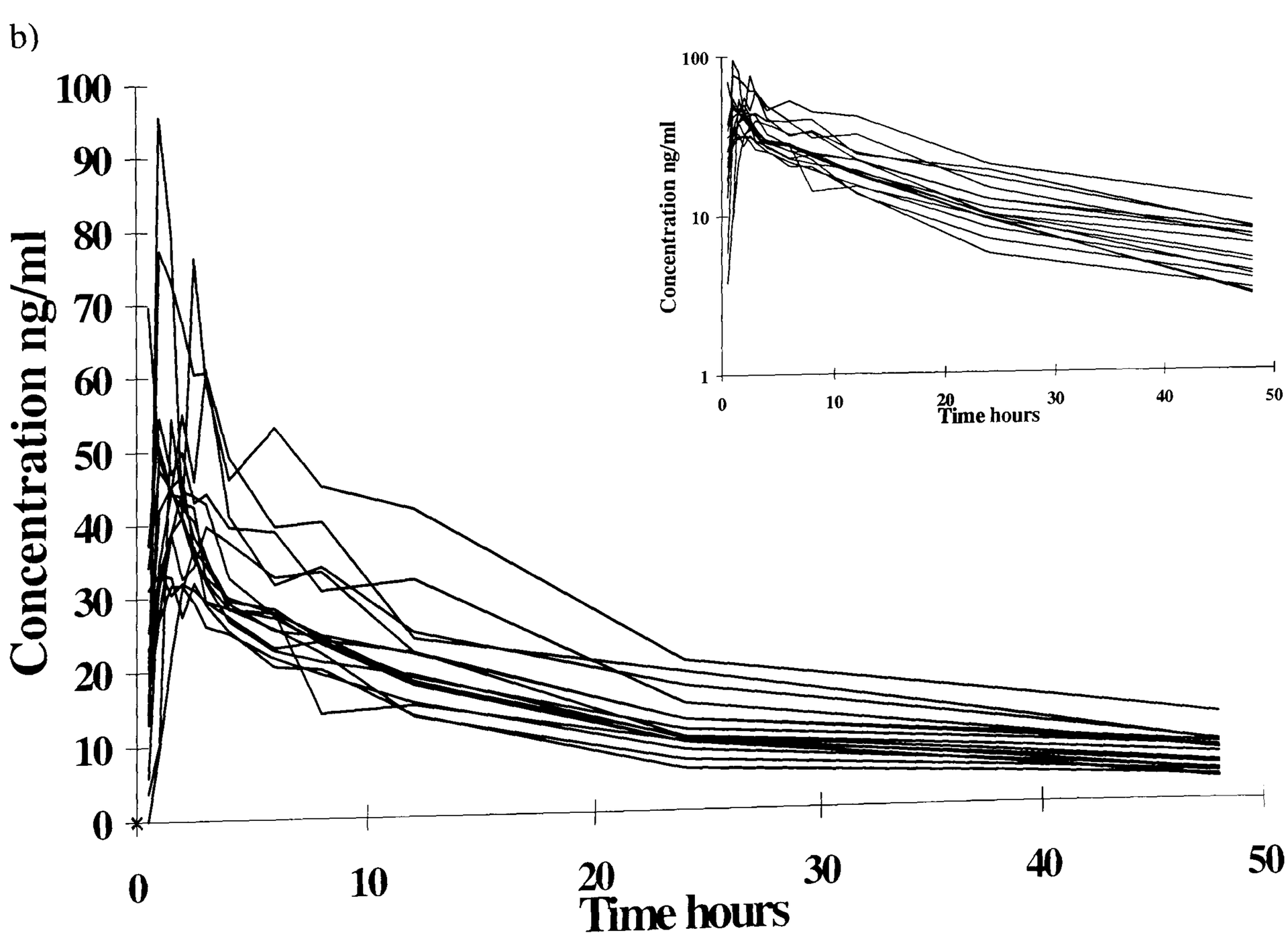
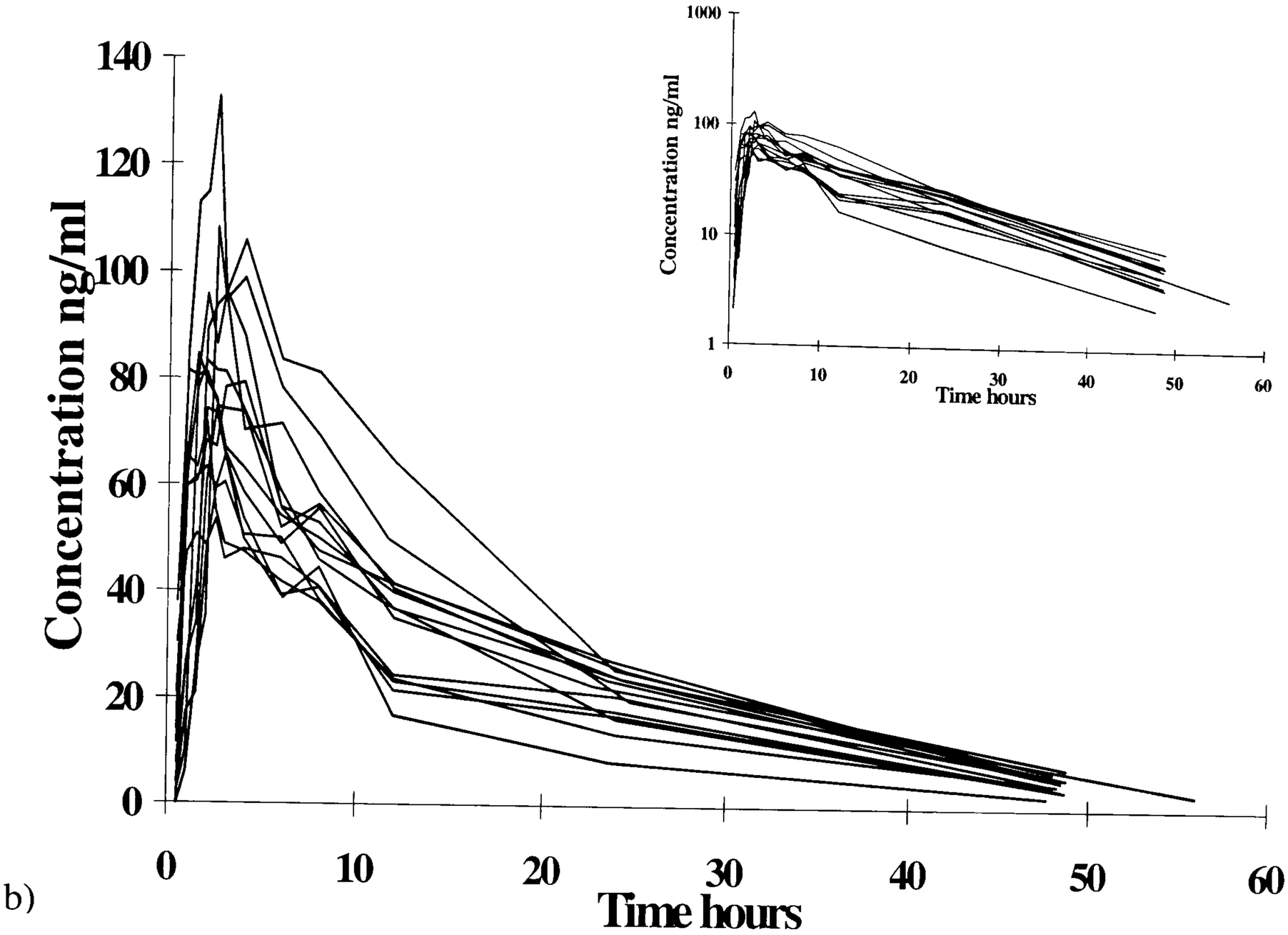
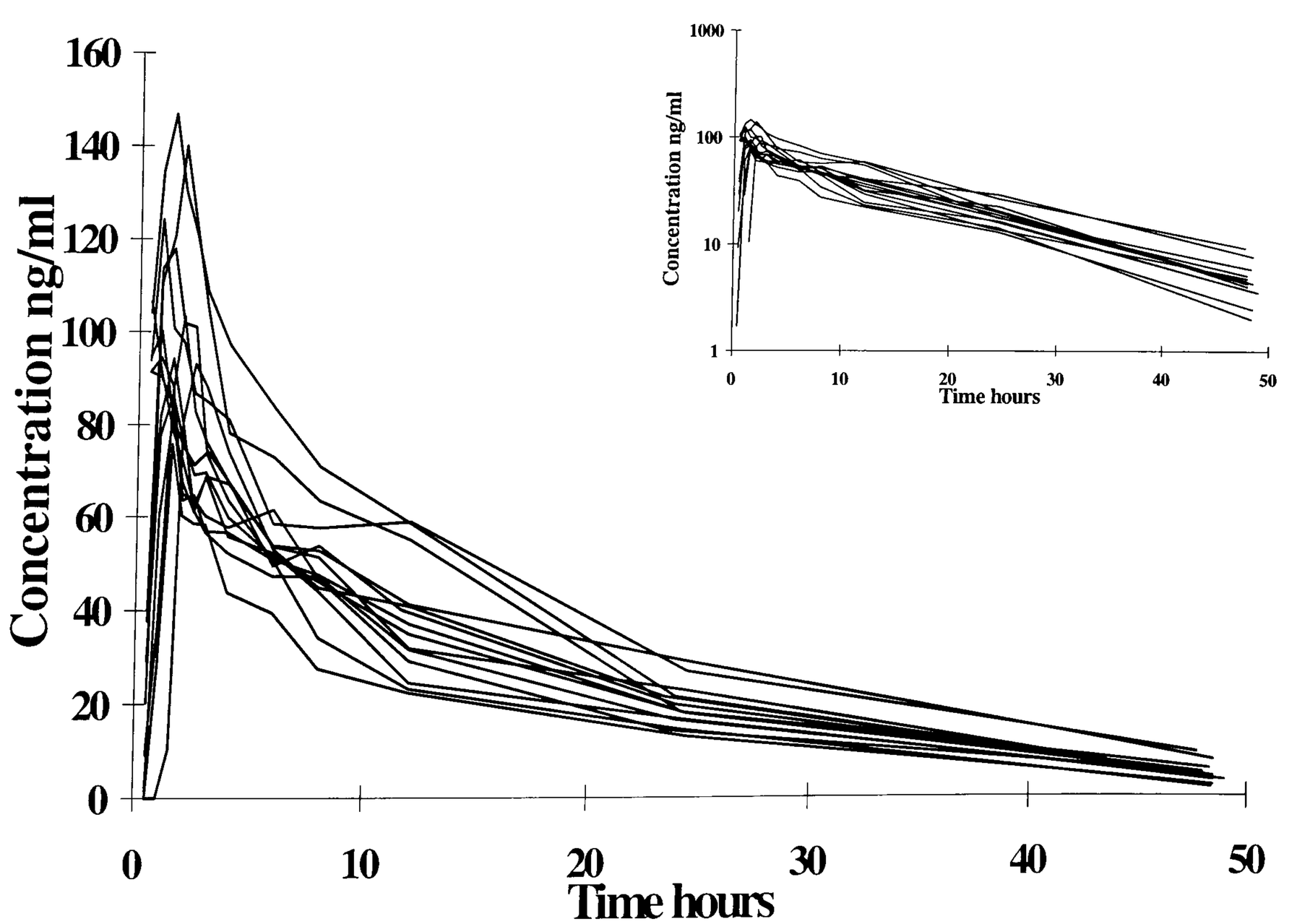


Figure 5.2 Individual concentration and log concentration time profiles for a) The reference and b) The test formulations after a 5mg dose



b)



5.4 Methods

5.4.1 Noncompartmental approach to bioequivalence testing

$AUC_{0-\infty}$ was calculated for each concentration time profile using the linear trapezoidal rule. Cmax was taken to be the highest observed concentration after each dose.

A two way ANOVA was performed separately for each study and then again for the total dataset after correcting for dose. The ANOVA analysis was repeated for both raw and ln-transformed $AUC_{0-\infty}$ and Cmax parameters. The point estimates and 90% confidence intervals for the absolute differences and θ_{RD} were calculated using the mean squared error (S^2) from the ANOVA and equation 5.1. The point estimates and 90% confidence intervals θ_{lnRD} were calculated using the mean squared error (S^2) from the ANOVA of the ln-transformed parameters and equation 5.5.

5.4.2 Population model dependent approach: Full model development

Using both FO and FOCE (with interaction) methods, one and two compartment models were compared. The absorption phase was characterised by either a first order (Ka) or a zero order (Ko) absorption model. The requirement for an absorption lag (Tlag) was tested in each case. Normal fitting criteria were used to compare the models (Chapter 3).

5.4.3 A population compartmental approach to bioequivalence testing

The following outlines how estimates of θ_{RD} and θ_{lnRD}, for $AUC_{0-\infty}$, ka and Cmax, were obtained for a one and two compartment model.

Estimation of θ_{RD} in $AUC_{0-\infty}$ for a one and two compartment model

The population approach to estimating θ_{RD} in the $AUC_{0-\infty}$ utilises the following relationship-

$$AUC_{0-\infty} = \frac{F.DOSE}{V_1.Ke} \qquad \text{Eq 5.6}$$

where formulation specific population mean estimates of $\frac{F}{V_1}$ and Ke were used to provide the population average estimates of μ_R' or μ_T' for $AUC_{0-\infty}$, shown in Eq 5.1.

Since dose and Ke are independent of formulation, the θ_{RD} from Eq 5.1 on substituting in Eq 5.6, for each formulation, simplifies to-

$$\theta_{RD} = \frac{\frac{F}{V_{1T}} - \frac{F}{V_{1R}}}{\frac{F}{V_{1R}}} \qquad \text{Eq 5.7}$$

Rearranging for $\frac{F}{V_{1T}}$ gives

$$\frac{F}{V_{1T}} = \frac{F}{V_{1R}} .(\theta_{RD} + 1) \qquad \text{Eq 5.8}$$

This relationship can be utilised in NONMEM to obtain parameter estimates of θ_{RD} by using

$$\frac{F}{V_1} = \frac{F}{V_{1R}} .(1 - Form) + \frac{F}{V_{1R}} .(1 + \theta_{RD}).(Form) \qquad \text{Eq 5.9}$$

where the indicator variable Form, takes a value of 0 for all concentration associated with the reference formulation and 1 for all concentrations associated with the test formulation.

$\frac{F}{V_1}$ is the reciprocal of the population parameter for volume of distribution divided by the bioavailability, and provides NONMEM with estimates of scale factor for the conversion of compartment amounts into observed concentrations. The following can be estimated-

$$\frac{V_1}{F} = \frac{1}{\frac{F}{V_{1R}} .(1 - Form) + \frac{F}{V_{1R}} .(1 + \theta_{RD}).(Form)} \qquad \text{Eq 5.10}$$

Estimation of θ_{RD} in Ka for a one and two compartment model

Bioequivalence in the rate of absorption can be obtained by comparing the absorption rate constants for each formulation. Coding the θ_{RD} as previously shown in Eq 5.9, gives-

$$Ka = Ka_R.(1 - Form) + Ka_R.(1 + \theta_{RD}).(Form) \qquad \text{Eq 5.11}$$

where Ka_R is the first order absorption rate for the reference formulation

Estimation of θ_{RD} in Cmax for a one compartment model

The estimated population parameters can be used to obtain estimates of Cmax for a one compartment model. A one compartment model with first order absorption can be described by the equation-

$$C(t) = \frac{F.Dose.Ka}{V_1.(Ka - Ke)}.\left(e^{-Ke.t} - e^{-Ka.t}\right) \qquad \text{Eq 5.12}$$

and may be written in the form -

$$C(t) = A.e^{-Ke.t} - A.e^{-Ka.t} \qquad \text{where } A = \frac{F.Dose.Ka}{V_1.(Ka - Ke)}. \qquad \text{Eq 5.13}$$

The maximum concentration occurs when the derivative of Eq 5.13 with respect to t is equal to zero -

$$\frac{dC(t)}{dt} = -Ke.A.e^{-Ke.Tpk} + Ka.A.e^{-Ka.Tpk} = 0 \qquad \text{Eq 5.14}$$

Solving for the peak time Tpk gives the followng equation -

$$Tpk = \frac{\ln\left(Ka/Ke\right)}{(Ka - Ke)} \qquad \text{Eq 5.15}$$

So Cmax can be estimated from the model parameters using the relationship-

$$Cmax^D = \frac{F.Dose.Ka}{V_1.(Ka - Ke)}.\left(e^{-Ke.Tpk} - e^{-Ka.Tpk}\right) \qquad \text{Eq 5.16}$$

where $Cmax^D$ is the estimate of Cmax derived from the modelled parameters. The θ_{RD} between $Cmax_R{}^D$ and $Cmax_T{}^D$ can be calculated using the formulation specific estimates of Ka, Tpk, $\frac{F}{V_1}$ and the population mean estimate of Ke.

However, it is also possible to estimate Cmax explicitly, by rearranging Eq 5.16 and substituting it into Eq 5.12, to give the following relationship -

$$C(t) = \frac{C\max^E}{e^{-Ke.Tpk} - e^{-Ka.Tpk}} \cdot \left(e^{-K.t} - e^{-Ka.t}\right) \qquad \text{Eq 5.17}$$

In this case $Cmax^E$ is an estimated model parameter, and the θ_{RD} between $Cmax_R{}^E$ and $Cmax_T{}^E$ can be estimated from the relationship -

$$C\max^E = C\max_R^E.(1-Form) + C\max_R^E.(1+\theta_{RD}).(Form) \qquad \text{Eq 5.18}$$

Estimation of θ_{RD} in Cmax for a two compartment model

A two compartment model with first order absorption is described by -

$$C(t) = \frac{F.Dose.Ka}{V_1}.\left[\frac{(K_{21}-\alpha)}{(Ka-\alpha)(\beta-\alpha)}e^{-\alpha.t} + \frac{(K_{21}-\beta)}{(Ka-\beta)(\alpha-\beta)}e^{-\beta.t} + \frac{(K_{21}-ka)}{(\alpha-Ka)(\beta-Ka)}e^{-Ka.t}\right] \qquad \text{Eq 5.19}$$

which may be abbreviated to-

$$C(t) = \frac{F.Dose.Ka}{V_1}.\left[A^*.e^{-\alpha.t} + B^*e^{-\beta.t} - (A^*+B^*).e^{-Ka.t}\right] \qquad \text{Eq 5.20}$$

Where A^* and B^* are constants.

$$A^* = \frac{(K_{21}-\alpha)}{(Ka-\alpha)(\beta-\alpha)} \qquad B^* = \frac{(K_{21}-\beta)}{(Ka-\beta)(\alpha-\beta)} \qquad A^*+B^* = \frac{(K_{21}-ka)}{(\alpha-Ka)(\beta-Ka)}$$

The maximum concentration occurs when $\frac{dC(t)}{dt} = 0$ i.e.

$$\frac{dC(t)}{dt} = \frac{F.Dose.Ka}{V_1}.\left[-\alpha.A^*.e^{-\alpha.t} - \beta.B^*e^{-\beta.t} + Ka.(A^*+B^*).e^{-Ka.t}\right] = 0 \qquad \text{Eq 5.21}$$

However, this cannot be solved analytically for Tpk, so the value of Tpk has to be estimated numerically. The θ_{RD} for the derived Cmax ($Cmax_D$) can then be estimated using the formulation specific estimates of Tpk, Ka and $\frac{F}{V_1}$ and population estimates of α and β.

Since Eq 5.21 cannot be solved, it is not possible to estimate Cmax directly. However, by adopting the methods proposed by Wagner and Nelson (Wagner, 1975) it is possible to obtain an approximation of Tpk. The Wagner Nelson method can be used to characterise the absorption profile of a drug product. If absorption is governed by a first order process, then the absorption profiles for drugs with mono and bi-exponential distributions can be predicted. With a two compartment model it can be shown that -

$$A_T/V_1 = \frac{F.D}{V_1}.\left[\frac{K_{12}}{\alpha} + \frac{1}{\alpha(Ka-\alpha)}.\left\{Ka.(\alpha - K_{21}).e^{-\alpha.T} - \alpha(Ka - K_{21}).e^{-Ka.T}\right\}\right] \quad \text{Eq 5.22}$$

where A_T is the total amount of drug in the central compartment at time T, assuming that no elimination has occurred i.e. β=0. A_T/V_1 is this amount as a concentration. As $T \rightarrow \infty$, A_T approaches the asymptotic value, $\frac{F.D}{V_1}.\frac{K_{12}}{\alpha}$. Also when $\frac{d(A_T / V_1)}{dT} = 0$ i.e. at time TApk, A_T/V_1 reaches a maximum. Differentiating Eq 5.22 with respect to T and solving for TApk gives-

$$TApk = -\frac{1}{(Ka-\alpha)}.\ln\left[\frac{(\alpha - K_{21})}{(Ka - K_{21})}\right] \quad \text{Eq 5.23}$$

Where-

$$K_{21} = \frac{(A.\beta + B.\alpha)}{(A+B)} = \frac{(A/B.\beta + \alpha)}{(A/B + 1)} = \frac{(A^*/B^*.\beta + \alpha)}{(A^*/B^* + 1)} \quad \text{Eq 5.24}$$

TApk is an approximation of Tpk and is the time at which the accumulative amount absorbed into the central compartment divided by V1 reaches a maximum. The concentration at this time (CAmax) is an approximation of Cmax and is given by

$$CA\max = \frac{F.Dose.Ka}{V_1}\left[A^*.e^{-\alpha.TApk} + B^*.e^{-\beta.TApk} - (A^*+B^*).e^{-Ka.TApk}\right]$$

Eq 5.25

By rearranging Eq 5.25 and substituting in to Eq 5.19 $CAmax^E$ can be explicitly estimated, using the relationship-

$$C(t) = \frac{CA\max^E\left[A^*.e^{-\alpha.t} + B^*.e^{-\beta.t} - (A^*+B^*).e^{-Ka.t}\right]}{\left[A^*.e^{-\alpha.TApk} + B^*.e^{-\beta.TApk} - (A^*+B^*).e^{-Ka.TApk}\right]}$$

Eq 5.26

These relationships hold providing $Ka > \alpha > \beta$. Also, $CAmax^E$ approaches $Cmax^E$ when the fraction of drug eliminated by TApk is small i.e. when the elimination rate is very small in comparison to Ka and α.

Obtaining population estimates of θ_{lnRD}

From Eq 5.5, the relationship between θ_{lnRD} and $\frac{F}{V_1}$ becomes-

$$\theta_{\ln RD} = \ln\left(\frac{F}{V_{1\,T}}\right) - \ln\left(\frac{F}{V_{1\,R}}\right)$$

Eq 5.27

As before, to provide a scale for the conversion of compartmental amounts, in NONMEM, the relationship can be expressed as -

$$V_1/F = \frac{1}{e^{\left[\ln(\frac{F}{V_{1\,R}}) + (\theta_{\ln RD}.Form)\right]}}$$

Eq 5.28

Similarly, the relationship between $Cmax^E$ and θ_{lnRD} becomes-

$$C\max^E = e^{\left[\ln(C\max_R^E) + (\theta_{\ln RD}.Form)\right]}$$

Eq 5.29

Obtaining confidence interval for the population estimates of θ_{RA} and θ_{lnRD}

The CI for the θ_{RD} or θ_{lnRD} in $\frac{F}{V_1}$ or Ka can be estimated directly using the SE for the θ_{RD} or θ_{lnRD} parameter estimates. The CI for the θ_{RD} or θ_{lnRD} in $Cmax^D$ cannot be obtained directly. However, the limits of $Cmax_T{}^D$ can be calculated using the upper and lower limits of the Ka_T. The approximate confidence intervals for the θ_{RD} in $Cmax^D$ can be estimated by dividing the limits by $Cmax_R{}^D$ (Kaniwa et al., 1990). In comparison, the 95%CI for the θ_{RD} or θ_{lnRD} in $Cmax^E$ or $CAmax^E$ can be directly obtained using the SE for the θ_{RD} or θ_{lnRD} parameter estimates.

The 95% CI derived from extended least squares asymptotic SE estimates calculated using NONMEM have been shown to contain the true value of the parameter estimate on less than 95% of occasions (Sheiner & Beal, 1987). In fact 90% was the best performance. Thus, in this study the 95% confidence intervals from NONMEM are taken to be equivalent to the nominal 90% confidence intervals used to perform the hypothesis tests.

5.5 Results

5.5.1 Bioequivalence assessment using noncompartmental estimates

Figures 5.3 and 5.4 show the untransformed and ln-transformed individual dose corrected AUCo-∞ and Cmax estimates. The ln-transfomation reversed the skew on the distribution of AUCo-∞ and normalised the distribution of Cmax. The bioequivalence of the test and reference formulations ($AUC_{0-\infty}$ and Cmax) was assessed using the data for each study separately and also the combined dataset. Both the untransformed (additive bioequivalence model), and the ln-transformed (multiplicative bioequivalence model) data were used. The point estimates and 90% CI for the relative differences are presented in Tables 5.1 to 5.4,

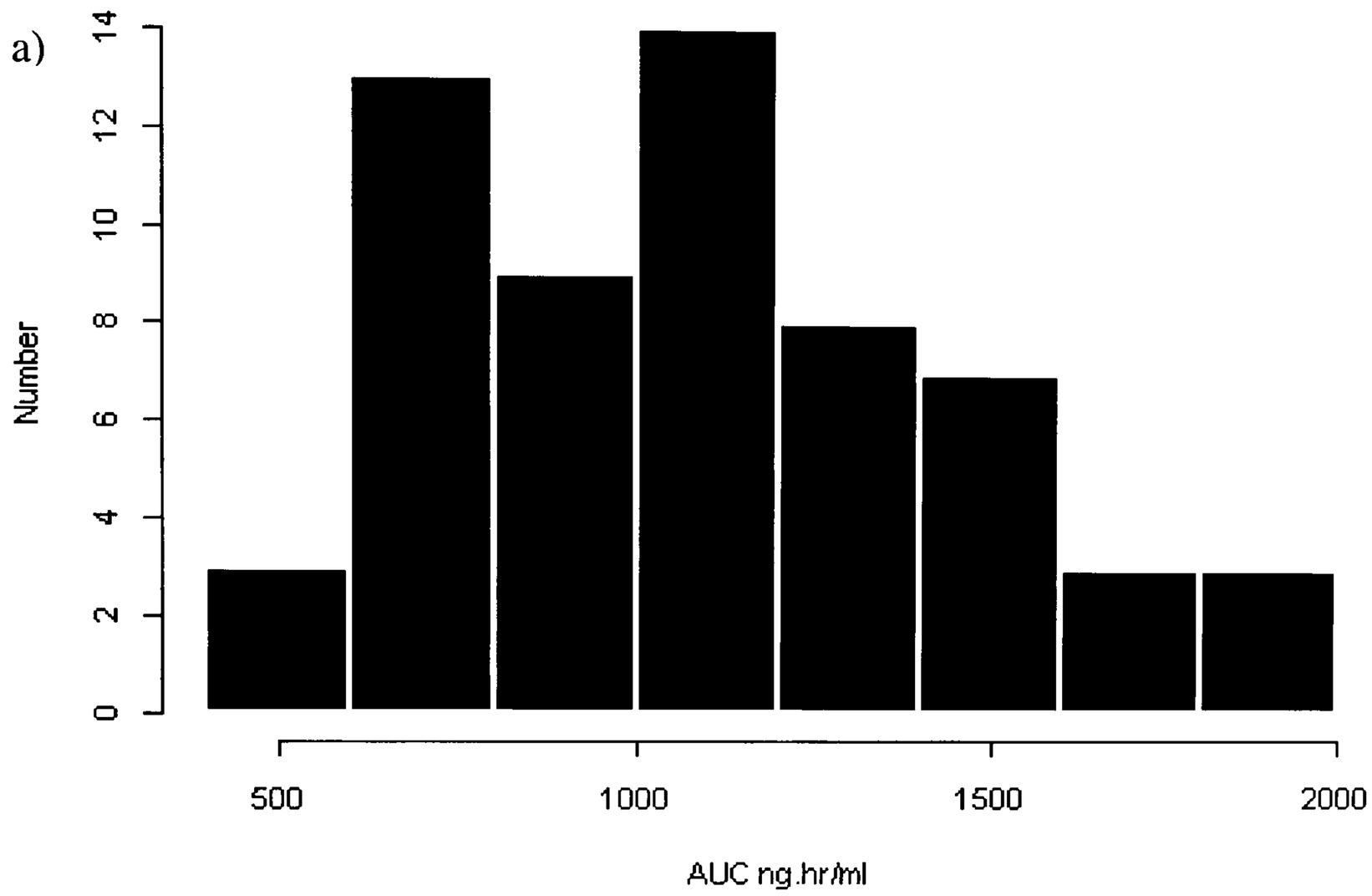
along with the ANOVA which was used to provide the Error MS (S^2) for the calculation of the confidence intervals.

Point and confidence interval estimates for the relative difference in $AUC_{0-\infty}$

Using an additive model

The point and 90% CI estimates for the θ_{RD} in AUC o-∞ were similar for the two studies and none of the confidence intervals overlapped the ±20% limits (Table 5.1). The Null Hypothesis of bioinequivalence could be rejected, so the formulations were considered to be bioequivalent in AUC o-∞.

Figure 5.3 a) Untransformed and b) Ln-transformed distributions of dose corrected AUCo-∞ (corrected to 5mg)



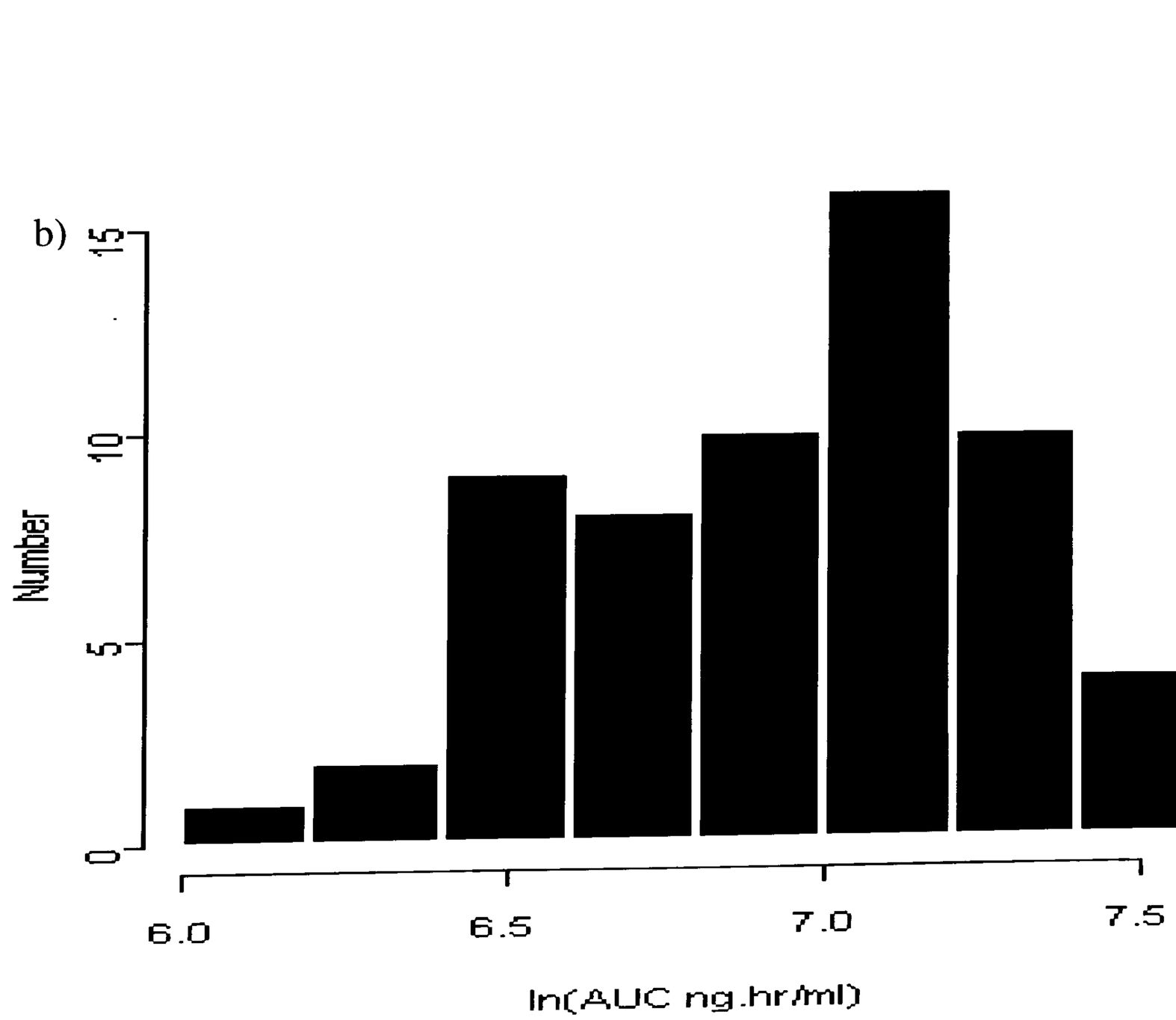
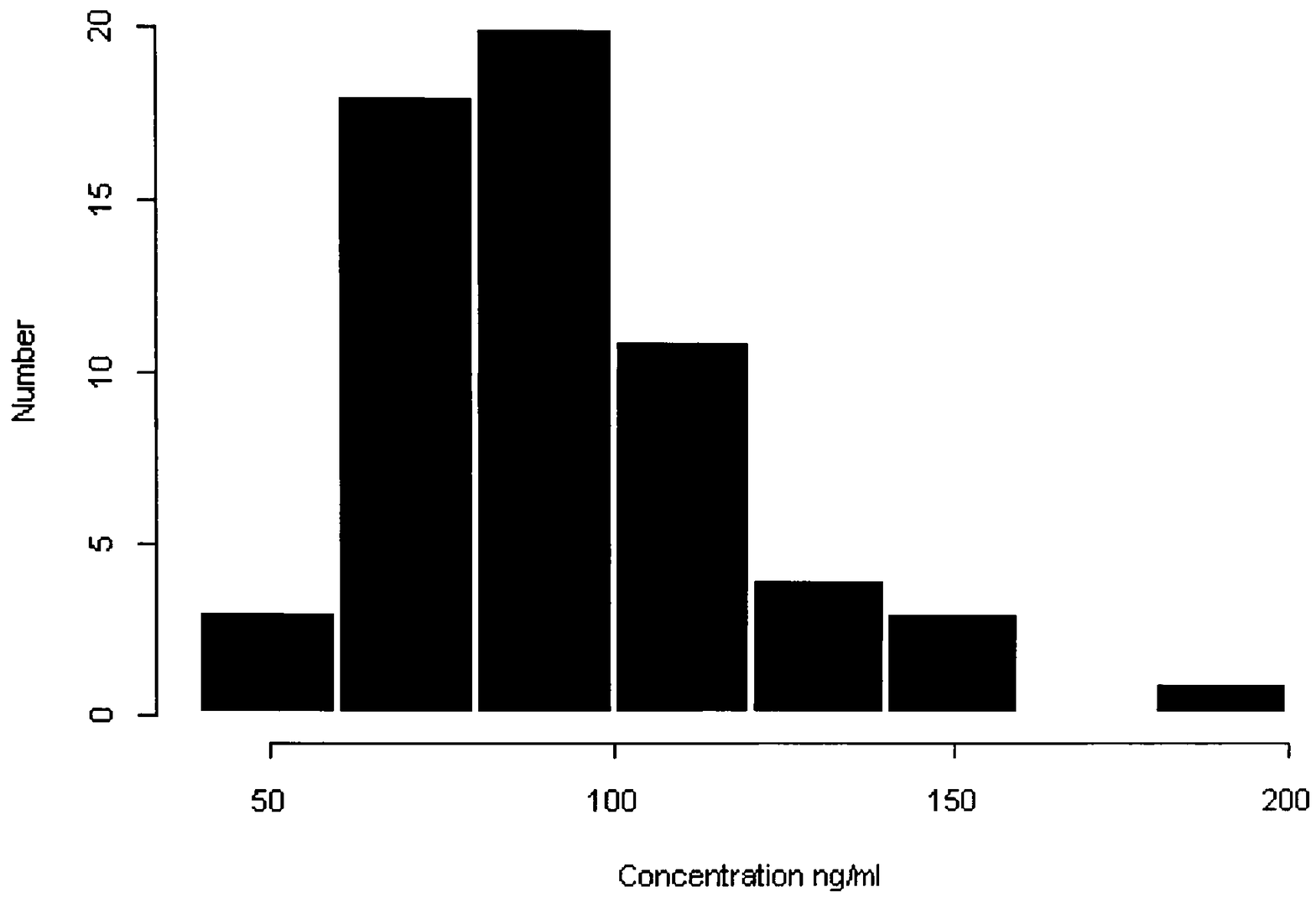


Figure 5.4 a) Untransformed and b) Ln-transformed distributions of dose corrected Cmax (corrected to 5mg)



b)

Table 5.1 ANOVA of $AUC_{0-\infty}$,absolute and relative mean differences (θ_{RD}) in $AUC_{0-\infty}$, between test and reference formulations for the 2.5 mg study, the 5mg study and for both studies combined

2.5mg Study					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between patients	2004580	15	133639	9.18	0.0001
Between formulations	2860	1	2860	0.20	0.6600
Error	218285	15	14552		
Total	2225724	31			
Mean difference($ng.ml^{-1}.hr$)	18.9		90% CI	-56.2	94.0
Relative mean difference	2.2%		90% CI	-6.4%	10.7%
≤ -20% or ≥+20%					No

5.0mg Study					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between patients	2344107	13	180316	9.09	0.0002
Between formulations	8198	1	8198	0.41	0.5300
Error	257739	13	19826		
Total	2610043	27			
Mean difference ($ng.ml^{-1}.hr$)	34.2		90% CI	-60.6	129.1
Relative mean difference	2.6%		90% CI	-4.6%	9.8%
≤ -20% or ≥+20%					No

Both 2.5 & 5.0mg Studies	Dose corrected (2.5mg $AUC_{0-\infty}$ x 2)				
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between patients	13116118	29	452279.9	11.60	0.00000
Between formulations	19588	1	19587.9	0.50	0.48400
Error	1130926	29	38997.5		
Total	14266632	59			
Mean difference ($ng.ml^{-1}.hr$)	36.1		90% CI	-50.6	122.9
Relative mean difference	2.3%		90% CI	-3.3%	7.9%
≤ -20% or ≥+20%					No

Table 5.2 ANOVA of Ln(AUC $_{0-\infty}$), absolute and relative mean differences (θ_{lnRD}) in Ln(AUC $_{0-\infty}$), between test and reference formulations for the 2.5 mg study, the 5mg study and for both studies combined

Dose 2.5mg					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between patients	2.520	15	0.170	8.60	0.0001
Between formulations	0.002	1	0.002	0.11	0.7400
Error	0.290	15	0.020		
Total	2.820	31			
Relative Mean difference	1.7%		90% CI	-7.1%	10.4%
≤ -20% or ≥+25%					No

Dose 5.0mg					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between patients	1.370	13	0.110	8.93	0.0002
Between formulations	0.006	1	0.006	0.51	0.4900
Error	0.150	13	0.010		
Total	1.53	27			
Relative Mean difference	2.9%		90% CI	-4.3%	10.2%
≤ -20% or ≥+25%					No

Dose 2.5mg & 5.0mg		Dose corrected (2.5mg AUC$_{0-\infty}$ x 2)			
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between patients	4.912	29	0.169	11.00	0.000
Between formulations	0.008	1	0.008	0.50	0.489
Error	0.448	29	0.015		
Total	5.367639	59			
Relative Mean difference	2.2%		90% CI	-3.2%	7.7%
≤ -20% or ≥+25%					No

Table 5.3 ANOVA of Cmax , absolute and relative mean differences (θ_{RD}) in Cmax, between test and reference formulations for the 2.5 mg study, the 5mg study and for both studies combined

2.5mg Study					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between patients	3847	15	256	2.08	0.08
Between formulations	939	1	939	7.62	0.01
Error	1847	15	123		
Total	6632	31			
Mean difference ($ng.ml^{-1}$)	10.8		90%CI	3.9	17.7
Relative Mean difference	26.8%		90%CI	9.7%	44.0%
≤ -20% or ≥+20%					Yes

5.0mg Study					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between patients	9520	13	732	6.08	0.0013
Between formulations	3035	1	3035	25.21	0.0002
Error	1565	13	120		
Total	14120	27			
Mean difference ($ng.ml^{-1}$)	20.8		90%CI	13.4	28.2
Relative Mean difference	25. 0%		90%CI	16.2%	33.9%
≤ -20% or ≥+20%					Yes

2.5 & 5.0mg Study	Dose corrected (2.5mg Cmax x 2)				
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between patients	24965	29	861	2.79	0.00367
Between formulations	6786	1	6786	21.98	0.00006
Error	8955	29	309		
Total	40706	59			
Mean difference ($ng.ml^{-1}$)	21.3		90%CI	13.6	29.0
Relative Mean difference	26.0%		90%CI	16.6%	35.4%
≤ -20% or ≥+20%					Yes

Table 5.4 ANOVA of Ln(Cmax), absolute and relative mean differences (θ_{lnRD}) in Ln(Cmax), between test and reference formulations for the 2.5 mg study, the 5mg study and for both studies combined

Dose = 2.5mg					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between patients	1.57	15	0.10	2.08	0.08
Between formulations	0.36	1	0.36	7.23	0.02
Error	0.75	15	0.05		
Total	2.68	31			
Relative Mean difference	21.3%		90%CI	7.3%	35.2%
< -20% or >+25%					yes

Dose = 5.0mg					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between patients	0.97	13	0.07	4.52	0.0053
Between formulations	0.37	1	0.37	22.59	0.0004
Error	0.21	13	0.02		
Total	1.55	27			
Relative Mean difference	23.1%		90%CI	14.5%	31.6%
< -20% or >+25%					yes

Dose =2.5 & 5.0mg			Dose corrected (2.5mg Cmax x 2)		
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between patients	2.55	29	0.09	2.64	0.00550
Between formulations	0.73	1	0.73	21.98	0.00006
Error	0.98	29	0.03		
Total	4.26	59			
Relative Mean difference	22.1%		90%CI	13.8%	30.4%
< -20% or >+25%					yes

Point and confidence interval estimates for the relative difference in $AUC_{0-\infty}$

Using the multiplicative model

The analysis was repeated using the logarithms of AUC o-∞ and Cmax, respectively (Table 5.2). Subtraction of the sample means provided estimates of the relative difference (θ_{lnRA}). To maintain a symmetrical interval on the logarithmic scale, the upper bioequivalence limit was set to +25%. The point and 90% CI estimates for AUCo-∞ were relatively unchanged from the θ_{RD} estimates discussed above. Therefore bioequivalence in AUCo-∞ could again be concluded.

The point and confidence interval estimates for absolute and relative difference in Cmax

Using an additive model

Although the point estimates for the θ_{RD} in Cmax were similar across both studies, the 90%CI estimates for the θ_{RD} in Cmax were wider for the 2.5mg study (Table 5.3). The point estimates were 26.8 and 25.0 % for the 2.5mg and the 5 mg studies, respectively. Both were greater than the upper limit (+20%). Accordingly, the Null Hypothesis could not be rejected and bioinequivalence in Cmax was concluded.

Using a multiplicative model

Bioinequivalence in Cmax was concluded when the multiplicative model was used (Table 5.4). However, in comparison to the additive model, the point estimates were all within the upper limit (+ 25 %). The confidence intervals were also smaller with the multiplicative model. In particular, the +9.7 to +44% confidence interval for the 2.5mg study using the additive model, was decreased to +7.3% to +35.2% when the multiplicative model was used. This result was consistent with the distribution of Cmax being characterised as ln-normal.

5.5.2 Compartmental analysis: Development of the structural and variability model

The development of the population pharmacokinetic model, for the combined set of study data, is shown in Table 5.5. The structural and variability model was determined assuming that there was no difference between the formulations. Standard one and two compartment models, parameterised in terms of α, β, $\frac{V_1}{F}$ and A/B, with zero and first order absorption rates (with and without Tlag) were tested.

The FO method was used in runs 1 to 6. In run 1, a one compartment model with first order absorption using an exponential model for interindividual and intraindividual variability was fitted. In run2, a two compartment model was fitted, and the decrease in objective function in comparison to run1 was statistically significant ($P<0.001$). However, interindividual variability in A/B could not be estimated. In run 3, the previous model was repeated but with a expression combining additive and proportional error to estimate intraindividual variability. The decrease in objective function was not statistically significant, and the estimate of the additive component of intraindividual variability was not significantly different from zero (σ_{ADD}). In run 4, an absorption lag time was added to the model, but the parameter could not be estimated. Attempts to model a zero order absorption with the FO method resulted in rounding errors, and therefore, an aborted covariance step (runs 5 and 6). The higher objective function for run 6 in comparison to run 5 (which has fewer parameters) was mostly likely due to a local minimum, which could not be overcome by changing the starting estimates.

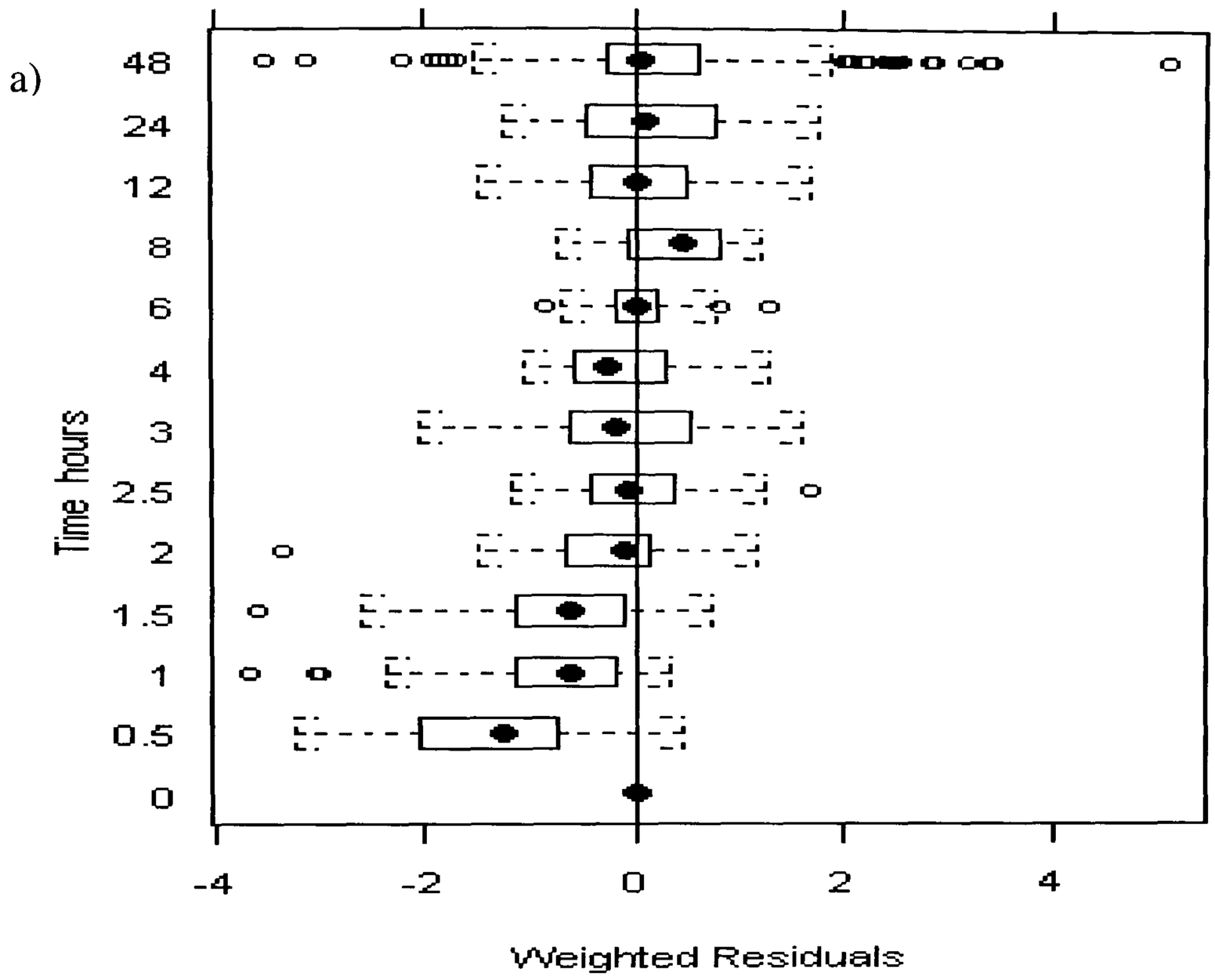
Table 5.5 Development of a compartment model for the combined study data

Run No	Distribution Model	Absorption Model	Obj. Fun.	β hr^{-1}	α hr^{-1}	Ka/ko $hr^{-1}/ng.hr^{-1}$	V_1/F L	A/B	Tlag hr	ω_β %	ω_α %	$\omega_{Ka/Ko}$ %	$\omega_{V1/F}$ %	$\omega_{A/B}$ %	ω_{Tlag} %	σ_{EXP} %	σ_{ADD} $ngml^{-1}$
FO Method																	
1	1 Comp	Ka	4034.033	0.05		4.75	64.3			21		250	21			25	
		SE %		4		27	4			20		83	28			12	
2	2 Comp	Ka	3854.412	0.04	0.21	3.27	54.20	0.99		28	53	159	20	ne		22	
		SE %		10	17	22	6	21		34	56	40	31			15	
3	2 Comp	Ka	3853.037	0.04	0.22	3.22	53.80	0.93		29	51	157	20	ne		22	0.61
		SE %		13	21	23	6	24		37	65	40	31			15	97
4	2 Comp	Ka &Tlag	3853.037	0.04	0.22	3.22	53.80	0.93	ne	29	51	157	20	ne	240	22	
		SE %		13	21	23	6	24	ne	37	65	40	30		42	15	
5	2 Comp	Ko No Cov	3884.069	0.04	0.83	114000	18.80	2.7		22	59	12369	24	ne		22	
6	2 Comp	Ko &Tlag No Cov	4031.071	0.05	7.21	6230	13.20	4.8	0.20	25	117	64	54	ne		25	
FOCE with Interaction																	
7	1 Comp	Ka	4120.152	0.05		2.33	65.8			53		19	20	ne		30	
		SE %		4		13	4			47		28	23			12	
8	2 Comp	Ka	4030.351	0.04	0.34	1.43	49.4	0.78		23	ne	50	19	ne		26	
		SE %		6	18	16	6	12		25	ne	44	26			14	
9	2 Comp	Ka &Tlag	4028.240	0.04	0.24	4.28	55.9	0.61	0.37	24	ne	88	19	ne	16	25	
		SE %		7	24	54	4	16	72	26	ne	60	26		193	14	
10	2 Comp	Ko No Cov	4027.284	0.05	0.28	3310	58.3	0.37		27	ne	60	19	ne		27	
11	2 Comp	Ko &Tlag No Cov	4092.931	0.03	0.135	8790	25.9	1.46	0.40	44	53	62	97	ne		27	

Repeating the analysis using the FOCE with interaction method, runs 7 to 11 (Table 5.5), showed similar but not identical results. As with the FO method, there was a statistically significant decrease in the objective function when a two compartment model was fitted (run 8 vs run 7), $p<0.001$. The intersubject variablity in α and A/B could not be estimated. In run 9, an absorption lag-time of 0.37 hrs was estimated and there was a further decrease in objective function. However, the estimates of Ka and Tlag were not significantly different from zero. Using the zero order absorption model with the FOCE method also resulted in rounding errors (runs 10 and 11, Table 5.5). The differences in the objective function and parameter estimates between runs 10 and 11 was again most likely due to run 11 terminating at a local minimum. Both the FO and FOCE methods indicated that a two compartment model with first order absorption best described the pharmacokinetics.

The weighted residual versus time plot for the two estimation methods showed that there was a bias in the fit during the absorption phase when the FO method was used (Figure 5.5a). In comparison, the weighted residual versus time plot for the FOCE method does showed no obvious bias. (Figure 5.5b). Splitting the weighted residuals versus time by formulation showed that during the absorption phase the concentrations for the test and reference products were under and over estimated, respectively.(Figure 5.6).

Figure 5.5 Weighted residual versus time after dose for a two compartment model fitted using a) FO method (run 2) b) FOCE with interaction method (run8)



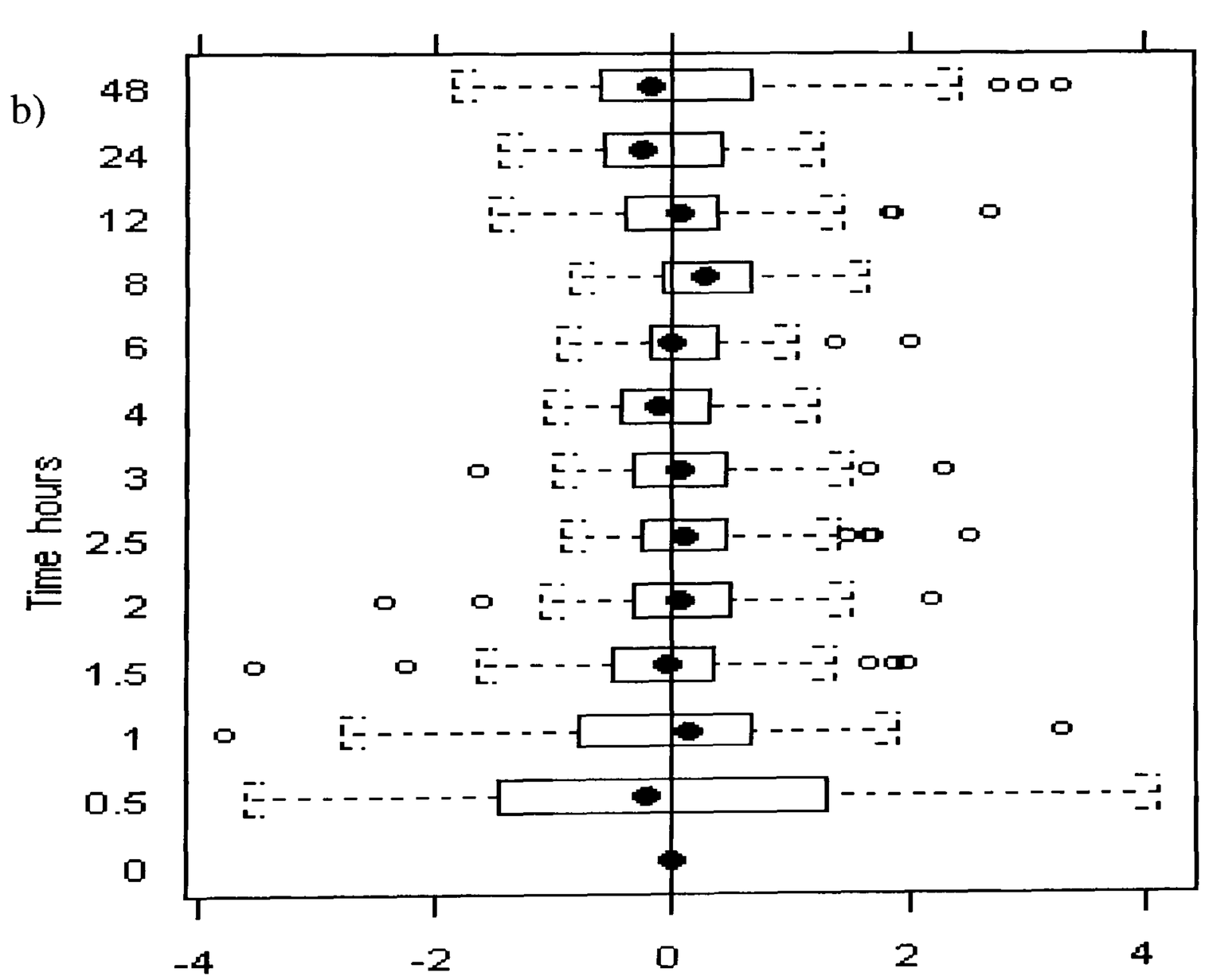
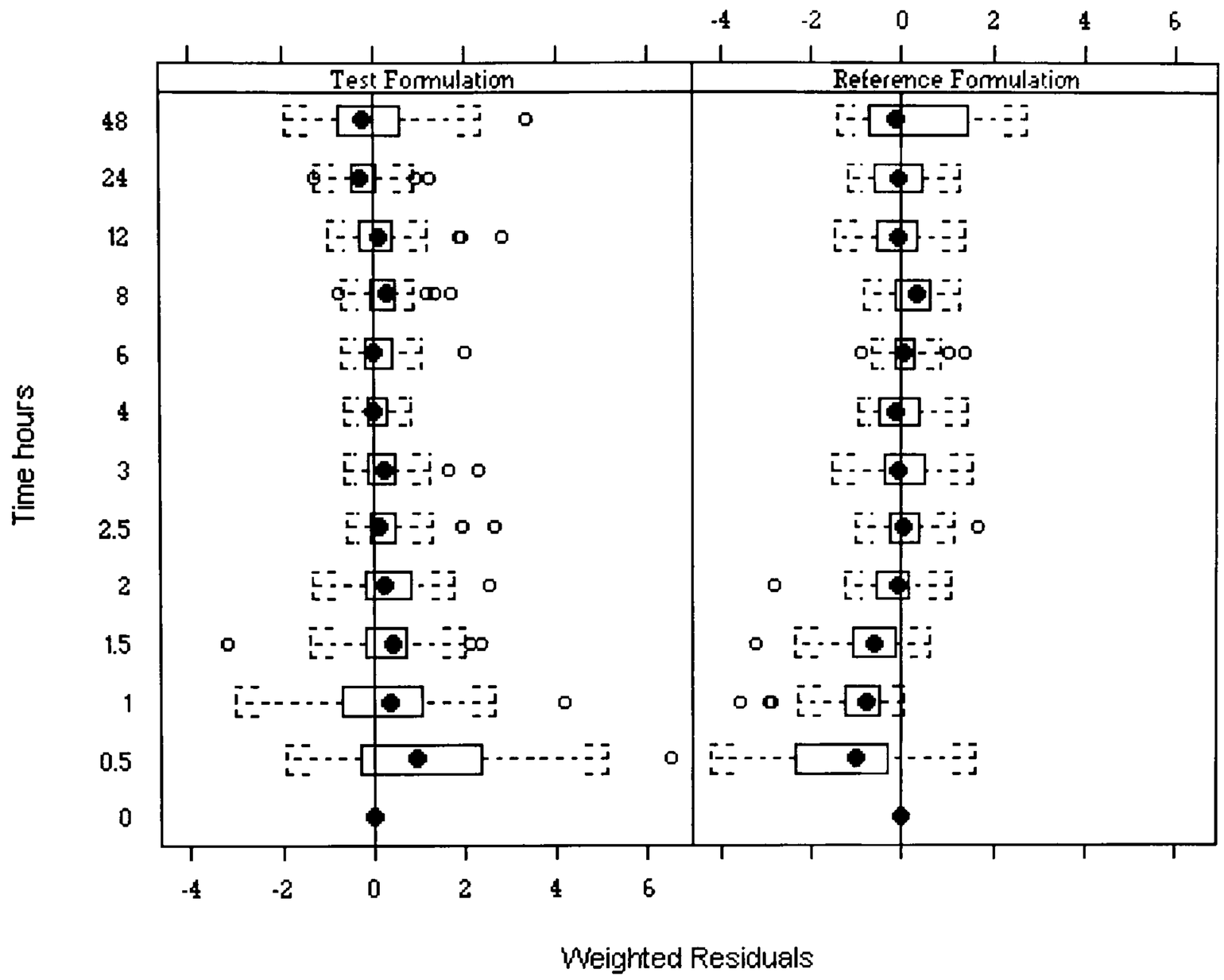


Figure 5.6 Weighted residual versus time for a two compartment model with first order absorption split by study and formulation



5.5.3 Estimation of the relative difference in $\frac{F}{V_1}$ Ka, Cmax^D and CAmax^E

Equations 5.10 and 5.11 were included in the model (Appendix 1.2) to allow the relative differences in $\frac{F}{V_1}$ and Ka to be estimated. Subsequently, the bias in the weighted residual versus time plot was reduced (Figure 5.7 vs Figure 5.6) and there was a further significant decrease in objective function ($P<0.001$) (run12 vs run 8 ,Table 5.6). When the θ_{RD} parameters were estimated the estimate of ω_{Ka} was reduced from 50% to 27%(run12 vs run 8). Parameterising the model in terms of CAmax (using E.q. 5.23, 5.26, 5.11 and 5.18-Appendix 1.2) resulted in a further small decrease in objective function (run 13 vs run 12 Table 5.6). The parameter estimates which were common to both models (α,β, ka and A/B) were very similar. The point estimates for θ_{RD} in CAmax^E and Cmax^D were also very similar i.e. the relative difference in the concentration at the point where the total amount absorbed reaches a maximum is very similar to relative difference in Cmax for this dataset. Figure 5.8 shows the senstivity of the θ_{RD} in CAmax^E and Cmax^D to individual changes in β, α and Ka^R, while other parameter estimates were fixed to those for run 12. As β approaches α, the θ_{RD} in CAmax^E becomes much greater than the θ_{RD} in Cmax. In comparison, the divergence when α approaches β or Ka^R, or Ka^R approaches α is smaller. Therefore, the equivalence of the θ_{RD} in CAmax and Cmax is dependent on β being small.

Runs 14 and 15 show the θ_{lnRD} estimates for $\frac{F}{V_1}$ and Cmax^E upon utilising E.q's 5.28 and 5.29 (Appendix 1.2), respectively. The other parameter estimates were unchanged.

Figure 5.7 Goodness of fit for two compartment model with first order absorption split by study and formulation after calculation of the relative difference in Ka and V_1/F

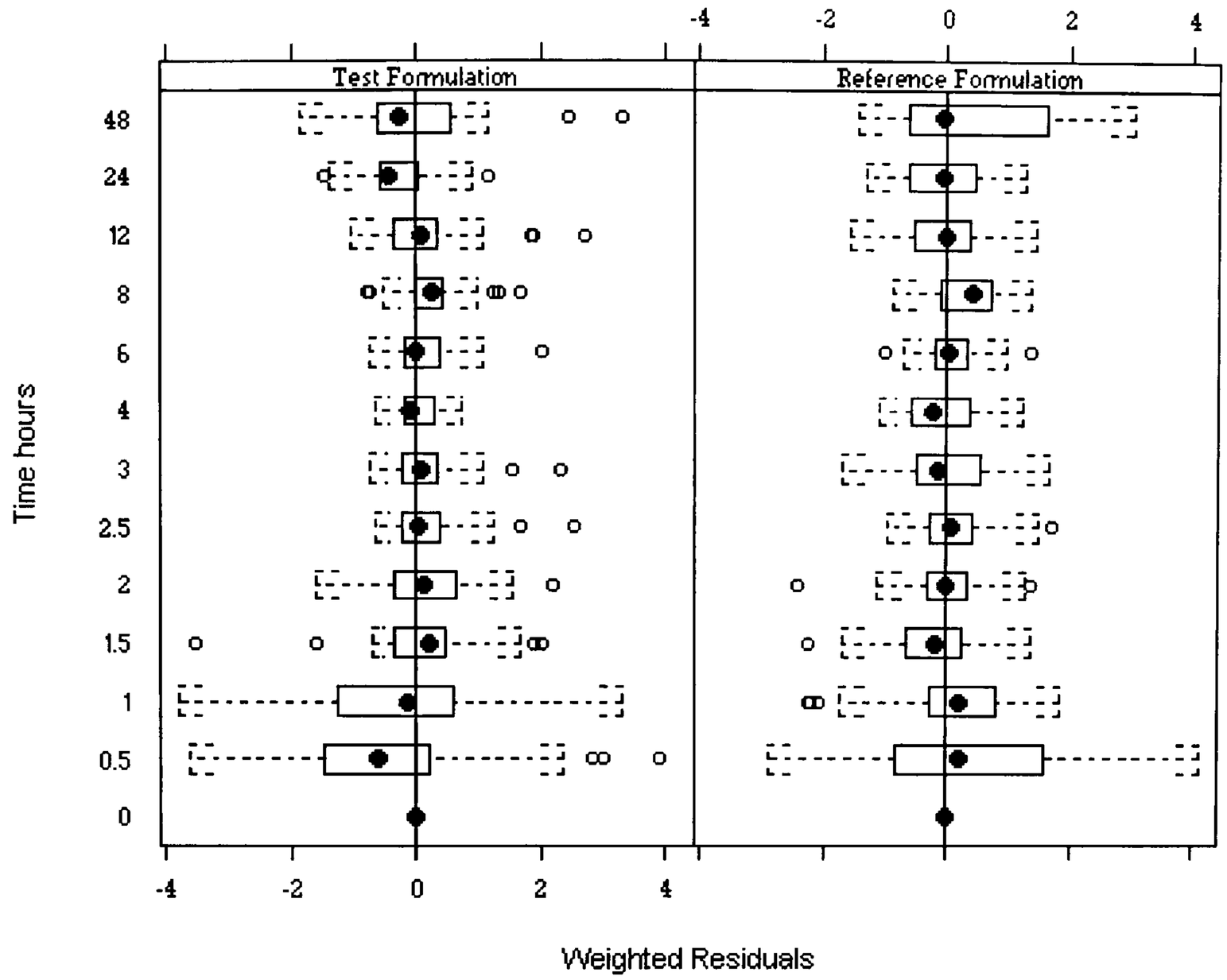


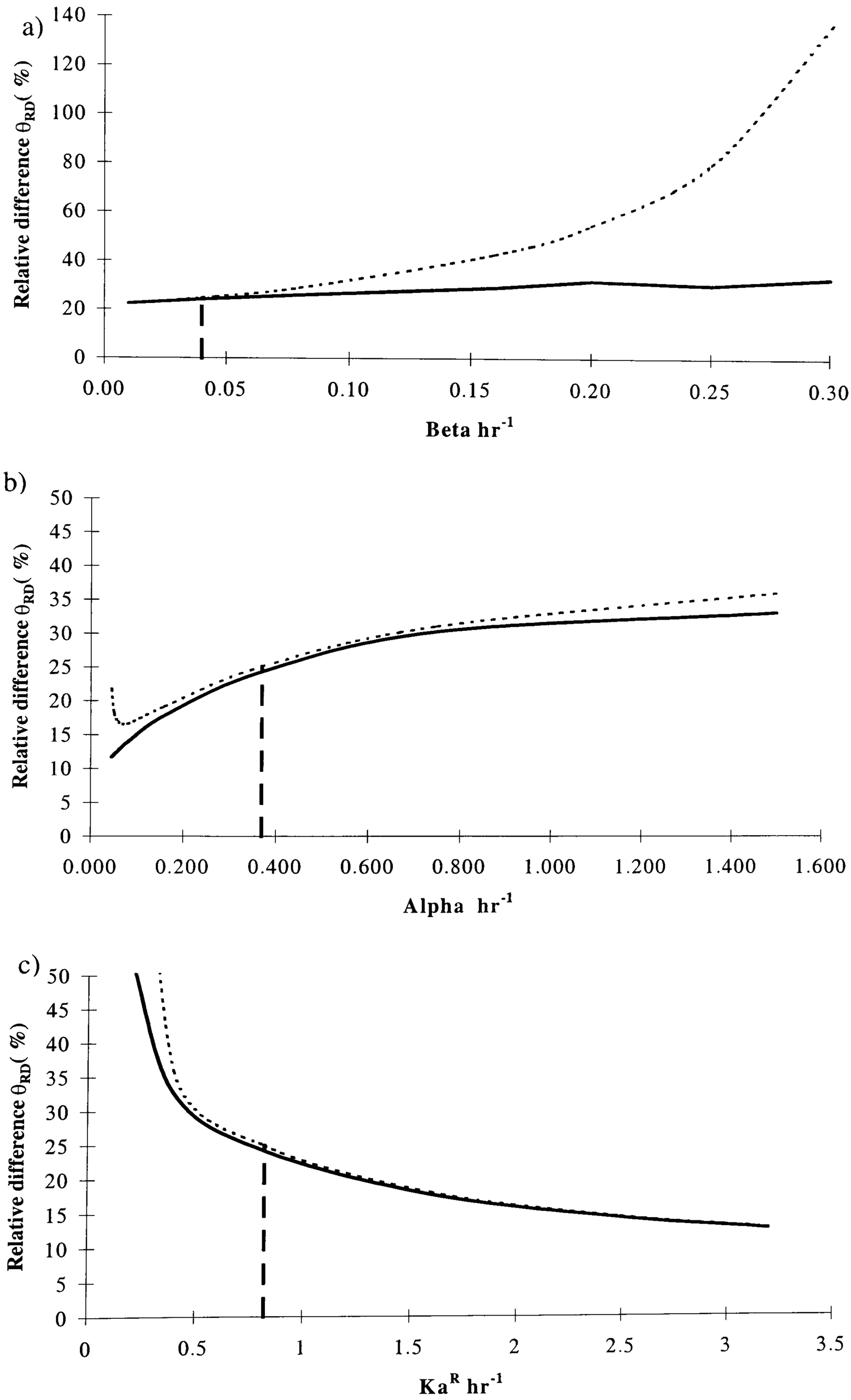
Table 5.6 Parameter estimates for a two compartment model with first order absorption, before and after estimation of the θ_{RD} and θ_{lnRD} V_1/F, Ka and Cmax using the FOCE method

Run	Obj.	β	α	Ka^R	See footnotes	A/B	θ_{RD}	θ_{RD}	θ_{lnRD}	θ_{RD}	θ_{RD}	θ_{lnRD}	ω_β	ω_α	ω_{Ka}	$\omega_{V1/F}$	$\omega_{A/B}$	σ_{EXP}
No	Fun.	hr^{-1}	hr^{-1}	hr^{-1}			Ka	$V/_1F$	$V/_1F$	$Cmax_D$	$CAmax_E$	$CAmax_E$	%	%	%	%	%	%
8	4030.351	0.04	0.34	1.43	49.4 [1]	0.78							23	ne	50	19	ne	26
	SE %	6	18	16	6	12							25	ne	44	26	ne	14
12	3943.041	0.04	0.37	0.82[1]	0.0209[2]	1.17	1.41	0.0322		0.228[6]			23	ne	27	19	ne	27
	SE %	6	16	10	7	11	30	88					24	ne	45	27	ne	14
13	3938.275	0.04	0.38	0.82[1]	68.4[3]	0.89	1.25				0.231		23	ne	31	19	ne	26
	SE %	6	14	10	4	10	25				22		23	ne	42	27	ne	14
14	3943.041	0.04	0.37	0.82	-3.87[4]	1.17	1.41		0.0317				23	ne	27	19	ne	27
	SE %	6	16	10	2	11	30		86				24	ne	45	27	ne	14
15	3938.275	0.04	0.38	0.82	4.22[5]	0.89	1.25					0.208	23	ne	31	19	ne	26
	SE %	6	14	10	1	10	24					20	23	ne	42	27	ne	14

Reference parameters 1) V_1/F 2) F/V_1 3) $Cmax_E$ 4) ln (F/V_1) 5) ln $(Cmax_E)$

6) No SE estimate since θ_{RD} was derived using test and reference formulation estimations of F/V_1 and Ka.

Figure 5.8 Comparison of relative difference in $Cmax_D$ (Solid line) and $CAmax_E$ (dashed Line) for a two compartment model with first order absorption across different estimates of α, β and Ka. The vertical lines indicate the actual population estimates for this dataset.



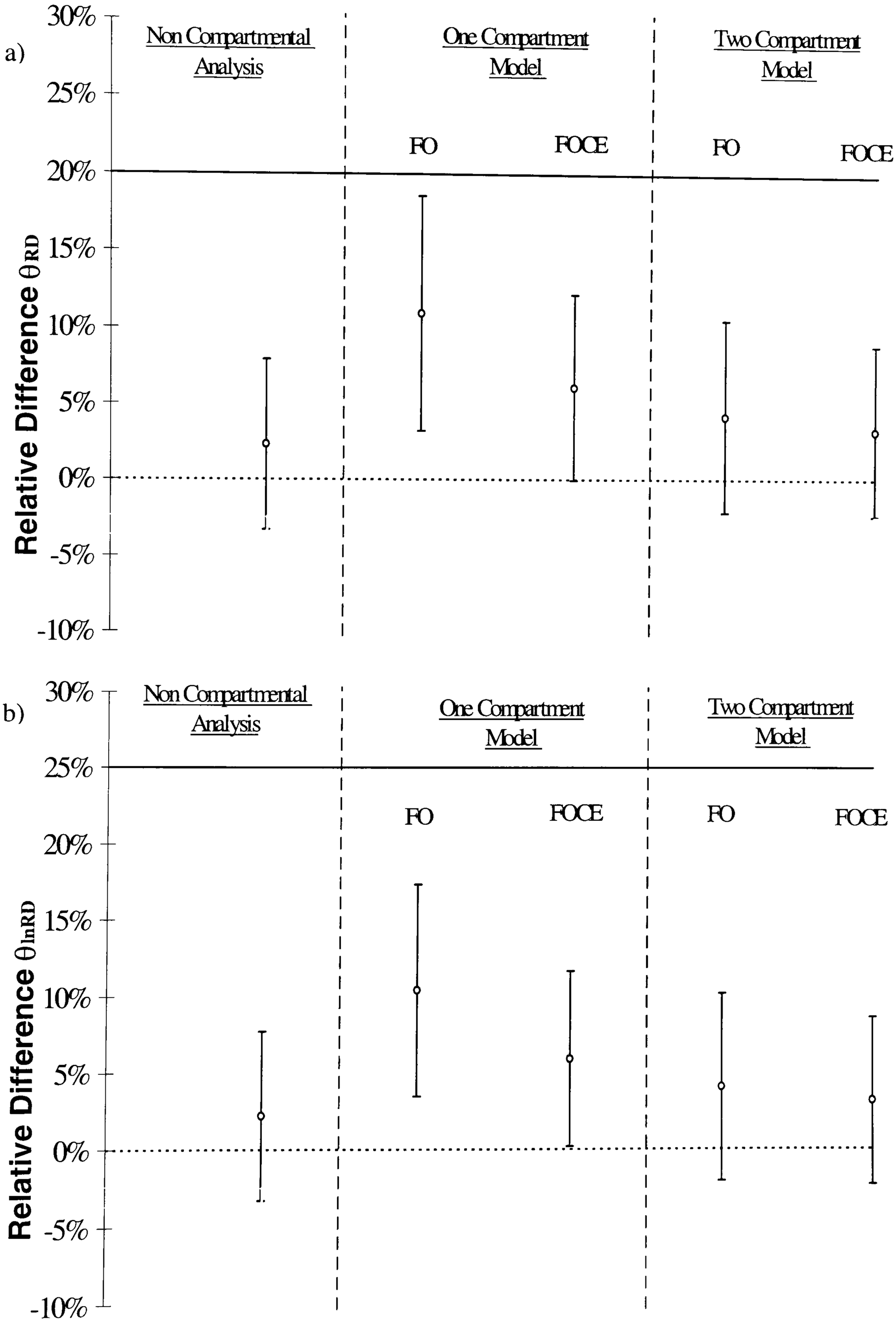
5.5.4 Comparison of compartmental and noncompartmental approaches to bioequivalence assessment

The point and CI estimates for the θ_{RD} and θ_{lnRD} in $\frac{F}{V_1}$, Ka, $Cmax^D$, and $CAmax^E$ for the two compartment model with first order absorption, using FOCE method (run 8 & 12, Table 5.6), are compared to the equivalent non-compartment estimates in Figures 5.9 to 5.11. The point and CI estimates for the θ_{RD} and θ_{lnRD} for the FO fit of the two compartment model and both FO and FOCE fit of the one compartment model, are included to demonstrate the effect of estimation method and model mispecification on the bioequivalence assessment.

Comparison of the point and confidence interval estimates for the relative difference in $\frac{F}{V_1}$

The Null Hypothesis could be rejected with either the one or the two compartment model, so bioequivalence was concluded in each case ($AUC0\text{-}\infty/F/V_1$) (Figure 5.9). The point and interval estimates were practically unchanged by using the multiplicative instead of the additive bioequivalence model. The lack of difference relates to there being no obvious improvement in the distribution upon assuming ln-normality (section 5.5.1). Point estimates obtained from the one compartment model were larger than those obtained using both the noncompartmental and two compartment methods. The CI estimates were largest when the FO method was used. The point and CI estimates were very similar to the noncompartmental estimates when the best fit two compartment model was fitted using the FOCE estimation method.

Figure 5.9 Comparison of the point and interval estimates for the a) θ_{RD} and b) θ_{lnRD} in $\frac{F}{V_1}$ / AUC0-∞



Comparison of the point and confidence interval estimates for the relative difference in Ka, $Cmax^D$ and $Cmax^E$ ($CAmax^E$ for two compartment model)

Ka

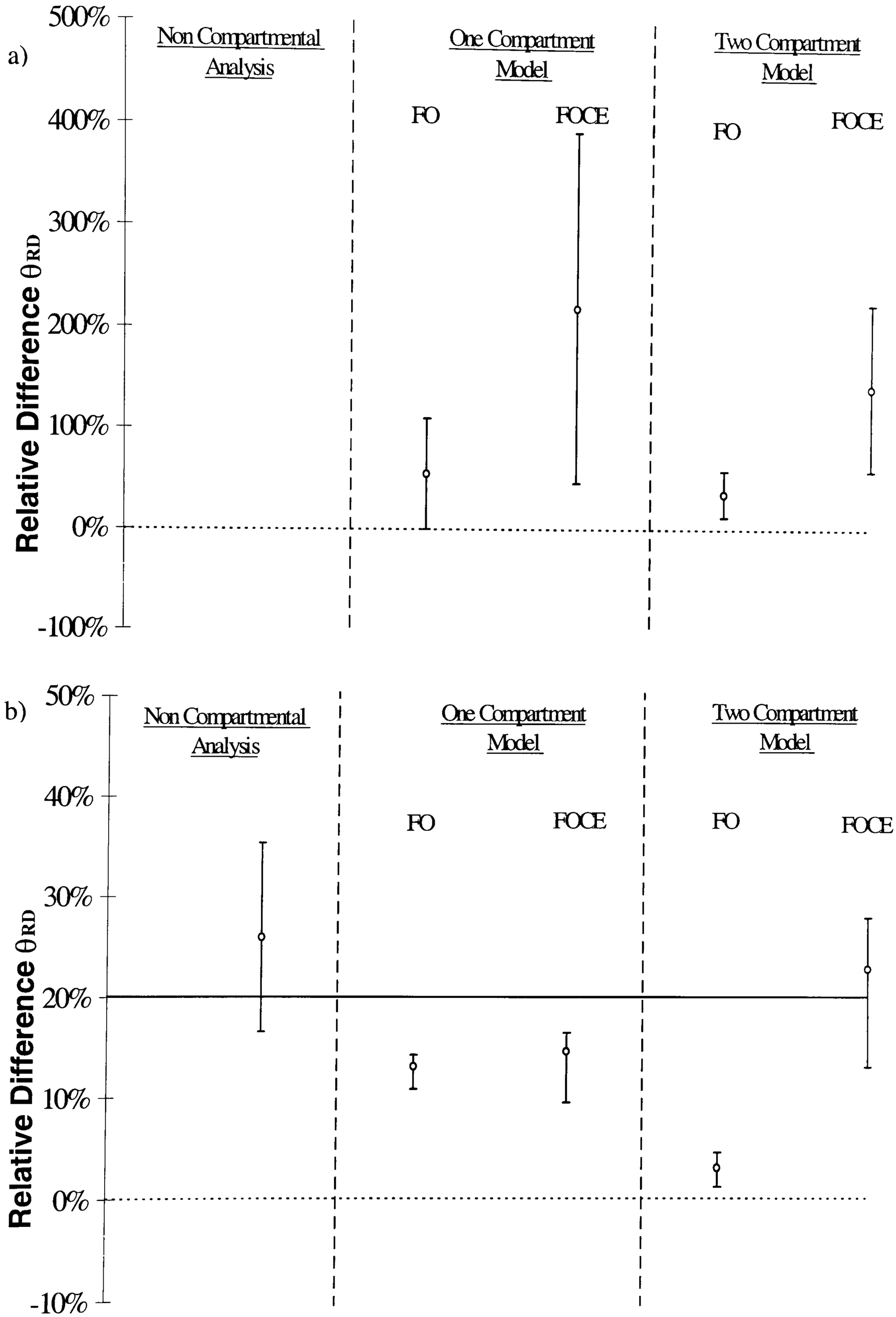
For all models, the point estimate for the relative difference in Ka was significantly different from zero, indicating that the absorption rate for the test formulation was significantly different from the reference formulation (Figure 5.10a). The point and CI estimates of this difference varied according to the estimation method and the model. For the same pharmacokinetic model, the point estimates obtained using the FOCE method were four times greater than those obtained using the FO method. Similarly, for the same estimation method, the CI estimates were greatest for the one compartment model.

$Cmax^D$

Point estimates for the relative difference in $Cmax^D$ were derived using the relative difference estimates for Ka and $\frac{F}{V_1}$ (Figure 5.10b). Corresponding asymmetrical confidence intervals were obtained using the bounds of the CI for the θ_{RD} in Ka (the method used by Kaniwa et al.(1990): the upper confidence intervals in $Cmax^D$ were shorter than the lower confidence intervals.

The Null Hypothesis could not be rejected when the two compartment model was fitted with the FOCE method. The Null Hypothesis of bioinequivalence could be rejected in all other cases where a compartment model was utilised. When the two compartment model was fitted using the FO method the $Cmax^D$ point estimate for the θ_{RD} was very small. In comparison to all other runs, Ka in this run was estimated to be smaller than α. Attempts were made to constrain $Ka > \alpha$ but the run did not successfully minimise.

Figure 5.10 Comparison of the point and interval estimates for the θ_{RD} in a) Ka and b) $Cmax^D$



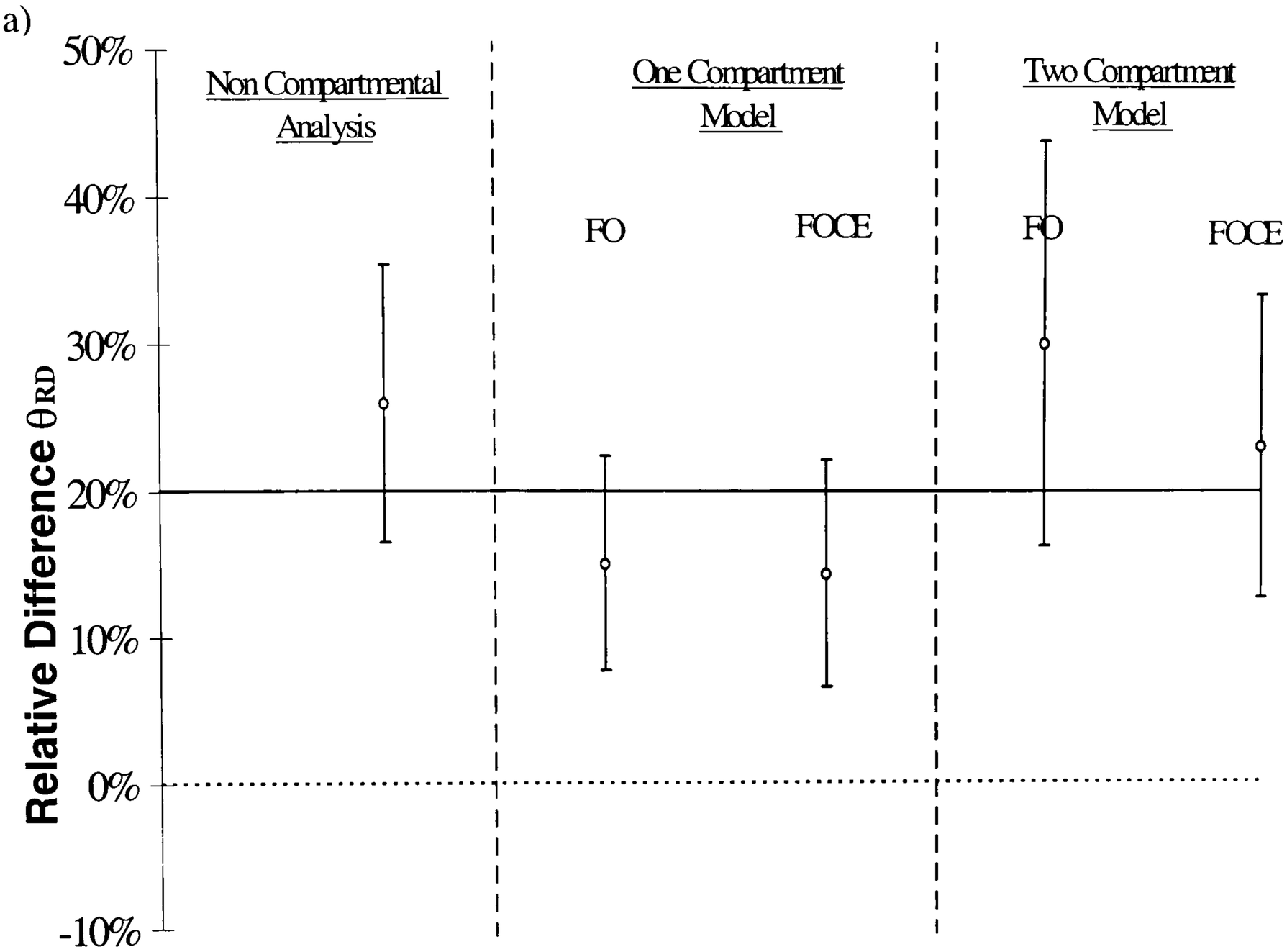
$Cmax^E$ ($CAmax^E$)

For both the $Cmax^E$ (one compartment model) and $CAmax^E$ (two compartment model) models, the Null Hypothesis of bioinequivalence could not be rejected when the additive bioequivalence model was used (Figure 5.11 a). The point and CI estimates for the relative difference using the best fit two compartment model and FOCE estimation were most similar to the noncompartmental estimate. As would be predicted from its distribution (section 5.5.1) all point and CI estimates were reduced when the multiplicative bioequivalence model was implemented (Figure 5.11 b). For the one compartment model, this resulted in rejection of the Null Hypothesis of bioinequivalence.

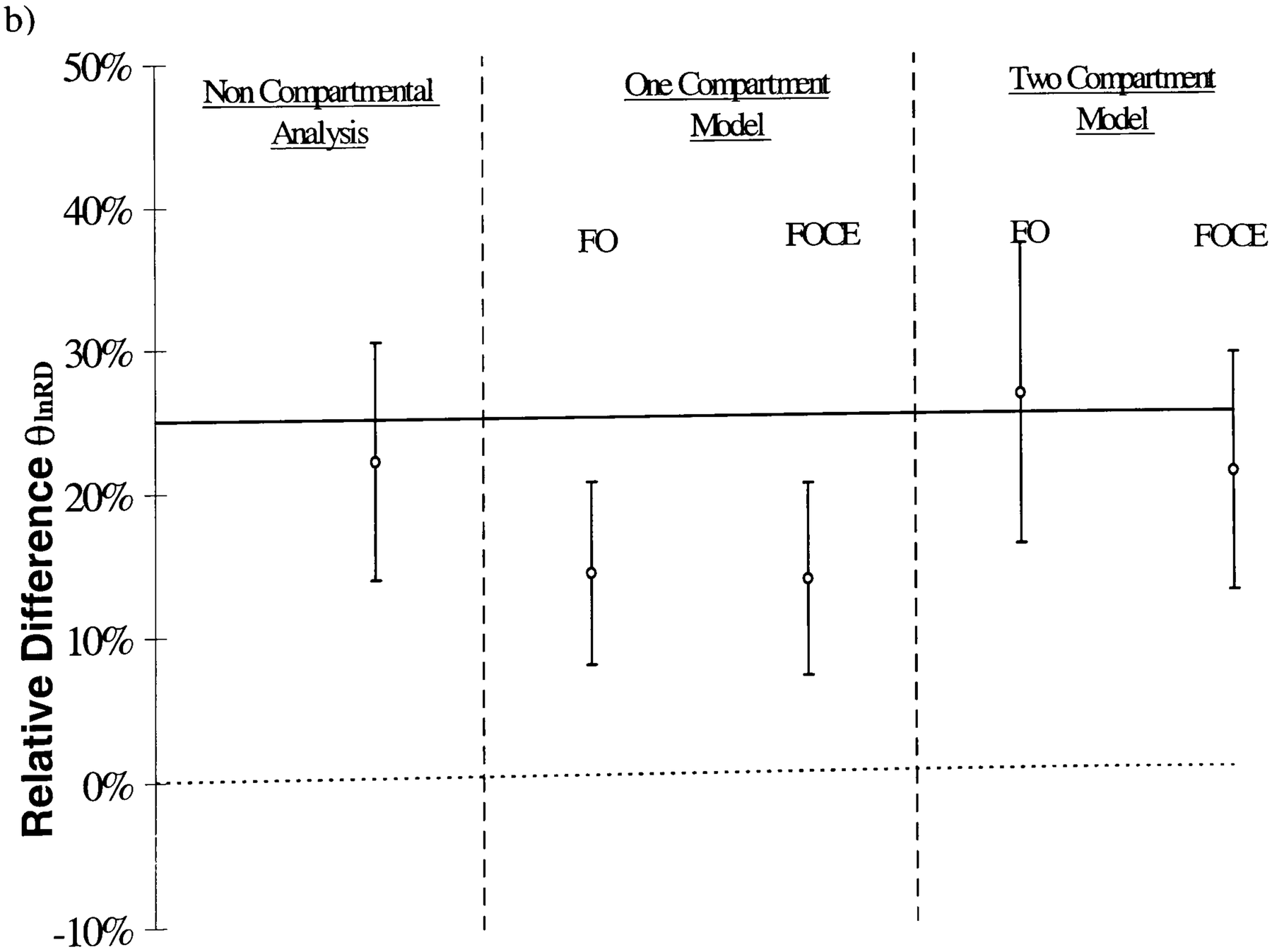
Overall, the results were found to be independent of estimation method. However, the point and confidence interval estimates were most similar to the non-compartmental estimates when the FOCE method was utilised. In this dataset, fitting a one compartment model reduced the ability of the compartmental approach to detect bioinequivalence in Cmax. This is not surprising, since it would be expected that accurate determination of the relative difference in Cmax would requires the biphasic distribution model to be well characterised i.e. use of the correct model.

Figure 5.11 Comparison of the point and interval estimates for the a) θ_{RD} and b) θ_{lnRD} in $Cmax^E$ /$CAmax^E$

a)



b)



5.5.5 Bioequivalence assessment of randomly reduced datasets using a compartmental approach

The ability of the population compartmental approach to conclude the correct result when the data was reduced by 80% was assessed using three different sampling strategies:

Full randomisation (FR): 20% of the concentration data from both studies was randomly sampled

Segmented randomisation (SR): 30% of concentrations before the 3hr time point and 10% of the data after the 3hr time from both studies was randomly sampled. The number of concentrations in each dataset was equivalent to 20% of the original data.

Matched randomisation (MR): 20% of concentrations following administration of the reference formulation were randomly sampled from both studies and then the same time points after the test formulation were extracted. The number of concentrations in each dataset was again equivalent to 20% of the original data.

Number of sampled datasets analysed

Ten datasets were obtained using each randomisation, providing 30 sparse datasets in total. The small number of randomisations used here was limited by time. The original initial plan was to use this investigation as a pilot for a larger simulation analysis.

Comparison of one and two compartment model fits to the sparse datasets (using the FOCE method)

A comparison between the fit of a one compartment model and a two compartment model with first order absorption to each of the 30 sparse datasets is shown on Table 5.7. The AIC criteria (Chapter 3) determined that 90% of the sparse data sets fitted a two compartment model better than a one compartment model.

Table 5.7 Comparison of the objective function and parameter precision from fitting one and two compartment models to the sparse datasets

Full Randomisation							
Objective Function				Parameter Precision			
	OBJ. Diff	Δ AIC	*½	β	α	ka^R	#½
1	19.01	15.01	2	ns	s	s	1
2	6.656	2.656	2	s	s	s	2
3	19.666	15.666	2	s	s	s	2
4	12.508	8.508	2	s	s	s	2
5	17.621	13.621	2	s	s	s	2
6	14.496	10.496	2	ns	ns	s	1
7	25.906	21.906	2	s	s	s	2
8	17.049	13.049	2	s	s	s	2
9	17.268	13.268	2	s	s	s	2
10	3.803	-0.197	1	ns	s	s	1
Match Randomisation							
Objective Function				Parameter Precision			
	OBJ. Diff	Δ AIC	*½	β	α	ka^R	#½
1	3.184	-0.816	1	s	ns	ns	1
2	14.623	10.623	2	s	ns	s	1
3	7.109	3.109	2	ns	s	s	1
4	11.136	7.136	2	s	s	s	2
5	13.681	9.681	2	s	ns	s	1
6	19.408	15.408	2	s	s	s	2
7	16.749	12.749	2	ns	s	s	1
8	23.752	19.752	2	s	s	s	2
9	19.005	15.005	2	s	s	s	2
10	10.166	6.166	2	s	ns	s	1
Segmental Randomisation							
Objective Function				Parameter Precision			
	OBJ.Diff	Δ AIC	*½	β	α	ka^R	#½
1	14.645	10.645	2	s	ns	s	1
2	3.183	-0.817	1	s	s	s	1
3	7.832	3.832	2	s	ns	s	1
4	11.144	7.144	2	s	s	s	2
5	6.877	2.877	2	s	s	s	2
6	10.142	6.142	2	s	ns	s	1
7	7.757	3.757	2	s	s	s	2
8	11.194	7.194	2	s	s	s	2
9	11.334	7.334	2	ns	s	s	1
10	6.663	2.663	1	ns	s	s	1

Δ AIC=change in the AIC between a 1 and 2 compartment model. s= Parameter estimate statistically different from zero ns= Parameter estimate not statistically different from zero.
*1/2 No of compartments for best fit model based on ΔAIC
#1/2 No of compartments for best fit model based on ΔAIC and parameter precision

However, if precision of the α ,β and Ka_R estimates (the parameters required to correctly characterise a two compartment model) were additionally considered in deciding which model was most appropriate, parameter estimates were only significantly different from zero in 50% of cases. Imprecise two compartment parameter estimates were obtained for 3 of the 10 FR datasets, 6 of the 10 MR and 6 of the 10 SR datasets.

Assessment of bioequivalence in the sparse datasets assuming a two compartment model

Figures 5.12 and 5.13 show the point and CI estimates for the θ_{RD} and the θ_{lnRD} in $\frac{F}{V_1}$ and $Cmax^E$, respectively, for the sparse datasets, using a two compartment model with first order input and the FOCE estimation method.

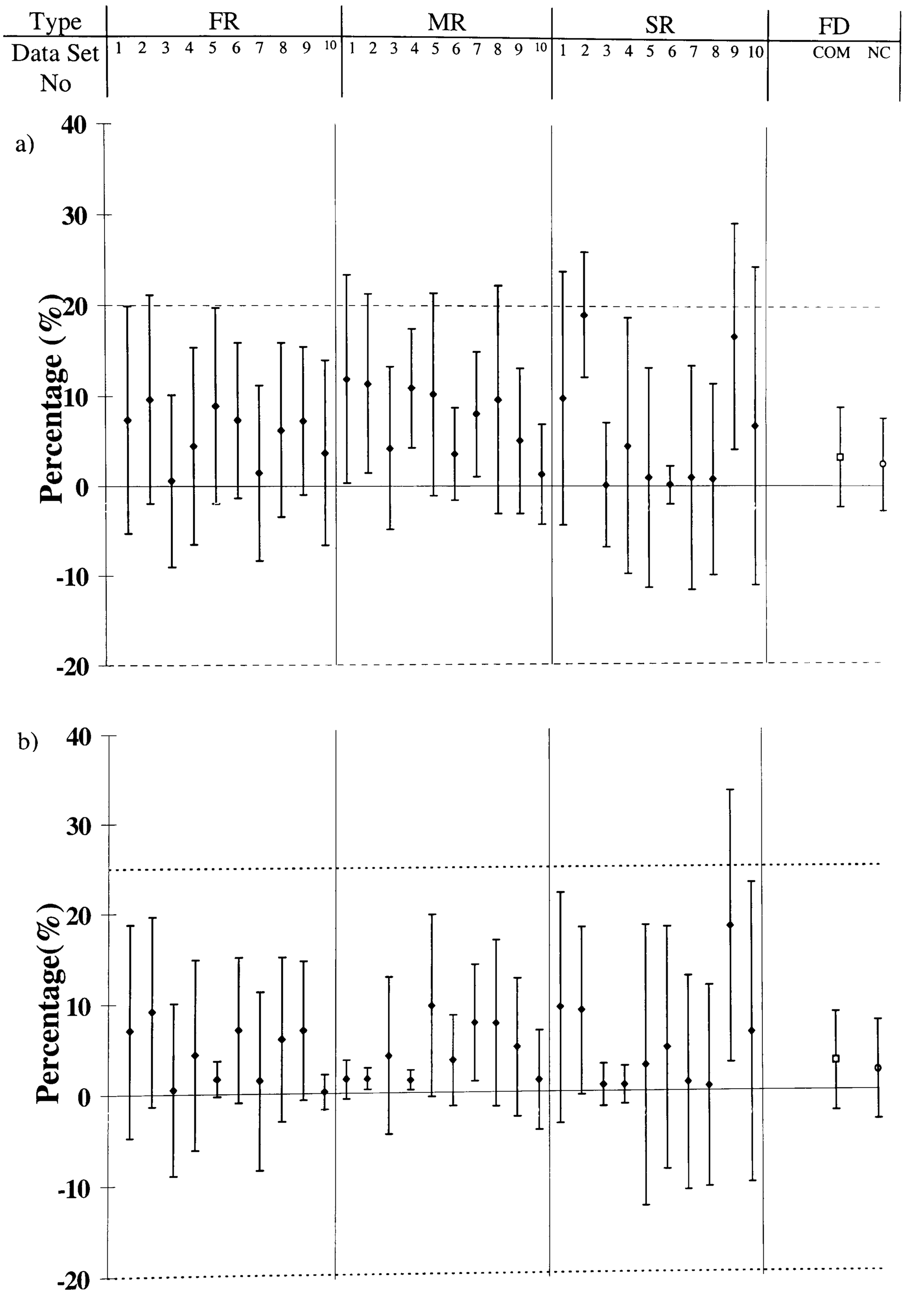
F/V_1

The average θ_{RD} point estimates across the FR, SR and MR datasets were +5.7% , +6.0% and +7.6%, respectively. The average for all 30 datsets was +6.4% which was 100% larger than the point estimate estimated using the full dataset (FD). The width of the average CI across all the sparse datasets was also almost twice that estimated for the full dataset (20 vs 11%, respectively).

The average θ_{lnRD} point estimates across the FR, SR and MR datasets, were +4.4% , +4.3% and + 5.4%, respectively. The average for all 30 datsets in this case was +4.7 %, which was 40% larger than the point estimate for the full dataset (+3.2 %).

The average width of the CI across all the sparse datasets was smaller when the multiplicative bioequivalence model was fitted (16% vs 20%) and therefore closer to that for the full data set (11%).

Figure 5.12 The point and interval estimates for a) θ_{RD} and b) θ_{lnRD} in F/V_1 for the sparse datasets. COM is the full dataset (FD) analysed using the compartmental approach and NC the full dataset (FD) analysed using noncompartmental approach



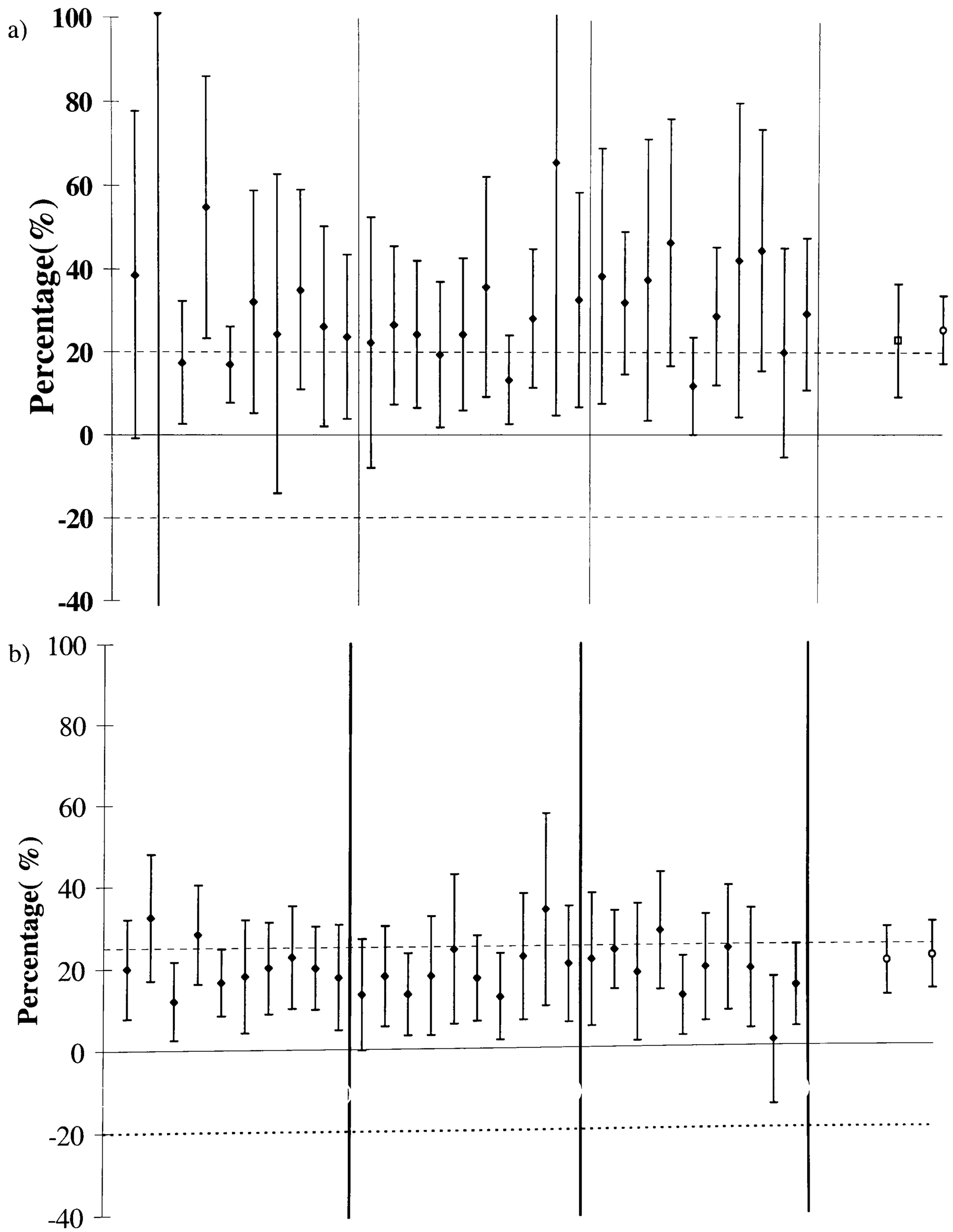
$Cmax^E$

The average θ_{RD} point estimates across the FR, SR and MR datasets were +37.1% , +29.3% and +33.2%, respectively. The average for all 30 datsets was +33%, which was 40% larger than the point estimate obtained using the full dataset (FD). The average width of the CI across all the sparse datasets was also almost 300% larger than that for the FD (59.5 % vs 20.7%, respectively).

The average θ_{lnRD} point estimates across the FR, SR and MR datasets, were +20.8% , +19.4% and + 18.4%, respectively. The average for all 30 datsets was +19.5%, which was very similar to the point estimate for the full dataset (+20.8). The average width of the CI was narrower and hence closer to the FD estimate when the muliplicative bioequivalence model was used (26.6% vs 16.8, respectively).

Figure 5.13 The point and interval estimates for a) θ_{RD} and b) θ_{lnRD} in $Cmax^E$\$CAmax^E$ for the sparse datasets. COM is the full dataset (FD) analysed using the compartmental approach and NC the full dataset (FD) analysed using noncompartmental approach

Type	FR_{FOCE}										MR_{FOCE}										SR_{FOCE}										FD	
Data Set No	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	COM	NC



Hypothesis testing: **Comparison with noncompartment estimates of bioequivalence**

Table 5.8 shows the number of datasets where the Null Hypothesis of bioinequivalence was not rejected, split by sampling scheme and repeated for additive (θ_{RD}) and multiplicative (θ_{lnRD}) bioequivalence models. The effect of choosing the most appropriatte model determined in Table 5.8 on the hypothesis test was also investigated.

Table 5.8 The number of Null Hypothesis of Bioinequivalence which were rejected, split by sparse sample scheme, compartmental model and bioequivalence model

	2 Compartment				**1/2 model**				**Total**	
	F/V_1		$Cmax_E$		F/V_1		$Cmax_E$		F/V_1	$Cmax_E$
	θ_{RD}	θ_{lnRD}	θ_{RD}	θ_{lnRD}	θ_{RD}	θ_{lnRD}	θ_{RD}	θ_{lnRD}	n/40	n/40
FR	9	10	0	2	9	10	0	2	38	4
MR	6	10	0	2	6	10	0	7	32	9
SR	6	9	0	3	6	9	1	3	30	6
Total for all sparse datasets	21	29	0	7	21	29	1	12		

1/2 model Relative difference when using the most appropriate model, as determined in Table 5.7

Effect of sampling scheme

When a two compartment model was used to estimate the θ_{lnRD} in $\frac{F}{V_1}$ and $Cmax^E$, the number of Null Hypotheses which were rejected was similar across the three sampling schemes. However, the total number of times the Null Hypothesis of bioinequivalence in $\frac{F}{V_1}$ was rejected was greatest for the FR sampling scheme. Similarly, the number of times the Null Hypothesis of bioinequivalence in $Cmax_E$ was rejected was also smallest when the FR sampling scheme was used.

Effect of bioequivalence model

More Null Hypotheses of bioinequivalence in $\frac{F}{V_1}$ were rejected when the multiplicative model was used. In contrast, more null hypotheses of bioinequivalence in $Cmax_E$ were not rejected when the additive bioequivalence model was used.

Effect of choosing best fit model rather than the two compartment model

Using the best fit model did not affect the number of Null Hypothesis of bioinequivalence in F/V_1 which were rejected. However, the number of times the Null Hypothesis of bioinequivalence in $Cmax_E$ was rejected increased from 0 to 1 for the additive bioequivalence model and from 7 to 12 for the multiplicative bioequivalence model.

5.6 Discussion

The results show that a population compartmental approach to bioequivalence can provide point and CI estimates for the relative difference in absorption rate and in the extent of absorption, similar to those estimated using the standard noncompartmental approach. The population approach may therefore have application in bioequivalence testing.

5.6.1 Data set and noncompartmental bioequivalence assessment

Both the additive and multiplicative bioequivalence models determined that the formulations were bioequivalent in $AUC_{0-\infty}$ and bioinequivalent in Cmax. The affect of the bioequivalence model on the point and CI estimates was investigated. The 90% CI for the relative difference in Cmax was narrower using the multiplicative bioequivalence model. Conversely, since ln-transformation did not normalise the distribution of $AUC_{0-\infty}$, the point and 90% CI estimates for the relative difference in $AUC_{0-\infty}$ were not affected by using the multiplicative bioequivalence model. The wider acceptance interval (-20% to +25%) used with the multiplicative bioequivalence model provided a greater chance of concluding bioequivalence (Chow & Liu, 1994). The point estimate for θ_{lnRD} in $Cmax^E$ was less than the upper limit of bioequivalence due to the wider acceptance intervals and the increased homogeneity of the variance upon ln-transformation. The similarity of the point and 90% CI, across the two studies, was expected, given that 2.5 mg tablets were used in both studies. The 90% CI for the θ_{RD} in Cmax was wider in 2.5mg study, but this was reduced on using the multiplicative model.

The single concentration measurements used for Cmax are generally considered to be more variable than the integrated $AUC_{0\infty}$, so wider acceptance limits for Cmax have been advocated and -30% to + 43 % have previously been accepted by the European regulators (Steinijans et al., 1992). However, Cmax often relates to adverse events, so the acceptance limits should probably be considered on a case by case basis, with variability and PK /PD

relationship being taken into account (Benet & Goyan, 1995). In this analysis the ± 20% acceptance intervals were used for Cmax to impose the strictest criteria and therefore the opportunity of showing a difference between the compartmental and noncompartmental assessments of bioequivalence.

5.6.2 Compartmental assessment of bioequivalence using the full dataset

The isolated bioinequivalence in Cmax coupled with the need for a bi-exponential equation to describe the drug distribution, represented a problem which was more complex than that previously investigated using a compartmental approach.

While a two compartment model was shown to be more appropriate than a one compartment model, the optimal model to describe the absorption was not as clearly determined. The FOCE method showed that there was little difference between the objective functions for the first and zero order absorption models. However, the rounding errors with the zero order model resulted in the failure to converge, so the first order absorption model was used to ensure that CI for the relative difference could be calculated. The small absorption lag time of 0.37 hr was less than the first sampled time point (0.5 hr) and not significantly different from zero. More intensive sampling to increase the precision in this estimate would probably not be practical. It is possible that the structure of the absorption model may differ between subjects. However, the lack of bias in the weighted residual versus time plots would indicate that the single first order rate model was the most appropriate model for the average subject.

The bias in the weighted residual versus time plot using the FO method was removed when the fit was repeated using the FOCE method. Therefore the advantage of the FOCE over the FO method was again demonstrated.

Mis-specifying the model, by using a one compartment model, resulted in under-estimation of Cmax, so it is not surprising that the point estimates for the relative differences, were found to be lower than those estimated using the two compartment model.

The model based approach provided estimates of the relative difference in the rate of absorption (Ka). Previous investigation of bioequivalence data using compartmental modelling has centred on making these comparisons (Graves & Chang, 1989; Piotrovskij et al., 1995). However, while identifying a difference in the rate of absorption can be useful for comparing the release profiles the clincal relevance of any difference is difficult to assess. On the other hand, due to its correlation with the extent of absorption, Cmax is not a good estimate of rate of absorption, but it has greater clinical relevance.

In this analysis, the work by Kaniwa et.al. (1990) has been extended to include the estimation of a more appropriate symmetrical confidence interval for the relative difference in Cmax. These were found to be very similar to those estimated using the noncompartmental method. The concentration at the time when the total amount absorbed per unit volume reaches a maximum (CAmax) was used as an approximation for Cmax. For this dataset, the point estimates for the relative difference in CAmax and Cmax were very similar. However, this approximation was shown to be highly dependent on the assumptions of $Ka > \alpha >> \beta$. In particular, β had to be small so that the amount eliminated up to Tpk was negligible. In this case the assumption was valid as the terminal half-life was 17 hours in comparison to the initial distribution and absorption half-lifes of 2 and 0.5 hours, respectively. The potential bias in the point estimate for relative difference was shown to increase dramatically as the half-lifes approach one another. On fixing all other parameters, a bias of 20% and 50% was demonstrated when $T_{1/2}\ \beta$ was set to 7 hours and 3.5 hours, respectively.

5.6.3 Bioequivalence assessment of randomly reduced datasets using a compartmental approach

The application of the population compartmental approach to bioequivalence testing was further investigated with the data randomly reduced by 80%. The robustness of the estimates under this reduction was investigated by repeating the reduction ten times for each of three sampling schemes. The bioequivalence in $AUC_{0-\infty}$ and bioinequivalence in Cmax was shown to be relatively robust to the sampling designs.

The noncompartmental and compartmental point and CI estimates of the relative difference in both rate and extent of absorption were similar using the multiplicative bioequivalence models. In comparison, the additive θ_{RD} point and CI estimates were inflated. The multiplicative model, may suggest that the modelling approach is more dependent on the ln-normality assumption when the data is reduced, however, this can only be confirmed by further investigation.

The increase in the number of Null Hypotheses of bioinequivalence in CAmax which were rejected when the multiplicative model was implemented, may be due to the wider acceptance limits and greater homogeneity in the variance estimate.

There was little difference between the sampling schemes in terms of the average point estimates for the relative difference. However, more datasets provided by the completely random sample scheme (FR) were appropriately described by the two compartment model. In addition, bioequivalence in extent of absorption and bioinequivalence in rate of absorption was more often demonstrated with the FR sampling scheme.

The most appropriate model for each of the datasets resulted in the Null Hypothesis of bioinequivalence in CAmax being rejected on more occasions than when the two compartment model was used. Based on this result, it would appear that prior knowledge of the distribution model is required, and should be used even when the sparse data seem to be appropriately described by a simpler model.

5.6.4 Application of population approach to bioequivalence testing

Standard bioequivalence data

While standard 2x2 cross-over studies, may not benefit from using a model dependent population approach, there may be some circumstances where modelling the data may offer additional information. Meta-analysis may be performed to investigate potential reasons for differences in Cmax, such as dose dumping (Graves & Chang, 1989).

Population pharmacokinetic bioequivalence investigation

The design of bioequivalence studies to utilise the population approach is more controversial. In particular, the transition from constrained experimental data to the "observational type" data used in population analysis may be too radical for the regulatory authorities and the pharmaceutical industry. However, a population pharmacokinetic approach may have application to situations where the standard 2x2 cross-over study is not appropriate.

Complex designs

The 2x2 cross-over design is most often considered as it removes interindividual variability from the comparison. However, when the intrinsic intrasubject variability is large, the power of the analysis is greatly decreased (Ekbomn & Melander, 1989). Since intrinsic intraindividual variability cannot be calculated from 2x2 cross-over studies, higher-order cross-over designs are required to estimate this variability (Kershner & Federer, 1981; Laska & Meisner, 1985; Jones & Kenward, 1989). If more than two formulations are to be compared, then the number of study days in each sequence can become very large. Higher order cross-overs can be time consuming and costly. In particular, they may require an unethical amount of plasma sampling and have an increased tendency for study dropouts (Westlake, 1973). By removing the need for full plasma concentration time profiles, the approach may make some of the higher cross-over designs easier to implement.

Patients vs Volunteers

Where bioequivalence testing is required for drugs which can only be ethically tested in patients, such as cytotoxics, high dose opiates, tamoxifen and flutamide etc., sparse sampling would allow the test to be conducted during the course of routine treatment. Application of the population approach may also allow testing of the assumption that pharmacokinetic equivalence is a substitute for therapeutic equivalence (Benet & Goyan, 1995; British Pharmaceutical Conference, 1995; Levy, 1995; Marzo, 1995). However, very few cases of clinical inequivalence have been identified. In one example, the concentration of cyclosporin after administration of its micro-emulsion formulation (Neoral) is reduced in liver transplant patients, the bile required for absorption is reduced as a result of choleostasis (Friman & Backman, 1996). Nevertheless, the limited number of identified clinical inequivalences may be due to the lack of prospective pharmacokinetic studies in this area. Even when a measurable and comparable response can be recorded, the large number of formulations available makes adhoc detection of defective formulations extremely difficult.

The population approach could be used to test for bioequivalence differences in formulations which are thought to show clinical inequivalence.

With the potential advent of new guidelines which separate population and individual bioequivalence, the importance of characterising the interindividual and intraindividual variabilities has become more important (FDA, 1997). As suggested by Sheiner (1992), the application of the population pharmacokinetic approach in this area requires further investigation.

5.7 Conclusions

In this chapter, a population compartmental approach to standard "full dataset" bioequivalence studies has been implemented. The most appropriate two compartment model provided point and CI estimates for the θ_{RD} and θ_{lnRA} in the "rate" and "extent" of absorption, which were almost identical to the noncompartmental estimates. The FOCE method should be utilised in preference to the FO approach, since it improved the model fit and provided the θ_{RD} and θ_{lnRA} point and CI estimates which were most similar to the noncompartmental estimates.

Symmetrical confidence intervals were successfully estimated by parameterising for Cmax within the compartment model. The compartmental bioequivalence models were extended to allow the present requirements for ln-transformation of parameters to be met. The application of the population approach to bioequivalence testing was robust even when the data was reduced by 80%. A complete randomised (FR) sample scheme performed best, but further investigation is required.

The application of the population approach may be useful in helping to reduce the amount of plasma sampling when complex designs are required. Furthermore, when patients have to be used for bioequivalence testing, the population approach may allow this to be implemented as part of routine clinical practice.

5.8 Future work

Confidence intervals

Exact 95% CIs for the hypothesis test have previously been based on NONMEM's maximum likelihood estimate (Combrink et al., 1997). The shift in parameter estimate which causes an objective function change of +3.68 corresponds to the 95% CI limit. A full simulation or bootstrapping technique has also been used to estimate the CI for the

relative difference in F/V_1 and Cmax (Pentikis et al., 1996). Further work using these approaches to calculate the confidence intervals is required.

Sample Size

A sample size of 20% of the original data was used in this study to represent the 4 to 5 samples per patient (at least two per formulation per patient in the majority of patients), and is consistent with that used by Kaniwa at al. (1990). It was proposed here as an arbitrary defined minimum that a prospective population bioequivalence investigation would require. Large simulation studies are required to explore the pharmacokinetic, variability and sampling issues identified in this analysis. The different randomisations represent potential study designs for the collection of sparse bioequivalence data, so the requirement for further work on optimal designs for a sparse data approach to bioequivalence testing is also highlighted.

CHAPTER 6

THE DOSE RESPONSE RELATIONSHIP FOR THE HMG COA REDUCTASE INHIBITOR SIMVASTATIN

In this chapter, mixed effect modelling is utilised in the assessment of the dose response relationship for the HMG-CoA reductase inhibitor simvastatin. The change in total cholesterol and each of its various subfractions is investigated using a set of hierarchical models. A covariate analysis is undertaken to determine which factors most influence the lipid response. The consequence of the apparent inappropriateness of the current recommended dosage regimen is discussed and alternative dosing strategies are compared through simulation.

6.1 Introduction

6.1.1 Dose ranging studies

The primary aim of phase II studies is to confirm the efficacy and tolerability of a new drug in the larger patient population and provide the PK/PD information for development of a dosing regimen for the Phase III programme. Three basic study designs which are generally utilised to provide this information are discussed below.

Dose escalation design

This design has been considered to most closely resemble clinical practice since the dose is increased until a desired response is obtained. However, it has been associated with an overestimation of the minimum dose required to produce this response (Temple, 1982; Freston, 1986) and has been linked to the introduction of atenolol, captopril (Temple, 1982; Reid & Meredith, 1990) chlorothalidone (Tweeddale et al., 1977; Materson et al., 1978) and chlorthiazide (Berglund & Andersson, 1976) at doses which were ultimately found to be higher than those required to treat the majority of patients. As suggested by Sambol et al., (1991), the expectation of unrealistic therapeutic responses may have exacerbated the underlying design problem. Sheiner et al.,(1989, 1991) have suggested that more appropriate interpretation of the data may have prevented this overestimation.

They highlighted, that since sensitive subjects achieve an acceptable response at lower doses, the mean response at the higher doses only reflects the response for the insensitive subjects. They proposed that the inherent bias in the escalation design could be largely avoided by utilising a mixed effects modelling approach. While this approach does not fully account for the observations missing from the sensitive patients at higher doses, they suggested that maintaining these subjects on their final dose throughout study period would further help to reduce the bias in estimation of the "typical" dose response relationship. Unfortunately, the sequential nature of the design makes it prone to bias when there are carry-over effects or time dependent changes in the disease state (Girard et al., 1995).

Parallel group design

In this case, subjects are randomised to receive either placebo or one of several selected doses. The advantage of this design is that it only lasts for one or two (if the study utilises an additional placebo run in phase) treatment periods. Therefore, the study is easy to manage, and patients are not lost on follow-up. While it was proposed that this design avoided the bias inherent in the escalation studies (Temple, 1982), Sheiner et al. (1989, 1991) and Sambol et al. (1991) disputed its appropriateness for dose ranging studies in general. Interpolation between the pre-selected doses may be prone to bias since the dose response relationship is only based on one dose response measurement per subject. Thus, the selection of the minimum effective starting dose for future studies is most often taken to be the lowest effective dose in comparison to placebo and therefore pre-selected in the study design. The single administration also prevents the estimation of intraindividual variability, so there is limited information on which to build a model for the individualisation of dose after observation of initial response.

Cross-over design

This design although potentially more complicated and problematic from an ethical stand point has been shown to be very robust in the estimation of dose response relationships

(Girard et al., 1995). In this design every subject receives every dose, so the ratio of sensitive to insensitive patients is the same at each dose level. Furthermore, the study design allows information to be gained by utilising both analysis of variance (ANOVA) and population dose response modelling. This chapter utilises both approaches in the establishment of a dose response relationship for simvastatin.

6.1.2 Hypercholesterolaemia: Clinical consequence and treatment

It is well established that hypercholesterolaemia is a major risk factor for the development and progression of atherosclerotic cardiovascular disease and it is also generally accepted that lipid lowering strategies are indicated for its prevention. In most instances, dietary intervention remains the recommended first step towards cholesterol reduction. When drug treatment is indicated, the 3-hydroxy-3-methylglutaryl-Coenzyme A (HMGCoA) reductase inhibitors, including simvastatin, have become established as effective and well tolerated treatments. The main goal in the treatment of hypercholesterolaemia is to reduce the risk of the premature development (primary intervention) or the recurrence (secondary prevention) of vascular events, and the HMG CoA inhibitors have now been shown to be beneficial in reducing the incidence of both (MAAS Investigators, 1994; Pederson, 1994; Shepherd et al., 1995).

Common or "polygenic" hypercholesterolaemia arises from a combination of genetic, dietary and environmental factors, and is usually mild to moderate in degree. The more serious genetic or "familial" hypercholesterolaemia affects about 1 in 500 people and is associated with a greater CHD risk. The treatment strategy is similar for both forms, with a more aggressive approach being adopted when a genetic predisposition is known. Patients with a cholesterol greater than 5.2 $mmol.l^{-1}$ are initiated on a lipid lowering diet and, depending on the response and presence of other risk factors, drug treatment may be considered. At present the accepted target range in control of hypercholesterolaemia is a

total cholesterol < 5.6 mmol.l^{-1} where it is an isolated risk factor and <5.2 mmol.l^{-1} where there are multiple risk factors present.

6.1.3 Lipoproteins and cholesterol: Classification

Plasma lipoproteins are water-soluble complexes composed of lipids (triglycerides, cholesterol and phospholipids) and one or more specific proteins, called apolipoproteins. They are broadly classified as high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) andchylomicrons. Each has a particular role in the transportation and utilisation of lipids.

The chylomicrons, pass dietary cholesteryl esters from the gastro-intestinal tract to the liver, where the cholesterol is stored or oxidised to form bile acids. Cholesterol can also be synthesised from acetyl CoA in the liver. The rate determining step for this process is the conversion of HMG-CoA to mevalonate and this is controlled by the HMG-CoA reductase enzyme. Sixty to seventy percent of the total plasma cholesterol is contained within circulating LDL particles. Cells requiring cholesterol synthesise receptors and take up LDL by receptor-mediated endocytosis. Free cholesterol from dead cell membranes is adsorbed onto HDL particles and esterified with long chain fatty acids. The resulting cholesteryl esters are subsequently transferred to LDL or VLDL (when triglycerides are present) particles and redistributed.

6.1.4 Simvastatin

Simvastatin is a semisynthetic prodrug, which is a structural analogue of lovastatin, a fermentation product of Aspergillus terrus (Hoffmann et al., 1986).

Mechanism of action

Several of its metabolites, most notably simvastatin acid are active and capable of competitively and reversibly inhibiting HMG CoA reductase, (Mauro, 1993). Inhibition of hepatic cholesterol biosynthesis, gives rise to an increased expression of LDL receptors.

These receptors bind LDL particles and remove them from circulation thus lowering total circulating cholesterol (Plosker & McTavish, 1995).

Pharmacokinetics

Simvastatin is well absorbed (approximately 60-80%) but undergoes extensive first pass metabolism. The bioavailability of simvastatin acid has been shown to be less than 5% (Todd & Goa, 1990; Mauro, 1993). Several studies in healthy volunteers (Todd & Goa, 1990; Pentikainen et al., 1992; Mauro, 1993) and in patients with hypercholesterolaemia (Cheng et al., 1992) have demonstrated that the peak concentrations of the active metabolites occur after 1 to 3 hours. Animal enzyme inhibition and radiolabelled drug studies have demonstrated that simvastatin and its metabolites concentrate in the liver (Todd & Goa, 1990; Mauro, 1993). Correspondingly, the circulating levels of simvastatin are lower than that for other less lipophilic statins (Pentikainen et al., 1992). At least five metabolites including the simvastatin acid have been identified by animal microsomal studies (Plosker & McTavish, 1995). The hepatic extraction ratio for simvastatin is large (93%) and the majority of the metabolites are found in the bile (Todd & Goa, 1990; Mauro, 1993). The total body clearance for simvastatin is approximately 31.8L.hr^{-1} and the elimination half-life for simvastatin acid is around 1.9 hours. Less than 10% of the peak HMG CoA reductase activity remains after 12 hours (Mauro, 1993).

Similar effects of gender and age on the pharmacokinetics of the HMG CoA reductase inhibitors were noted following a single dose of atorvastatin (Gibson et al., 1996) and multiple doses of simvastatin and lovastatin (Cheng et al., 1992). After 17 days of simvastatin (40mg per day), the mean plasma concentration was 40-60% higher in elderly patients than young patients and 20%-50% higher in females than males (Walker, 1989).

Tolerability and adverse effects

Adverse events with simvastatin are usually mild and transient with treatment related discontinuation rates being between 2% and 6% (Todd & Goa, 1990; Pederson, 1994;

Pedersen & Tobert, 1996). Creatinine kinases are raised in about 5% of patients, but the incidence of myopathy and rhabdomyolysis is rare (Thompson, 1993; Plosker & McTavish, 1995).

Clinical efficacy

Decreases of 20 to 40% in total serum cholesterol, 35 to 45% in LDL cholesterol, 20 to 40%in LDL : HDL cholesterol and 10 to 20% in total triglycerides, as well as increases of 5 to 15% in HDL cholesterol have been shown (Plosker & McTavish, 1995). The dose escalation studies which have been undertaken were not placebo controlled i.e. (Molgaard et al., 1988; Leclercq & Harvengt, 1989; Sirtori et al., 1989; French et al., 1990). The placebo controlled dose ranging trials are summarised in Table 6.1. Although the lipid lowering efficacy of simvastatin is well recognised, it appears that the current dose range was based mainly on parallel dose studies. Furthermore, although dose ranging studies have been performed, there do not appear to be any published account of attempts to model the dose response relationship.

Factors important in the prediction of clinical response

It has been shown that the pre-treatment level may be correlated with the percentage reduction in both total cholesterol and LDL cholesterol (Molgaard et al., 1988; Miserez et al., 1994). However, other studies have suggested that the percentage change is independent of baseline level (Farish et al., 1990; Todd & Goa, 1990; Frohlich et al., 1993).

Table 6.1 Placebo controlled dose ranging studies for simvastatin

Reference	Patient Type	Duration (weeks)	Study design	No Patients	Dosage Regimen (mg) *	Total-C (%)Δ	LDL-C (%)Δ	HDL-C (%)Δ	Tri-gylcerides (%)Δ	LDL /HDL-C (%)Δ
Mol et al. (1986)	Familial	4	Parallel Group	8	Placebo	-5	-6	-1	+2	-5
				8	2.5	-16	-18	+2	-17	-19
				4	5	-22	-27	-2	+5	-8
				8	10	-25	-28	+10	-26	-34
				4	20	-25	-30	+11	-14	-36
				7	40	-32	-37	+21	-34	-47
				4	80	-36	-42	+8	-11	-47
Sim ons et al. (1987)	Familial & poly-genic	4	Parallel group	5	Placebo	+5	+8	0	-20	+8
				15	2.5-10	-20	-20	0	-24	-20
				10	20-80	-30	-37	+8	-44	-42
Kuhn et al. (1989)	Elderly	4	Parallel group	4	Placebo	+1	+2	+11	-12	-9
				5	2.5	-10	-19	16	+16	-23
				4	5	-14	-20	-2	+8	-18
				6	10	-21	-30	+9	-15	-35
				4	20	-35	-49	+4	-14	-51
Nakaya & Goto (1989)	Not defined	4	Parallel group	5	Placebo	+3	+2	+12	-13	
				5	1.25	-8	-8	+4	-13	
				5	2.5	-13	-20	+18	-25	
				5	5	-20	-27	+4	-14	
				5	10	-24	-33	+4	-16	
				5	20	-26	-40	+12	-10	
Goto et al. (1989)	Not defined	12	Parallel group	72	placebo	-3	-6	-2	+9	
				72	2.5	-15	-24	+6	-9	
				72	5	-21	-30	+6	-14	
Walker et al. (1990)	Elderly	4	Parallel group	31	placebo	-3	-3			
				32	2.5	-17	-23			
				32	5	-19	-27			
				32	10	-23	-31	+11	-15	
				32	20	-28	-37	+7	-20	
Keech et al. (1994)	Poly-genic	8	Parallel Group	207	placebo					
				208	20	-27	-38	+5	-17	
				206	40	-29	-41	+6	-19	
Tuomilehto (1994) et al.	Not defined	8	Parallel Group	28	placebo	-3	-5			
				28	2.5	-16	-21			
				28	5	-20	-25			
				27	10	-22	-28			
				26	20	-25	-33			
				29	40	-30	-41			

* Daily dose Δ % change from baseline

While age has been shown to affect the pharmacokinetics of simvastatin (6.1.4), the influence of age on response could not be shown (Antonicelli et al., 1990; Plosker & McTavish, 1995). In one prospective cross-over study an interaction between gender and response was demonstrated (Clifton et al., 1994). Similarly, in a large study of 2083 patients, gender was identified as a significant predictor of the reduction in LDL cholesterol (Miserez et al., 1994). Therefore, the variability in response to simvastatin may be partially explained by patient characteristics, and the potential for dose adjustment on this basis requires further investigation.

6.2 Aims

In this chapter, data from a phase II dose ranging study was used to establish the dose response relationship for simvastatin. The primary aims were as follows-

1) Use ANOVA and standard statistical tests to determine which doses were associated with significant changes in total cholesterol and its various subfractions.

2) Establish the relationships governing the changes in cholesterol and its various subfractions by determining the most appropriate pharmacodynamic model for each.

3) Investigate for relationships between demographic covariates and the parameters of the established models.

4) Compare the results and extrapolations from the models with other dose ranging studies.

5) Use the models to propose suitable dosing strategies for future treatment

6) Discuss the limitations of the study design and the potential for using more intuitive designs for dose ranging studies

6.3 Study data

The study was part of a wider investigation into the tolerance, safety and efficacy of HMGCoA inhibitors in a West of Scotland population. Other studies from the programme investigating the management of patients with the co-existence of hypercholesterolaemia and hypertension have previously been reported (Farish et al., 1990; Macdonald et al., 1990, 1991). The aim of this particular study was to investigate the lipid response to a range of simvastatin doses. All patients had co-existing hypertension and had previously completed the initial 12 week tolerance and efficacy study (Macdonald et al., 1991). The standard lipid lowering diet, recommended by the European Artherosclerosis Society (Study Group, 1988) and blood pressure control were maintained throughout the study. In a randomised single blind Latin square crossover design, patients received either placebo.

10mg, 20mg or 40mg as a single daily dose for 12 weeks. The treatment sequences were as follows.

	Period time (weeks)			
	0-12	12-24	24-36	36-48
Group A	Placebo	10mg	40mg	20mg
Group B	10mg	40mg	20mg	Placebo
Group C	40mg	20mg	Placebo	10mg
Group D	20mg	Placebo	10mg	40mg

The demographics for the 41 patients who completed the study are shown in Table 6.2. The demographics were similar across the four treatment groups. The population comprised 30 females (mean age 58 years and mean body weight 70 kg) and 11 males (mean age 56 years and mean body weight 82 kg). All doses of simvastatin were well tolerated and no significant abnormalities were detected on routine laboratory testing.

6.4 Assay procedures

The total triglycerides and total cholesterol were measured by recognised enzymatic methods (Bucolo & David, 1973; Allan et al., 1974). The various lipoprotein subfractions were first separated by preparative ultracentrifuge and precipitation techniques (Farish et al., 1983). Inter assay coefficients of variation were 2.0% for the measurement of total cholesterol, 2.5% for the total triglycerides and between 2.5 - 9.0% for the measurement of the total cholesterol subfractions. Total cholesterol and total triglyceride concentrations were determined every six weeks, and the concentrations of the lipid subfractions: LDL cholesterol, HDL cholesterol and VLDL cholesterol were determined every 12 weeks.

Table 6.2 Summary of patient demographics

	Group				
Data	A	B	C	D	All Groups
FEMALES					
No	9	7	7	7	30
AGE years					
Mean	57.2	59.1	60.1	55.1	57.9
SD	6.4	1.9	5.1	8.7	6.3
MAX	69	62	67	67	69
MIN	48	56	52	41	41
Weight Kg					
Mean	66.5	72.1	65.1	78.2	70.2
SD	12.1	9.3	9.1	13.5	12.3
MAX	91.0	85.4	84.4	107.2	107.2
MIN	52.8	50.8	51.6	60.8	50.8
MALES					
No	3	3	3	2	11
AGE years					
Mean	54.7	55.0	58.3	53.5	55.5
SD	7.7	5.1	3.8	4.6	5.8
MAX	60.0	62.0	63.0	58.0	63.0
MIN	44.0	51.0	54.0	49.0	44.0
Weight Kg					
Mean	83.9	86.4	81.1	71.5	81.6
SD	10.2	13.5	8.6	16.5	13.0
MAX	99.5	104.4	93.8	90.7	104.4
MIN	72.0	69.6	72.6	55.0	55.0
ALL					
AGE years					
Mean	56.6	57.9	59.6	54.8	57.2
SD	6.8	3.7	4.8	7.9	6.2
MAX	69.0	62.0	67.0	67.0	69.0
MIN	44.0	51.0	52.0	41.0	41.0
Weight Kg					
Mean	70.9	76.4	69.9	76.7	73.3
SD	13.9	12.5	11.6	14.4	13.4
MAX	99.5	104.4	93.8	107.2	107.2
MIN	52.8	50.8	51.6	55.0	50.8

6.5 Methods

6.5.1 ANOVA and statistical tests

The influence of dose, period and group on each of the lipid measurements was initially investigated using ANOVA. Since there were unequal numbers in each group, this was accomplished using general linear modelling (GLM). For the total cholesterol and total triglycerides measurements, an additional factor was included to investigate for differences between week 6 and week 12 samples. When the F ratio test demonstrated that there was a significant difference ($P<0.05$), between the levels of a factor, Tukey's tests with error rates of 0.05, 0.01, 0.001 (to adjust for multiple comparisons) were used to determine which levels were significantly different.

6.5.2 Population dose response models

Population dose response relationships were determined using the FO and FOCE estimation methods (Chapter 3). Each lipid subfraction and subfraction ratio was modelled as a separate lipid response variable (LR). In each case, a hierarchy of models, similar to those of Sambol and Sheiner (1991) were fitted to determine the most appropriate relationship.

The placebo measurements (PM) were modelled as:

$$LR_{ij} = \left(\theta_1 \cdot \left(1+\eta_{i1}\right)\right) \cdot \left(1+\varepsilon_{ij}\right) \qquad \text{Eq 6.1}$$

where θ_1 is the average PM, $\bar{PM}$. For active drug, the response was modelled using one of the following expressions:

$$LR_{ij} = \left[\left(\theta_1 \cdot \left(1+\eta_{i1}\right)\right) - \left(\theta_2 \cdot \left(1+\eta_{i2}\right), If\left(Dose>0\right)\right)\right] \cdot \left(1+\varepsilon_{ij}\right) \qquad \text{Step model} \qquad \text{Eq 6.2}$$

$$LR_{ij} = \left[\left(\theta_1 \cdot \left(1+\eta_{i1}\right)\right) - \left(\left(\theta_2 + \theta_3 . Dose\right) \cdot \left(1+\eta_{i2}\right)\right), If\left(Dose>0\right)\right] \cdot \left(1+\varepsilon_{ij}\right) \quad \text{Steplinear model Eq 6.3}$$

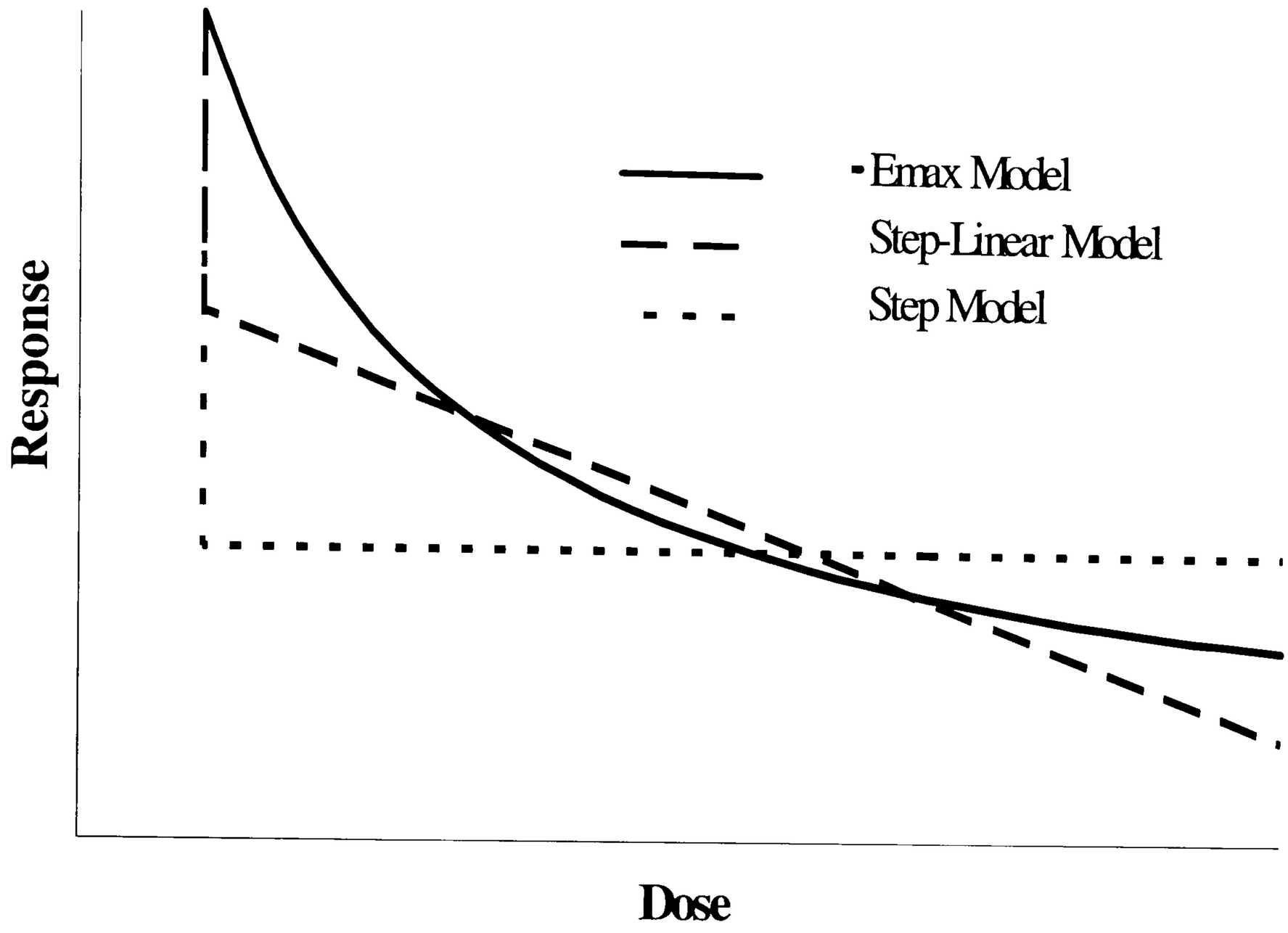
$$LR_{ij} = \left[\left(\theta_1 \cdot \left(1+\eta_{i1}\right)\right) - \left(\frac{\left(\theta_2 \cdot \text{Dose}\right)}{\left(\theta_3 + \text{Dose}\right)} \cdot \left(1+\eta_{i2}\right)\right)\right] \cdot \left(1+\varepsilon_{ij}\right) \qquad \text{Emax model} \qquad \text{Eq 6.4}$$

Where LR_{ij} is the lipid subfraction response for the ith individual at the jth observation, θ_1 is the typical patient's estimated placebo measurement, θ_2 is Emax, θ_3 is D_{50} and η_{ik} is the random interindividual error. Interindividual variability estimates were initially obtained for both the placebo response (k=1) and the reduction component of the model (k=2). The variance of the intraindividual errors (ε_{ij}) was also estimated. A proportional error model was initially used for all random effects. The NMTRAN user supplied PRED subroutines used to implement these models are shown in Appendix 1.3.

A graphical representation of the three models is shown in Figure 6.1. The step model, which describes an all or nothing response (Eq 6.2), was compared to the step-linear model which models the step response but has an additional term to describe a subsequent change in response with increasing dose (Eq 6.3). Therefore, the step-linear model (full model) has one more parameter than the step-model (reduced model). The full-reduced pair can be tested using a likelihood ratio test (Chapter 3).

If a graded response could be established, then the more physiological Emax model was fitted. In this case, structural parameters for Emax and D_{50} were estimated (Eq 6.4). As the step-linear model and the Emax model are different models with the same number of parameters, they do not form a full/reduced pair. The AIC (Chapter 3) was therefore used to compare the step-linear and Emax models. Since the number of parameters is the same for each model the AIC is simply equal to difference between the objective functions.

Figure 6.1 Graphical representation of the Step model Step-Linear model and Emax model



6.5.3 Covariate analysis and further model development

Individual placebo estimates were obtained (Chapter 3) and associations with patient covariates were investigated graphically. For Eq 6.3 and 6.4 interindividual variability was estimated on the whole "reduction portion" of the model, so individual parameter estimates of θ_2 and θ_3 were not available. Instead, individual specific responses at each dose level were plotted against the patient covariates to investigate for possible relationships. Potential covariates were formally tested using the likelihood ratio test (Chapter 3).

The appropriateness of the interindividual and intraindividual variability models was also tested. In particular, an additive and additive plus proportional model for intraindividual variability was also tested. Since relationships between the drug response and the measurement on placebo were detected, covariance between the two η's was also tested. Lipid response in terms of reduction in total cholesterol or LDL cholesterol is most often communicated in terms of a percent reduction from the pre-treatment baseline level i.e.

Table 6.1. While these percentages can be calculated from the model parameters of Eq 6.2 to 6.4, the percentage reductions at each dose level can themselves be modelled. The percentage reduction model equivalent to Eq 6.4 is as follows

$$\%LR_{ij} = \left[100\% - \left(\frac{\theta_1 . Dose}{\theta_2 + \text{Dose}}\right) \cdot \left(1 + \eta_1\right)\right] \cdot \left(1 + \varepsilon_{ij}\right) \quad \text{Percentage reduction model Eq 6.5}$$

Where $\%LR_{ij}$ is the lipid subfraction response for the ith individual at the jth observation as a percent change from the placebo measurement. This model reduces the number of structural parameters by one, thus simplifying the model and variance structure. This model was also used in the investigation of covariate relationships. The NMTRAN user supplied PRED subroutine used to implement this models is shown in Appendix 1.3.

6.5.4 Predictions and simulations

Predictions and simulations were undertaken using the parameter estimates (structural and variance) from the final model. Since no pre-treatment data was available the placebo response could not be estimated. To allow extrapolation to the wider population initial (baseline) and placebo measurements were considered to be equivalent i.e. the placebo effect was assumed to be negligible in comparison to the drug effect.

6.6 Results

Forty one patients completed the study and provided 328 total cholesterol and triglyceride concentrations, and 164 measurements of the other subfractions.

6.6.1 Mean reductions and analysis of variance (ANOVA)

The mean absolute values and percent changes are summarised in Table 6.3. The mean (SD) total cholesterol and total triglycerides concentrations after 12 weeks at each dose level were very similar to those after 6 weeks. The mean concentration of total cholesterol and its constituent subfractions, with the exception of HDL cholesterol, decreased with

increasing dose. The concentration of HDL cholesterol was shown to increase with increasing dose. The maximum percentage changes from the placebo measurement (PM) for total, LDL and HDL cholesterol were -31(11.7) %, -41(12)% and +14(14)% respectively. The maximum reductions in the ratios of total to HDL cholesterol and LDL to HDL cholesterol were -39 and -47 %, respectively. The maximum fall in ratios were therefore greater than that for total and LDL cholesterol alone.

The ANOVA results for total cholesterol and each of the subfractions are shown in Table 6.4. With the exception of HDL cholesterol, a significant difference between the dose levels was detected for all measurement ($P<0.05$). There was no evidence for a period effect with any measurements. The similarity between the mean at 6 and 12 weeks for the total cholesterol and total triglycerides measurements was confirmed by the lack of week effect. The week 6 and 12 measurements were therefore considered as multiple observations for purposes of population dose response modelling. There was evidence of a group effect for the total cholesterol, total triglycerides and VLDL cholesterol responses. The results of the multiple comparisons between the response at each dose level are shown in Table 6.3 (see foot note for details). There was a significant reduction from the placebo concentrations at all dose levels for total, LDL, VLDL, LDL:HDL and total: HDL cholesterol. In comparison, a significant reduction from placebo in total triglycerides was only shown at the 40mg dose level. A significant reduction between the 10mg and the 40mg was shown for total (wk6 & wk12), LDL, and LDL:HDL cholesterol. The differences in concentrations between the 20 and 40mg doses was not significant for any measurements. Multiple comparisons showed that Group A was significantly different ($P<0.05$) from groups B, C and D for the total triglycerides and that Group A was also significantly different ($P<0.05$) from groups C and D for VLDL cholesterol. Group differences for total cholesterol were not found to be statistically different upon multiple comparison.

Table 6.3 Absolute mean concentration and mean percentage change in total cholesterol and other lipid concentrations after six and twelve weeks simvastain treatment.

		Total Cholesterol	Total Triglycerides	LDL Cholesterol	VLDL Cholesterol	HDL Cholesterol	Total: HDL Cholesterol	LDL: HDL Cholesterol	Total Cholesterol	Total Triglycerides	LDL Cholesterol	VLDL Cholesterol	HDL Cholesterol	Total : HDL Cholesterol	LDL: HDL Cholesterol
		$mmol.l^{-1}$	$mmol.l^{-1}$	$mmol.l^{-1}$	$mmol.l^{-1}$	$mmol.l^{-1}$	Ratio	Ratio	%	%	%	%	%	Ratio	Ratio
DOSE		Absolute concentration after six weeks							Percentage reduction from placebo after six weeks						
0	Mean	7.80	2.21												
	SD	1.13	1.05												
10	Mean	5.97 $\#_3$	1.90						-22.9	-15.1					
	SD	0.75	0.79						9.4	21.5					
20	Mean	5.73 $\#_3$	1.96						-26.0	-12.0					
	SD	0.73	0.97						9.3	25.6					
40	Mean	5.35$\#_3\forall_1$	1.77						-30.8	-18.6					
	SD	1.01	0.76						12.9	24.2					
		Absolute concentration after twelve weeks							Percentage reduction from placebo after twelve weeks						
0	Mean	7.77	2.36	5.37	1.05	1.35	5.97	4.11							
	SD	0.98	1.12	0.82	0.56	0.28	1.40	1.00							
10	Mean	5.95$\#_3$	1.87	3.78$\#_3$	0.72	1.45 $\#_2$	4.24$\#_3$	2.71$\#_3$	-22.9	-13.2	-28.6	-23.4	8.3	-24.3	-29.0
	SD	0.85	0.83	0.74	0.37	0.28	1.01	0.79	11.4	31.8	14.8	41.4	11.9	25.1	27.2
20	Mean	5.60$\#_3$	1.88	3.40$\#_3$	0.73	1.47$\#_2$	4.05$\#_3$	2.47$\#_3$	-27.7	-15.7	-35.8	-29.0	8.6	-31.6	-39.6
	SD	0.78	0.83	0.69	0.42	0.34	1.23	0.87	10.2	24.6	13.9	32.6	13.4	16.4	16.5
40	Mean	5.30$\#_3\forall_2$	1.77$\#_1$	3.13$\#_3\forall_3$	0.64$\#_1$	1.53$\#_3$	3.60$\#_3$	2.14$\#_3\forall_1$	-31.5	-20.4	-41.0	-35.3	13.8	-38.8	-47.5
	SD	0.76	0.86	0.68	0.32	0.29	0.88	0.66	9.9	25.6	12.5	27.8	13.7	12.0	13.4

Mean response is significantly different from placebo: $\#_1$ ($P< 0.05$) $\#_2$ ($P< 0.01$) $\#_3$ ($P< 0.001$) and
∀ Mean response is significantly different from 10mg: $\forall_1$ ($P< 0.05$)$\forall_2$ ($P< 0.01$)$\forall_3$ ($P< 0.001$)

Table 6.4 ANOVA for total cholesterol and its the various subfractions

Analysis of Variance for Total Cholesterol : Week 6 and Week 12 Measurements

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Dose	3	297.4	298.1	99.4	135.45	0.000
Group	3	16.8	16.8	5.6	7.64	0.000
Period	3	1.4	1.4	0.5	0.65	0.582
Week	1	0.3	0.3	0.3	0.38	0.536
Error	317	232.5	232.5	0.7		
Total	327	548.5				

Analysis of Variance for Total Triglycerides: Week 6 and Week 12 Measurements

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Dose	3	12.4	12.90	4.30	5.84	0.001
Group	3	30.2	30.18	10.06	13.67	0.000
Period	3	2.5	2.55	0.85	1.15	0.327
Week	1	0.0	0.00	0.00	0.01	0.938
Error	317	233.3	233.27	0.74		
Total	327	278.4				

Analysis of Variance for HDL cholesterol

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Dose	3	0.63	0.64	0.21	2.4	0.074
Group	3	0.21	0.21	0.07	0.8	0.511
Period	3	0.02	0.02	0.01	0.1	0.974
Error	154	13.87	13.87	0.09		
Total	163	14.73				

Analysis of Variance for LDL cholesterol

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Dose	3	123.4	122.6	40.9	74.6	0.000
Group	3	1.1	1.1	0.4	0.7	0.580
Period	3	0.6	0.6	0.2	0.4	0.771
Error	154	84.4	84.4	0.5		
Total	163	209.5				

Analysis of Variance for VLDL

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Dose	3	4.1	4.1	1.4	8.1	0.000
Group	3	2.9	2.9	1.0	5.7	0.001
Period	3	0.3	0.3	0.1	0.6	0.618
Error	154	26.1	26.1	0.2		
Total	163	33.3				

6.6.2 Population Dose Response Relationships

The objective functions and likelihood ratio tests for the model fits are shown in Table 6.5. The fit of the step-linear model was superior over the step-model in all cases except for the total triglycerides, where the response was adequately described by the simpler model ($p< 0.15$). With HDL and VLDL cholesterol, the decrease in objective function was considerably less in comparison to that for LDL cholesterol , total cholesterol and the ratio models. Only subfractions showing a superior fit to the step-linear model were considered further. On fitting the Emax models, only total (Run3) and LDL cholesterol (Run 6) showed a greater decrease in objective function over the steplinear model. Individual profiles for the total and LDL cholesterol responses are shown in Figures 6.2a and 6.3a. The estimated population Emax response relationships for these data with ±1 SD of inter-subject variability and ±1 SD inter-subject plus intraindividual variability are shown in Figures 6.2b and 6.3b. Estimated structural and variability parameters are shown with corresponding standard errors in Table 6.6. Emax values for total and LDL cholesterol were 2.7mmol.l^{-1} (95% CI 2.4 to 3.1mmol.l^{-1}) and 2.6mmol.l^{-1}(95% CI 2.2 to 2.9mmol.l^{-1}) with D_{50} of 5.0mg (95% CI 3.3 to 6.8mg) and 6.3mg (95% CI 3.5 to 9.0mg), respectively. There was little change in the variability to accompany the small drop in objective function between the Emax and step-linear models for total and LDL cholesterol reduction. For the HDL cholesterol, the objective function was increased slightly on fitting the Emax model (Table 6.5). The population average percentage reduction for competing models are also shown in Table 6.6 and there was little difference between the model predictions. However, the Emax model would be favoured for extrapolation outside the study dose range. The estimated population Emax profiles for total, LDL and HDL cholesterol are shown in Figure 6.4. The tested dose range was predicted to only cover 20% (70 to 90% of Emax) of the upper portion of total and LDL cholesterol response curves, and the middle 30% (40 to 70%) of the HDL response curve.

Table 6.5 Objective function changes and likelihood ratio tests for the hierarchical population dose response models

	Objective Function			Model Comparison		
	Step	Steplinear	Emax	Step vs Steplinear		Steplinear vs Emax
Lipid Subfraction				Objective Function Difference	Likelihood Ratio Test	Objective Function Difference
Total cholesterol	(Run1) 150.247	(Run2) 99.023	(Run3) 97.798	-51.2	P<0.0005	-1.2
LDL cholesterol	(Run4) 44.423	(Run5) 8.547	(Run6) 4.349	-35.9	P<0.0005	-4.2
VLDL cholesterol	(Run7) -189.406	(Run8) -194.285	(Run9) -150.682	-4.9	P<0.05	+43.6
HDL cholesterol	(Run10) -386.969	(Run11) -394.050	(Run12) -393.399	-7.1	P<0.01	+0.6
Total :HDL cholesterol Ratio	(Run13) 136.368	(Run14) 112.612	(Run15) 116.606	-23.7	P<0.0005	+4.0
LDL : HDL cholesterol Ratio	(Run16) 41.988	(Run17) 8.065	(Run18) 10.481	-33.9	P<0.0005	+2.4
Total Triglyceride	(Run19) 22.012	(Run20) 19.324	(Run21) 20.400	-2.7	P<0.15	+1.1

Figure 6.2 **a)** Individual total cholesterol profiles and b) Population predicted Response versus dose.

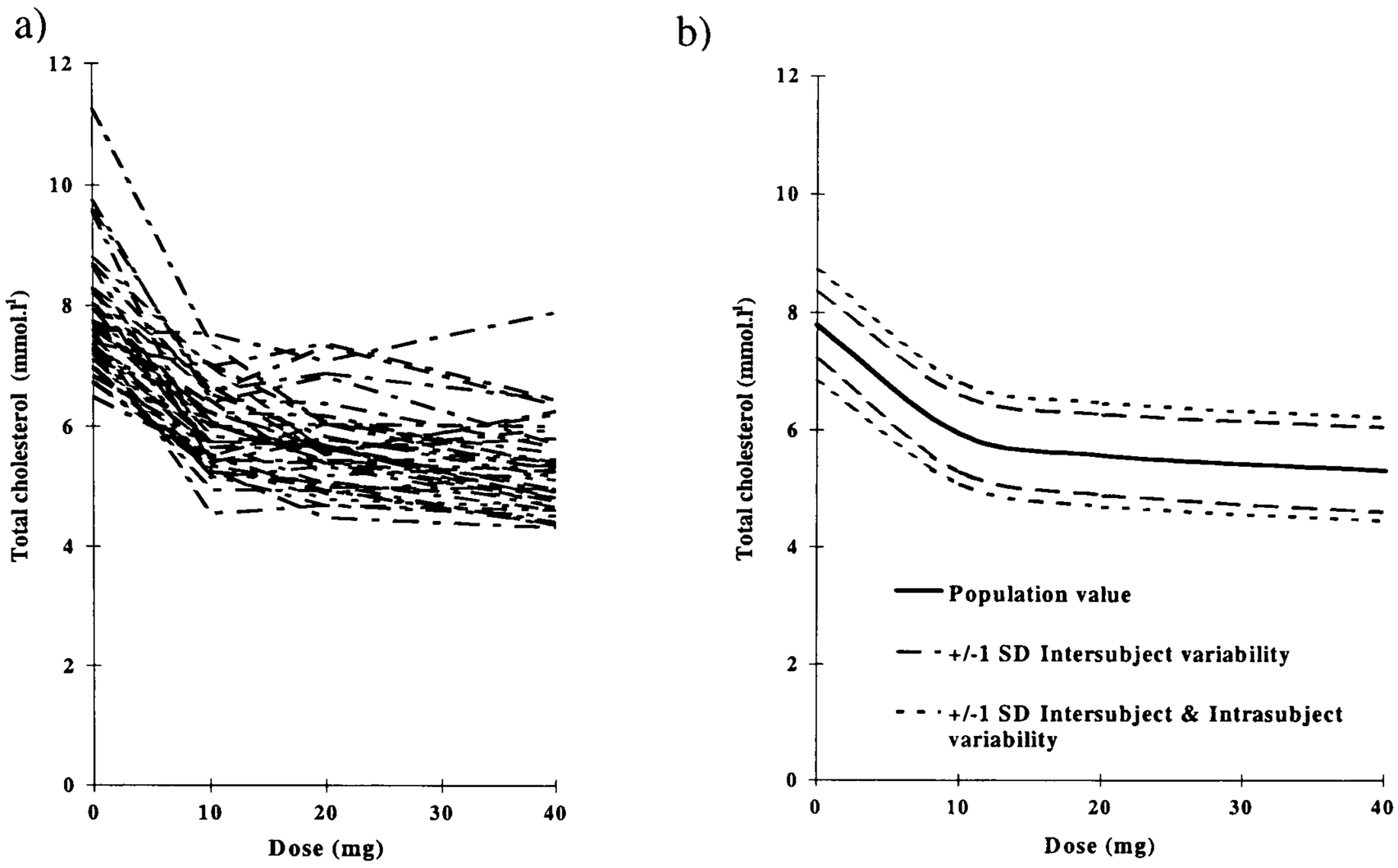


Figure 6.3 **a**) Individual LDL cholesterol profiles b) Population predicted Response versus dose

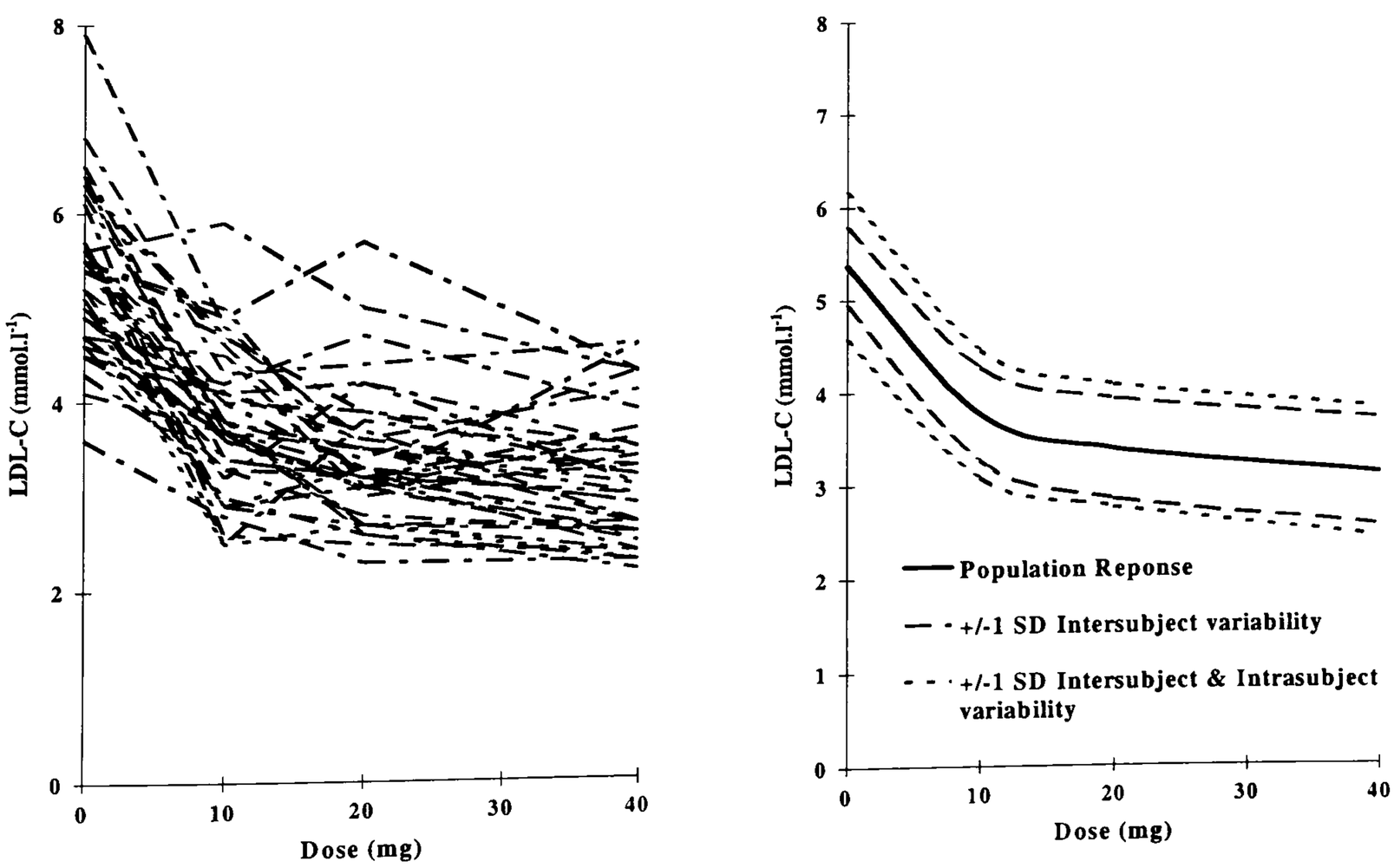
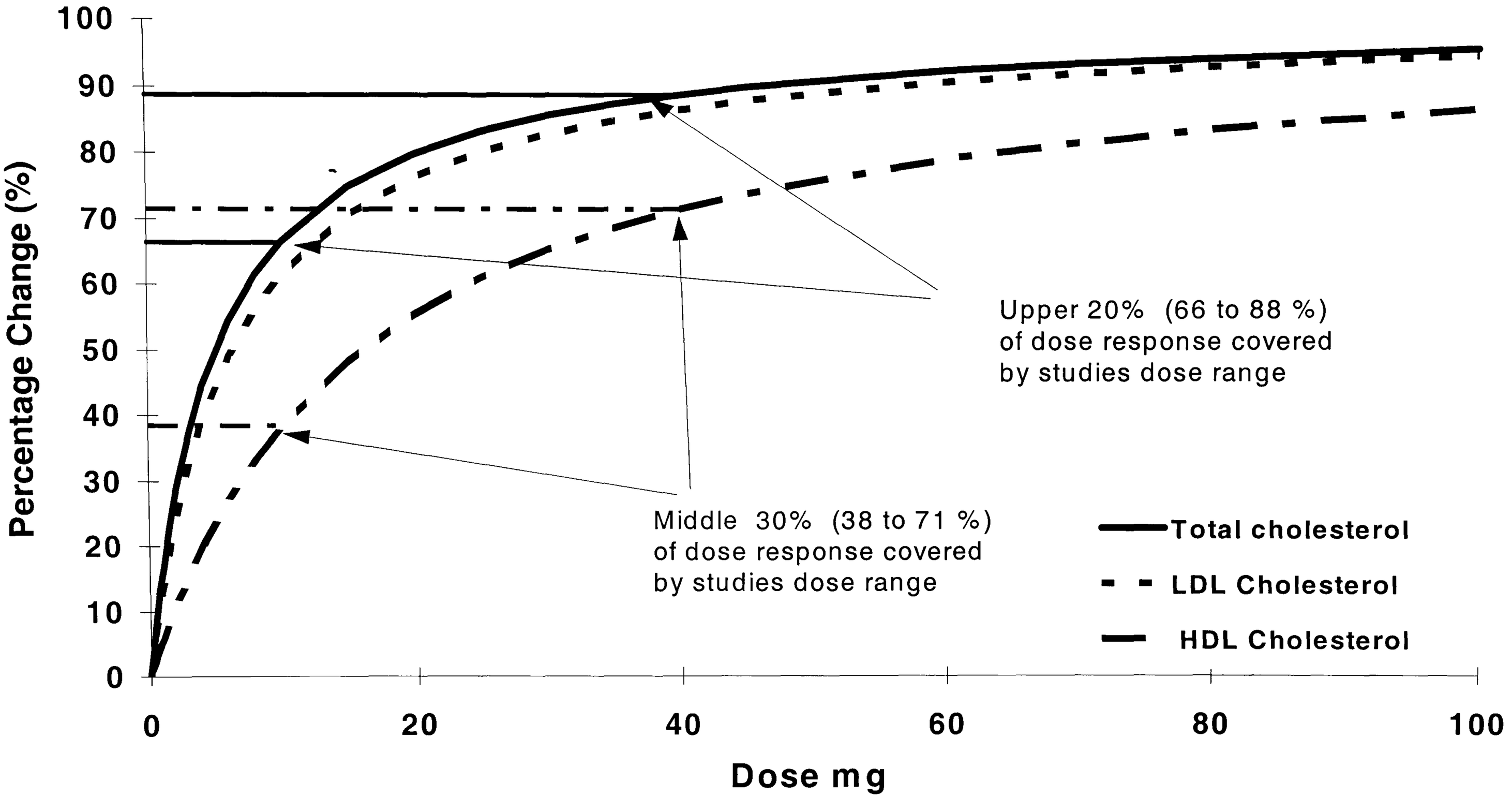


Table 6.6 Population parameter estimates and the predicted percentage change from placebo, for the hierarchical models associated with each of the lipid responses

	Best Model(s)	θ_1 mmol.l^{-1} (SE)	ω_1 %CV (SE)	θ_2 mmol.l^{-1} (SE)	θ_3 mg /mmol.l^{-1}.mg^{-1} (SE)	ω_2 %CV (SE)	σ %CV (SE)	Predicted change (%) 10mg	20mg	40mg
Total cholesterol	Emax (Run3)	7.8 (4.8%)	7.2 (30%)	2.7 (6.8%)	5.0 (17%)	19 (76%	9.7 (14%)	-23	-28	-31
	Step-linear (Run2)	7.8 (1.9%)	7.3 (29%)	1.7 (8.6%)	0.019 (13%)	19 (77%)	9.7 (14%)	-24	-26	-32
LDL cholesterol	Emax (Run6)	5.4 (2.3%)	7.8 (38%)	2.6 (21%)	6.3 (25%)	17 (62%)	13 (13%)	-30	-37	-42
	Step-linear (Run5)	5.4 (2.3%)	3.0 (40%)	1.5 (11%)	0.020 (14%)	18 (63%)	13 (13%)	-31	-35	-42
HDL cholesterol	Emax (Run12)	1.4 (3.2%)	20 (22%)	*0.23 (29%)	16.0 (74%)	ne	8.7 (28%)	*6.5	*9.4	*12
	Step-linear (Run11)	1.4 (3.2%)	20 (22%)	*0.072 (35%)	*0.0025 (32%)	ne	8.7 (28%)	*7.2	*9.1	*12
Total: HDL cholesterol Ratio	Emax (Run15)	6.0 (3.7%)	11 (250%)	2.7 (8.6%)	6.0 (30%)	28 (95%)	15 (28%)	-28	-35	-39
	Step-linear (Run14)	6.0 (3.7%)	14 (24%)	1.5 (14%)	-0.022 (18%)	ne	15 (26%)	-29	-33	-40
LDL: HDL cholesterol Ratio	Emax (Run18)	4.1 (3.8%)	16 (64%)	2.2 (7.7%)	6.5 (27%)	ne	17 (17%)	-33	-40	-46
	Step-linear (Run17)	4.1 (3.8%)	16 (25%)	1.2 (12%)	0.018 (16%)	ne	17 (17%)	-35	-39	-48
VLDL cholesterol	Step-linear (Run 8)	1.1 (9.7%)	28 (25%)	0.28 (32%)	0.0037 (49%)	ne	35 (17%)	-30	-33	-40

Where the Emax and Step-linear model parameters are equivalent to Eq 6.3 and 6.4, respectively i.e. θ_1 is the modelled average placebo value $\bar{PM}$, θ_2 is either Emax or the step response and θ_3 is the D_{50} or the linear response. σ = Intraindividual variability; ω_x = Interindividual variability (for PM or response); NE = Not estimable :data insufficient to support parameter estimation, and * indicates that the parameters estimate an increase in lipid subfraction concentration with increasing dose.

igure 6.4 Population predicted percentage change from the placebo estimate for total, LDL and HDL cholesterol versus dose of simvastatin



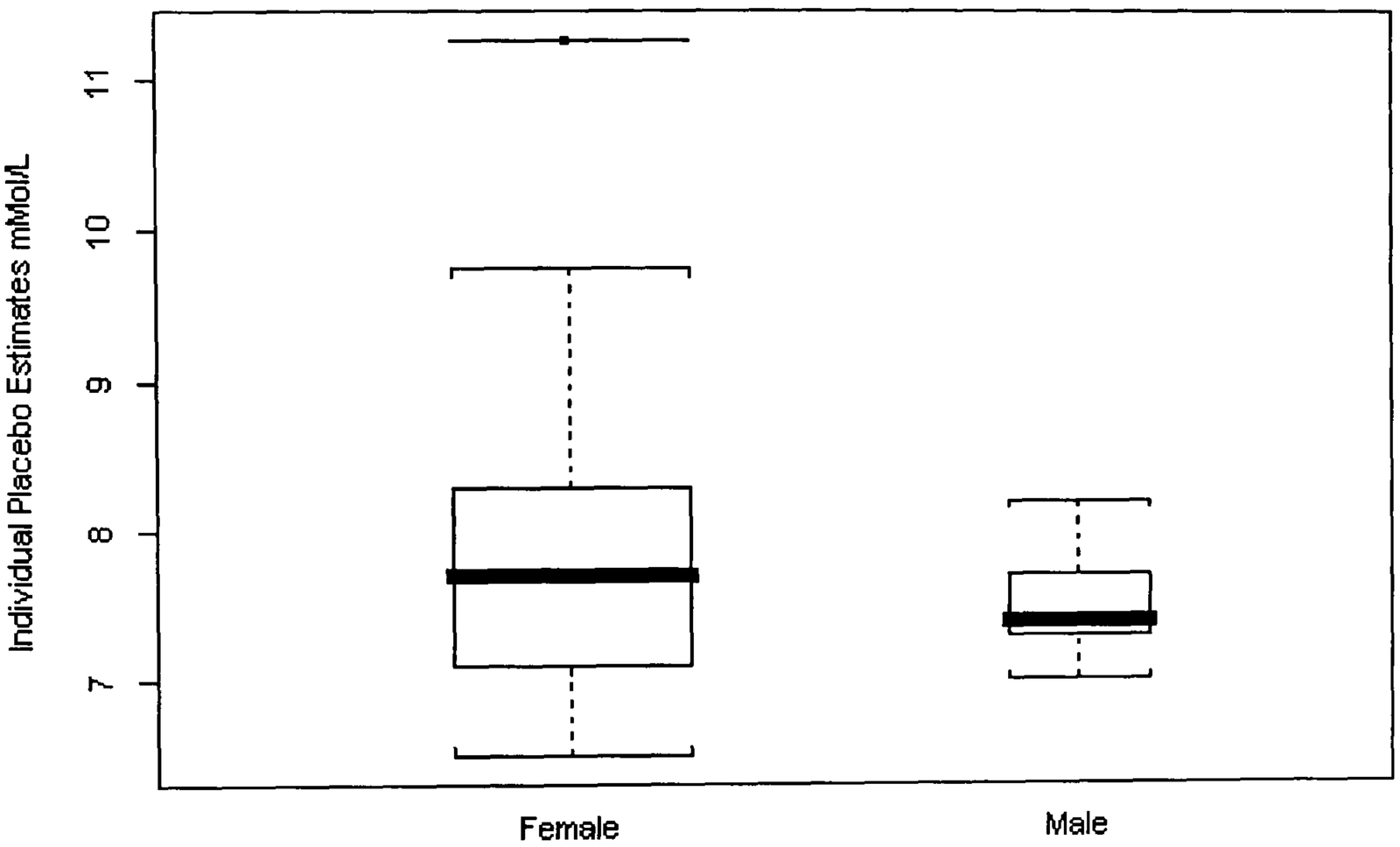
6.6.2 Covariate analysis

Figure 6.5 shows boxplots of both the modelled individual placebo estimates and the individual estimates of response by gender for the total cholesterol Emax model (Run 3). Similarly, the corresponding plots for the LDL cholesterol Emax model (Run 6) are shown in Figure 6.6. In both cases, males had a slightly lower median placebo estimate and slightly higher median response estimate. The differences were more obvious for LDL cholesterol Emax model (Figure 6.6).

Relationships between individual estimates of response and both the observed placebo measurements (PM) and body weight are shown in Figures 6.7 and 6.8. Patients with a high PM were shown to have a larger absolute response (Figure 6.7 a and Figure 6.8 a). In this case, the relationship for LDL cholesterol Emax model (Figure 6.7 a) was less apparent than that for total cholesterol Emax model (Figure 6.8 a). There was also some evidence of a linear relationship between body weight and response for both the total and LDL cholesterol Emax models (Figure 6.7b and 6.8b, respectively).

Figure 6.5 Covariate relationships for total cholesterol model: a) Modelled individual placebo estimates by gender and b) Modelled individual estimates of response by gender. Horizontal line and boxes indicate median and interquartile range (Q1-Q3), respectively. The whiskers extend to the lowest and highest values that are still inside the region defined by Q1 - 1.5.(Q3 - Q1) to Q3 + 1.5.(Q3 - Q1), showing the range of the data. The other lines indicate values which lie outside this interval.

a)



b)

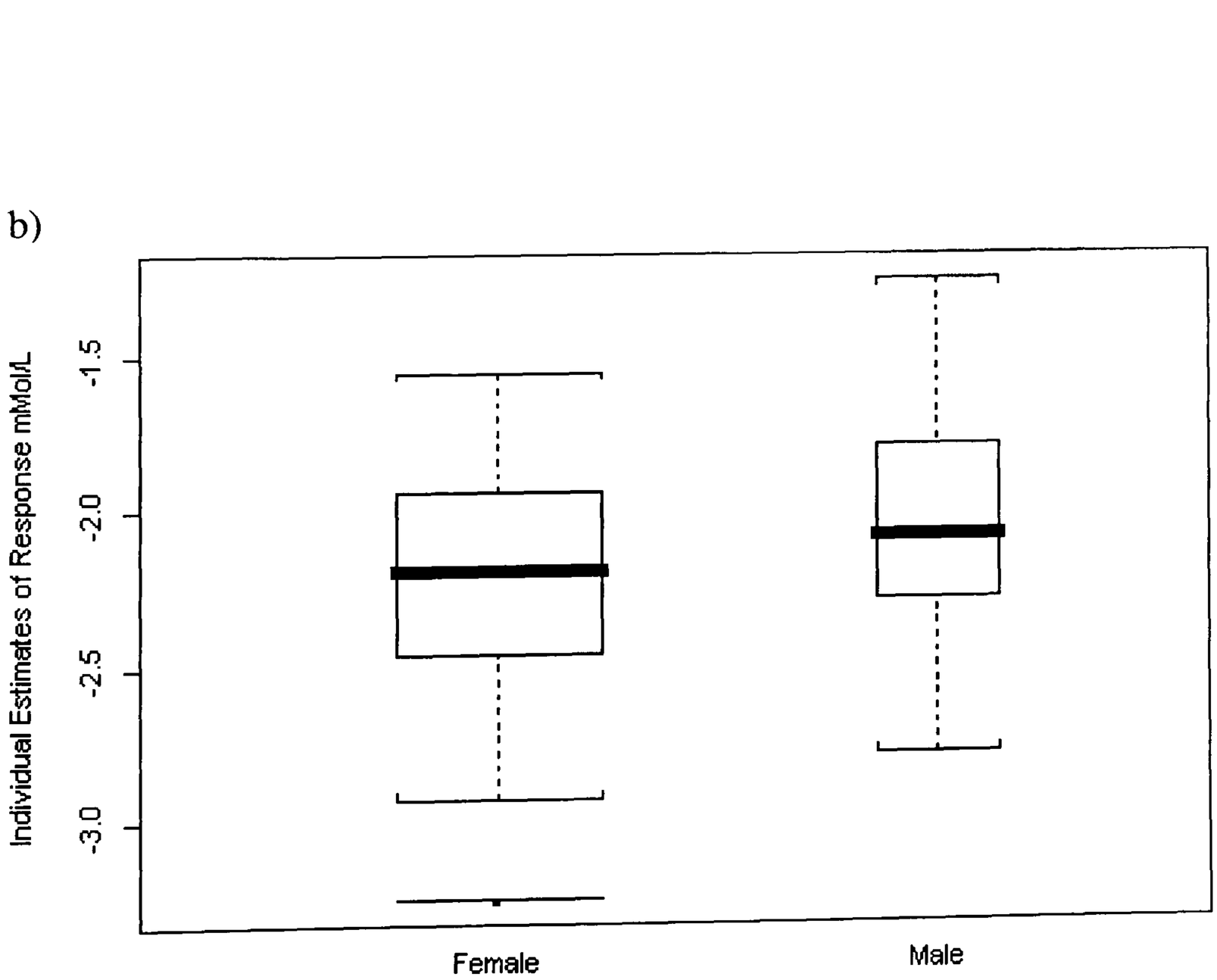
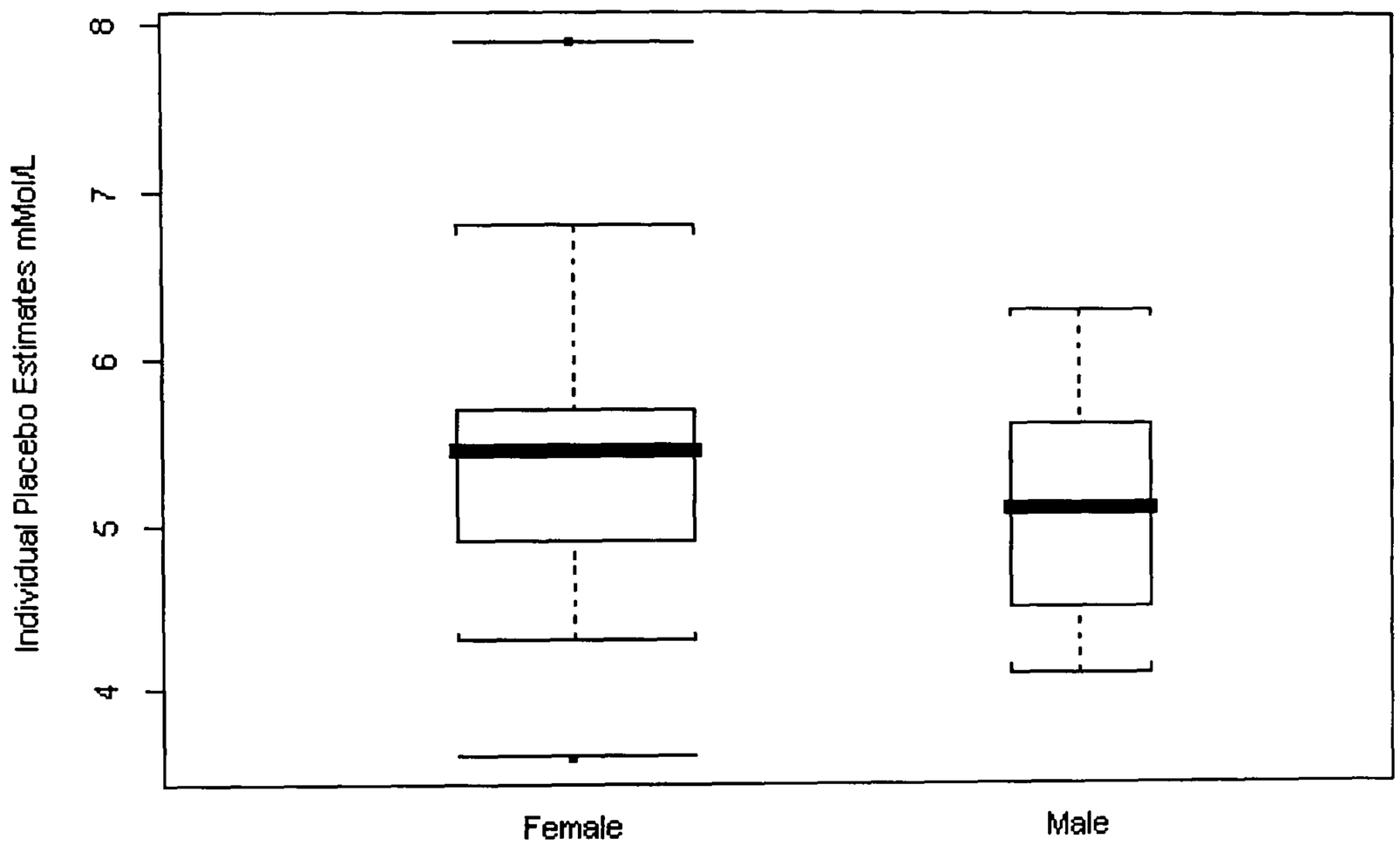


Figure 6.6 Covariate relationships for LDL cholesterol model: a) Modelled individual placebo estimates by gender and b) Modelled individual estimates of response by gender. Horizontal line and boxes indicate median and interquartile range (Q1-Q3), respectively. The whiskers extend to the lowest and highest values that are still inside the region defined by Q1 - 1.5.(Q3 - Q1) to Q3 + 1.5.(Q3 - Q1), showing the range of the data. The other lines indicate values which lie outside this interval.

a)



b)

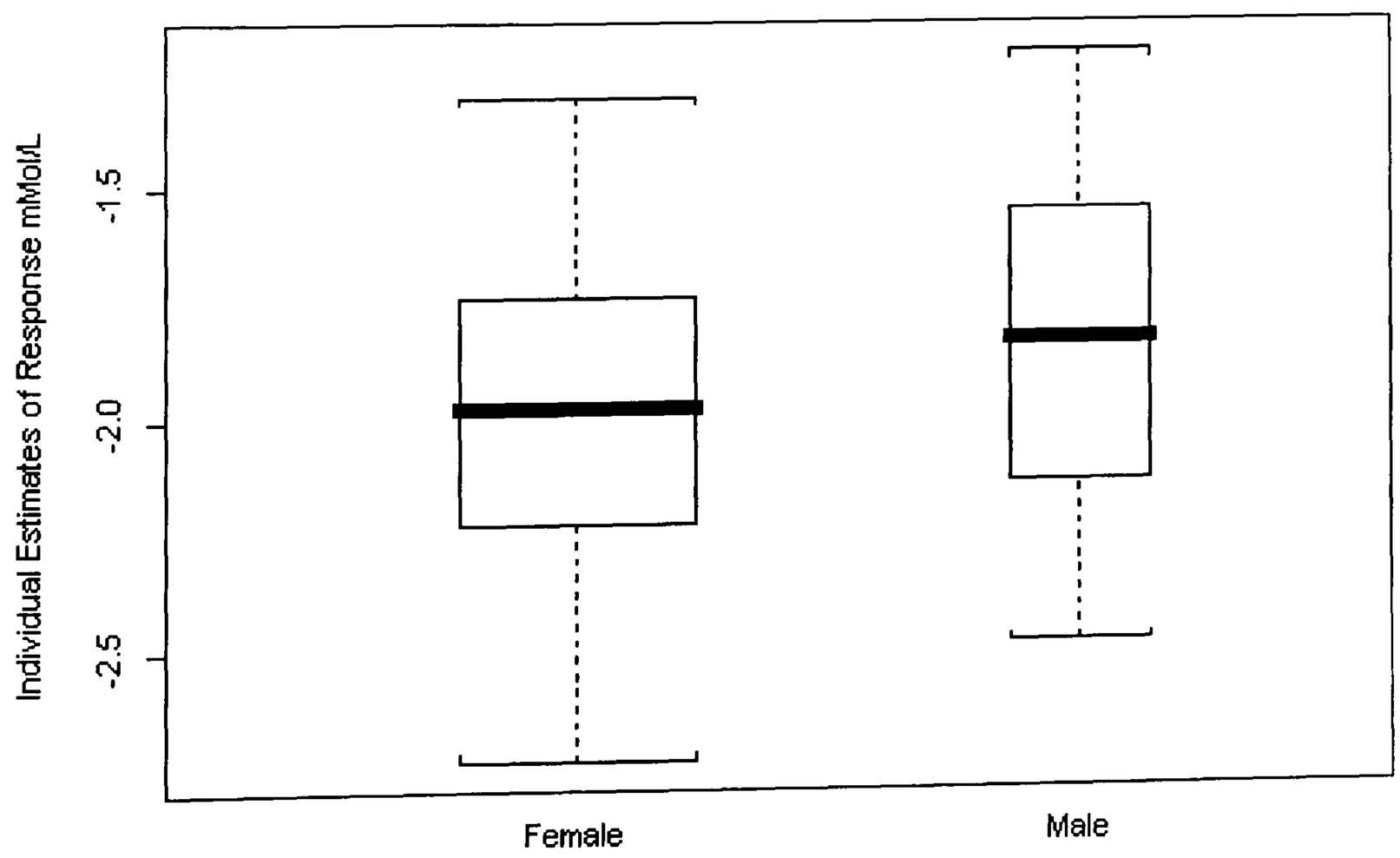


Figure 6.7 Covariate relationships for total cholesterol model: The modelled individual estimates of response at 40 mg versus a) The observed placebo measurement and b) Body weight

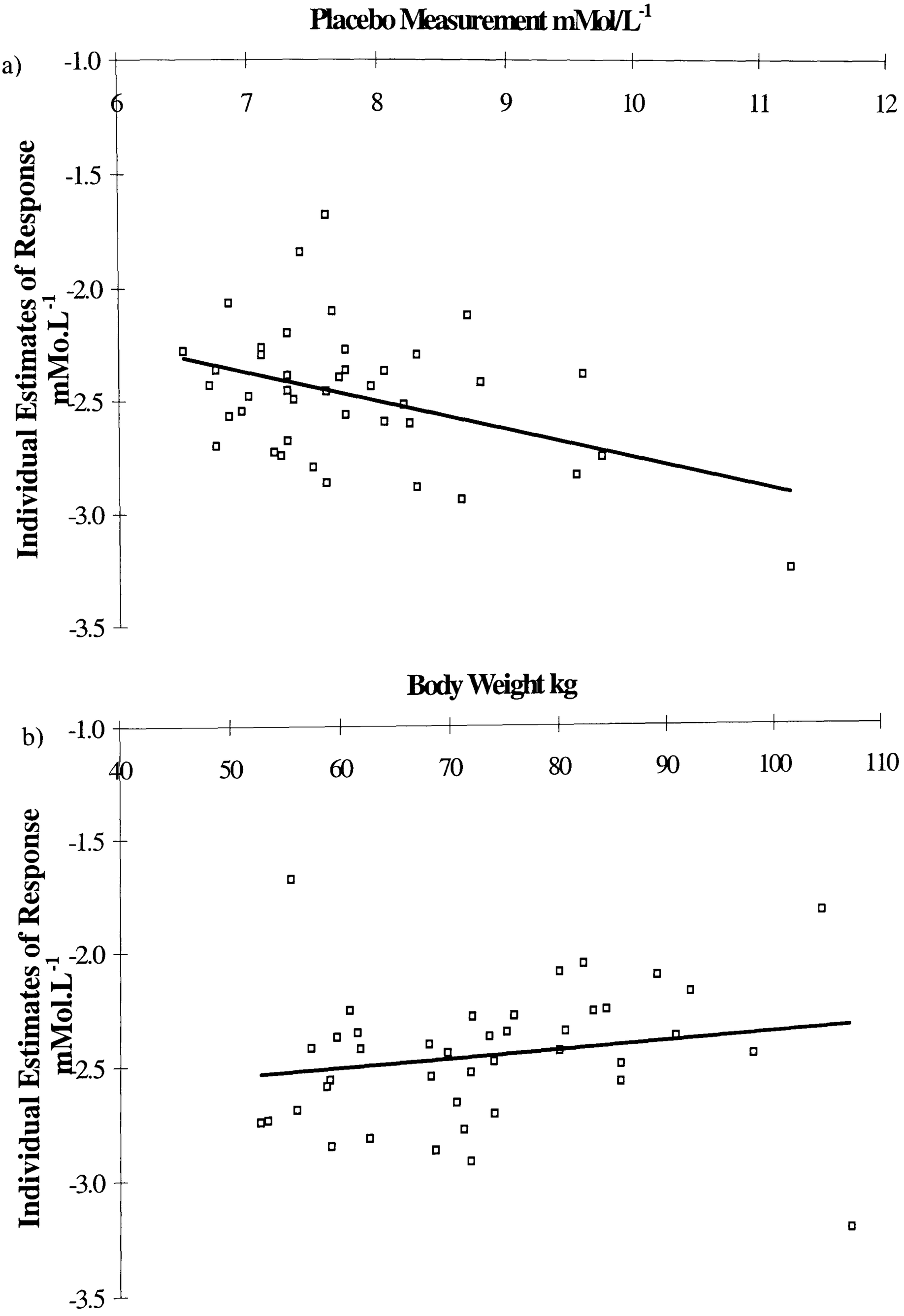
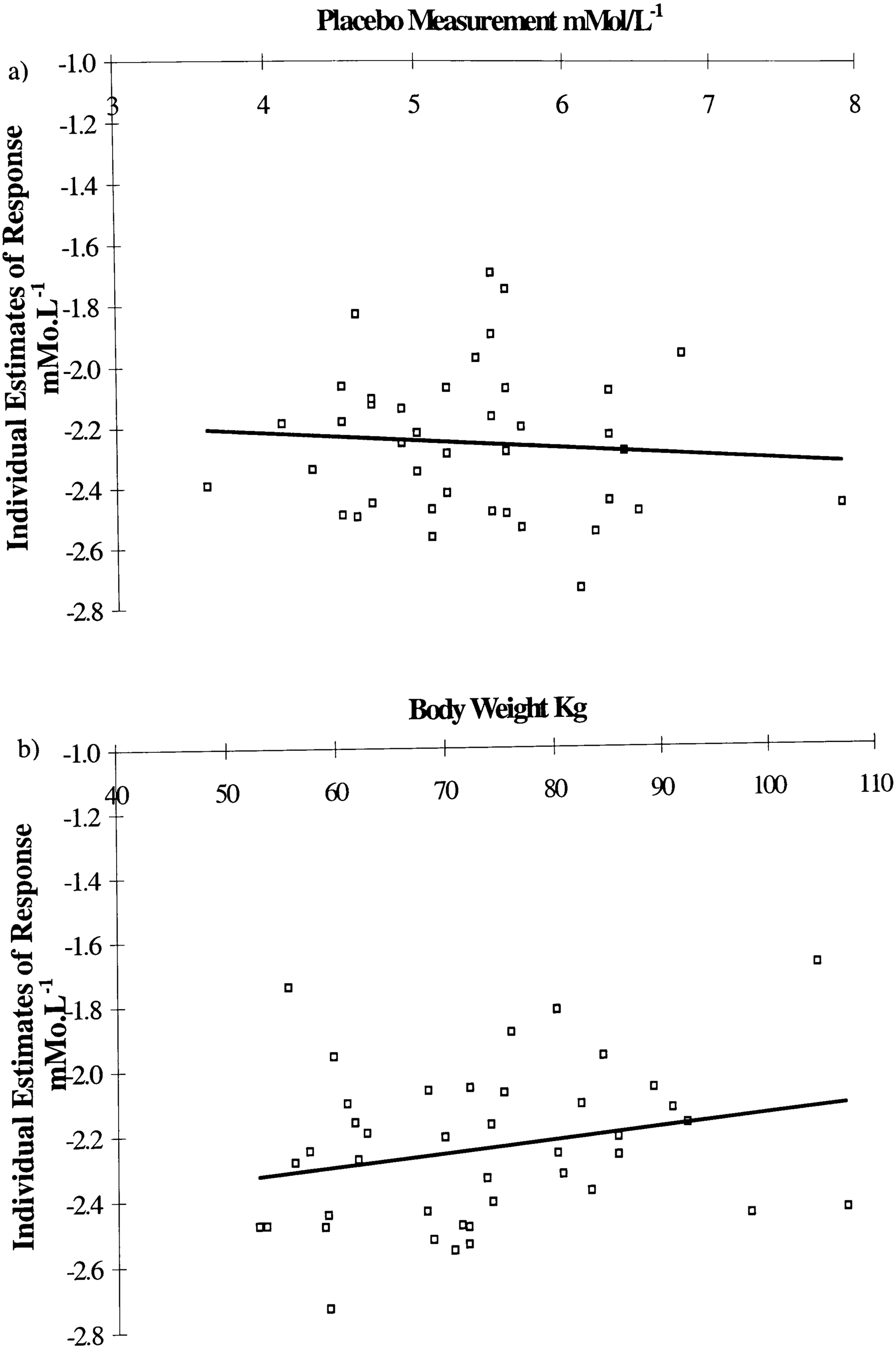


Figure 6.8 Covariate relationships for LDL cholesterol model: The modelled individual estimates of response at 40 mg versus a) The observed placebo measurement and b) Body weight



Model development and covariate effects for the total cholesterol response

Absolute reduction model

The development and covariate investigation for the total cholesterol using the absolute reduction model is shown in Table 6.7. The best structural model from the initial model development i.e. Run 3 Table 6.5, is included for comparison. The additive component, using a combined additive and proportional intraindividual variability model, was estimated to be zero. Re-running with an additive instead of a proportional model increased the objective function (Run 3b), so the proportional model was used for the covariate investigation.

In Run 22, the FOCE with interaction method was used instead of the FO method; the estimates of variability and precision were less and run times were reasonable, so further model development utilised this estimation method.

Estimating covariance between the modelled placebo measurement $\bar{PM}$ and the response, significantly reduced the objective function, $P<0.01$ (Run 23 vs Run 22). However, a further significant reduction was achieved when the observed PM was used as a covariate of Emax, $P<0.025$ (Run 24 vs Run 23) and the covariance term could be subsequently removed without significantly increasing the objective function (Run 25 vs Run 24). Including PM with D_{50} (Run 26), or gender with either Emax (Run27) or D_{50} (Run 28) also significantly decreased the objective function. However, the parameter estimates for the gender effects were not significantly different from zero. Including gender with the modelled $\bar{PM}$ (Run 28b) did not significantly decrease the objective function. Adding body weight with a linear or non-linear relationship did not improve the fit, but the use of weight-corrected dose did reduce the objective function (Run 29).

Table 6.7 Model development and covariate assessment for the absolute reduction in total cholesterol

Run No	Estimation Method	Covariate Model	OBJ. Fun	Model Comparison	$\bar{PM}$ (θ_1) mMol.l^{-1} (SE)	Emax (θ_2) mMol.l^{-1}	D_{50} (θ_3) mg / mg.kg^{-1} (SE)	θ_4 (SE)	θ_5 (SE)	$\omega\,\bar{PM}$ (SE)	ω_{Resp} (SE)	ω_{cov} (SE)	σ (SE)
Run 3	FO	None	97.798		7.79 (4.8%)	2.74 (7%)	5.05 (17%)			7% (30%)	19% (76%)		9.7% (14%)
Run3b	FO	None	105.732	vs 3 +7.3	7.79 (2%)	2.74 (6%)	5.12 (17%)			9% (46%)	25% (58%)		0.57 MMol.L^{-1} (17%)
Run22	FOCE INTER	None	67.113		7.74 (2%)	2.72 (6%)	5.34 (16%)			8% (26%)	16% (74%)		9.2% (13%)
Run23	FOCE INTER	None	61.131	vs22 P<0.01	7.73 (2%)	2.70 (6%)	5.27 (16%)			10% (39%)	23% (46%)	11% (73%)	9.0% (14%)
Run24	FOCE INTER	Emax=θ_1+(PM-7.79)* θ_4	55.978	vs23 P<0.025	7.74	2.79	5.37	0.611		10%	7%	8%	8.9%
Run25	FOCE INTER	Emax=θ_1+(PM-7.79)* θ_4	56.802	vs22 P<0.005	7.74 (2%)	2.76 (5%)	5.33 (16%)	0.429 (33%)		10% (34%)	10% (137%)		8.9% (13%)
Run26	FOCE INTER	D_{50}=θ_2+(PM-7.79)* θ_5	62.943	vs22 P<0.05	7.73 (2%)	2.75 (6%)	5.62 (17%)		-1.36 (28%)	9% (22%)	13% (94%)		9.1% (13%)
Run 27	FOCE INTER	Emax=θ_1+θ_4 (if male)	62.781	vs 22 P<0.05	7.74 (2%)	2.86 (5%)	5.29 (17%)	-0.49 (54%)		8% (26%)	14% (74%)		9.2% (13%)
Run 28	FOCE INTER	D_{50}=θ_2+θ_4 (if male)	62.969	vs 22 P<0.05	7.74 (2%)	2.75 (6%)	4.62 (19%)		4.30 (55%)	8% (26%)	15% (73%)		9.2% (13%)
Run28b	FOCE INTER	$\bar{PM}$=θ_1 +θ_4 (if male)	67.069	vs 22 P<0.85	7.73 (2%)	2.73 (6%)	5.34 (16%)	0.05 (384%)		8% (27%)	16% (72%)		9.2% (13%)
Run29	FOCE INTER	Dose/WT	64.293	vs22 -2.8	7.74 (2%)	2.74 (6%)	0.08 (17%)			8% (27%)	15% (88%)		9.2% (13%)
Run30	FOCE INTER	Emax=θ_1+(PM-7.79)* θ_4 D_{50}=θ_2+(PM-7.79)* θ_5	55.873	vs25 P<0.4	7.74 (2%)	2.76 (5%)	5.49 (16%)	0.376 (43%)	-0.677 (70%)	10% (33%)	10% (145%)		8.9% (13%)
Run 31	FOCE INTER	Emax=θ_1+(PM-7.79)* θ_4 Emax=θ_1+θ_5 (if male)	53.717	vs 25 P<0.4	7.74 (2%)	2.86 (4%)	5.28 (16%)	0.40 (36%)	-0.38 (67%)	10% (35%)	8% (174%)		9.0% (13%)
Run 32	FOCE INTER	Emax=θ_1+(PM-7.79)* θ_4 D_{50}=θ_2+θ_5 (if male)	53.335	P<0.4	7.74 (2%)	2.78 (5%)	4.69 (18%)	0.41 (34%)	3.77 (60%)	10% (35%)	9% (151%)		8.9% (13%)
Run33	FOCE INTER	Emax=θ_1+(PM-7.79)* θ_4 Dose/WT	53.124	vs25 -3.7	7.74 (2%)	2.77 (5%)	0.08 (17%)	0.44 (33%)		10% (34%)	9% (152%)		8.9% (13%)

Including PM with Emax provided the lowest objective function, and none of the possible two factor models (runs 30 to 32) resulted in a further statistically significant decrease in objective function. The weight correction of dose still decreased the objective function and was included in the final model (Run 33).

Percentage reduction model

The development and covariate investigation for the total cholesterol model using the percentage reduction model is shown in Table 6.8. For the basic model (Run 34), the population parameter estimates (SE) for Emax and D_{50} were 31.7 % (±7.6%) and 5.03mg (±17%), respectively. In this case, the additive model was shown to be more appropriate than the proportional model (Run 34b vs Run 34). The lower variability estimates and improved precision for the parameter estimates was again shown when the FOCE method was utilised (Run 35). Estimating interindividual variability on both Emax and D_{50} did not significantly decrease the objective function (Run 36). As expected, similar covariate relationships to those for the absolute reduction model were detected. Including PM with Emax (Run 37) or D_{50} (Run 38) significantly decreased the objective function. Similarly, including gender with D_{50} (Run 40) significantly reduced the objective function. However, the reduction in objective function when gender was included with Emax (Run 39) did not reach statistical significance, and neither of the estimates for the gender effects were significantly different from zero (Run 39 and Run 40). The addition of weight-corrected dose again reduced the objective function without increasing model complexity (Run 41).

Table 6.8 Model development and covariate assessment for the percentage reduction in total cholesterol

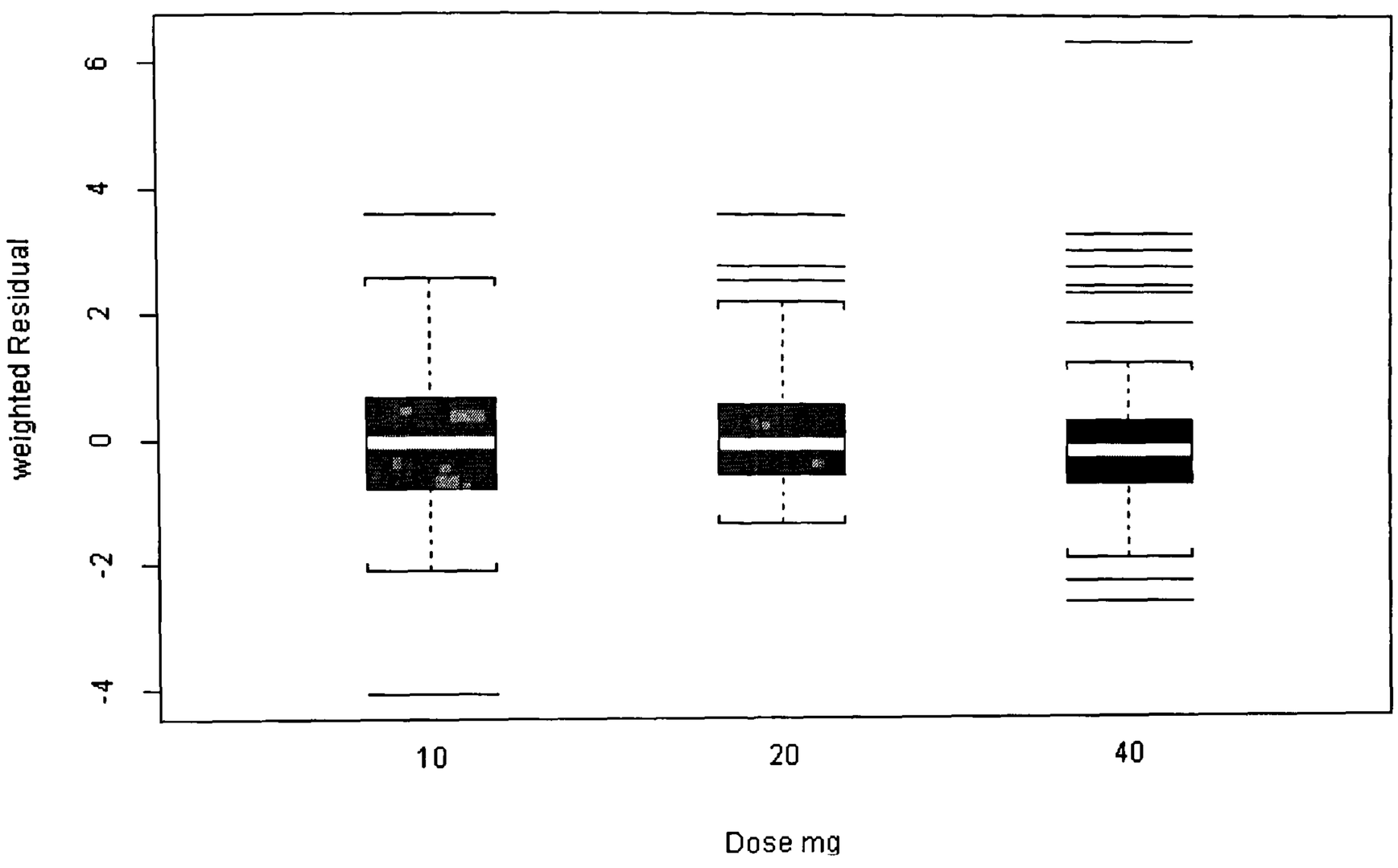
Run No	Estimation Method	Covariate Model	OBJ. Fun	Model Comparison	Emax (θ_1) % (SE)	D_{50} (θ_2) (SE)	θ_4 (SE)	θ_5 (SE)	ω_{Resp} (SE)	ω_{Emax} (SE)	ω_{D50} (SE)	σ (SE)
Run 34	FO	None	1636.61		31.7 % (7.6%)	5.03 (17%)			33% (45%)			7.7 % (14%)
Run34b	FO	None	1603.04		33.00 (6%)	5.06 (17%)			34% (53%)			5.9 mMol/L (16%)
Run 35	FOCE INTER	None	1584.15		34.50 (5%)	5.09 (17%)			30% (36%)			5.8 mMol/L (15%)
Run 36	FOCE INTER	None	1581.41	vs 35 P<0.15	34.80 (5%)	5.59 (18%)				28% (44%)	43% (59%)	5.7 mMol/L (17%)
Run 37	FOCE INTER	Emax=θ_1+(PM-7.79)* θ_4	1575.00	vs 35 p<0.005	34.40 (5%)	5.08 (17%)	4.70 (20%)		26% (43%)			5.8 mMol/L (15%)
Run 38	FOCE INTER	D_{50}=θ_2+(PM -7.79)* θ_5	1574.25	vs 35 p<0.005	35.00 (5%)	5.69 (16%)		-1.47 (33%)	27% (41%)			5.8 mMol/L (15%)
Run 39	FOCE INTER	Emax=θ_1+θ_4 (if male)	1581.85	vs 35 P<0.15	35.80 (5%)	5.09 (17%)	-5.34 (45%)		29% (36%)			5.8 mMol/L (15%)
Run40	FOCE INTER	D_{50}=θ_2+θ_5 (if male)	1579.47	vs 35 P<0.05	34.70 (5%)	4.45 (19%)		3.79 (50%)	29% (36%)			5.8 mMol/L (15%)
Run 41	FOCE INTER	Dose/WT	1580.47	vs 35 -3.7	34.60 (5%)	0.07 (16%)			29% (37%)			5.8 mMol/L (15%)
Run 42	FOCE INTER	Emax=θ_1+(PM -7.79)* θ_4 D_{50}=θ_2+(PM -7.79)* θ_5	1571.16	vs 38 P<0.1	34.70 (5%)	5.47 (16%)	3.05 (40%)	-1.09 (52%)	26% (43%)			5.8 mMol/L (15%)
Run 43	FOCE INTER	D_{50}=θ_2+(PM -7.79)* θ_4 D_{50}=θ_2+θ_5 (if male)	1571.82	vs 38 p<0.15	35.10 (5%	5.14 (18%	-1.29 (38%	2.84 (67%	27% (41%			5.7 mMol/L (15%
Run44	FOCE INTER	Emax=θ_1+(PM -7.79)* θ_4 D_{50}=θ_2+θ_5 (if male)	1571.53	vs 38 p<0.15	34.60 (5%)	4.53 (19%)	4.44 (21%)	3.06 (56%)	26% (42%)			5.8 mMol/L (15%)
Run 45	FOCE INTER	D_{50}=θ_2+(PM -7.79)* θ_4 Dose/WT	1568.51	vs 38 -5.7	35.40 (5%)	0.08 (15%)	-0.02 (24%)		26% (44%)			5.7 mMol/L (15%)
Run 46	FOCE INTER	Emax=θ_1+(PM -7.79)* θ_4 Dose/WT	1569.62	vs 37 -5.4	34.70 (5%)	0.07 (16%)	4.95 (18%)		25% (45%)			5.8 mMol/L (15%)

As with the absolute reduction model, none of the two covariate models significantly reduced the objective function further. However, the best single covariate model was less easy to determine, since the objective functions for PM with D_{50} and PM with Emax were very similar (Run 37 vs Run 38), even after correcting dose for body weight (Run 45 vs Run 46). There was no difference in the weighted residual versus dose plots for the two models (Figure 6.9). The lack of distinction between the two models is due to the high degree of correlation between Emax and D_{50}. Absolute reductions in ω_{RESP} of 4% (Run 45) or 5% (Run 46) corresponding to a decrease of 24% and 30% in the interindividual variability (ω_{RESP}^2) were achieved with the final models.

Using the parameters from Run 45, the predicted D_{50}, for a 75 kg person, with initial measurements of 6, 7.8, 10mMol.l^{-1} was 9.00, 6.26, 2.91 mg, respectfully. Similarly, using the parameters from Run 46, the predicted maximum reduction would be 25.8, 34.8 45.6%, respectfully. These correspond to absolute maximum reductions of 1.6, 2.7, 4.6mMol.L^{-1} and lowest possible values of 4.4, 5.1, 5.4mMol.l^{-1}, respectfully. Figure 6.10 shows the predicted percentage reductions for the three different initial measurements using the population typical parameter estimates from Run 45 (Figure 6.10 a) and Run 46 (Figure 6.10 b). The lines are the predicted responses for a 75kg person, and the upper and lower limits around each line correspond to the predictions for a 100kg and a 50kg person, respectfully. The predictions for average weight (75kg) and average initial measurement (7.8mMol.L^{-1}) were very similar for the two models, due to the high degree of correlation between Emax and D_{50}. However, as expected, the predictions for higher or lower initial values were very different. When the covariate effect was on Emax (Run 46) the maximum percentage reduction was predicted to increase as the initial measurement increased.

Figure 6.9 Weighted residuals versus dose for the percentage reduction model for total cholesterol a) Run 45 (PM as covariate of D_{50}) and b) Run 46 (PM as covariate of Emax)

a)



b)

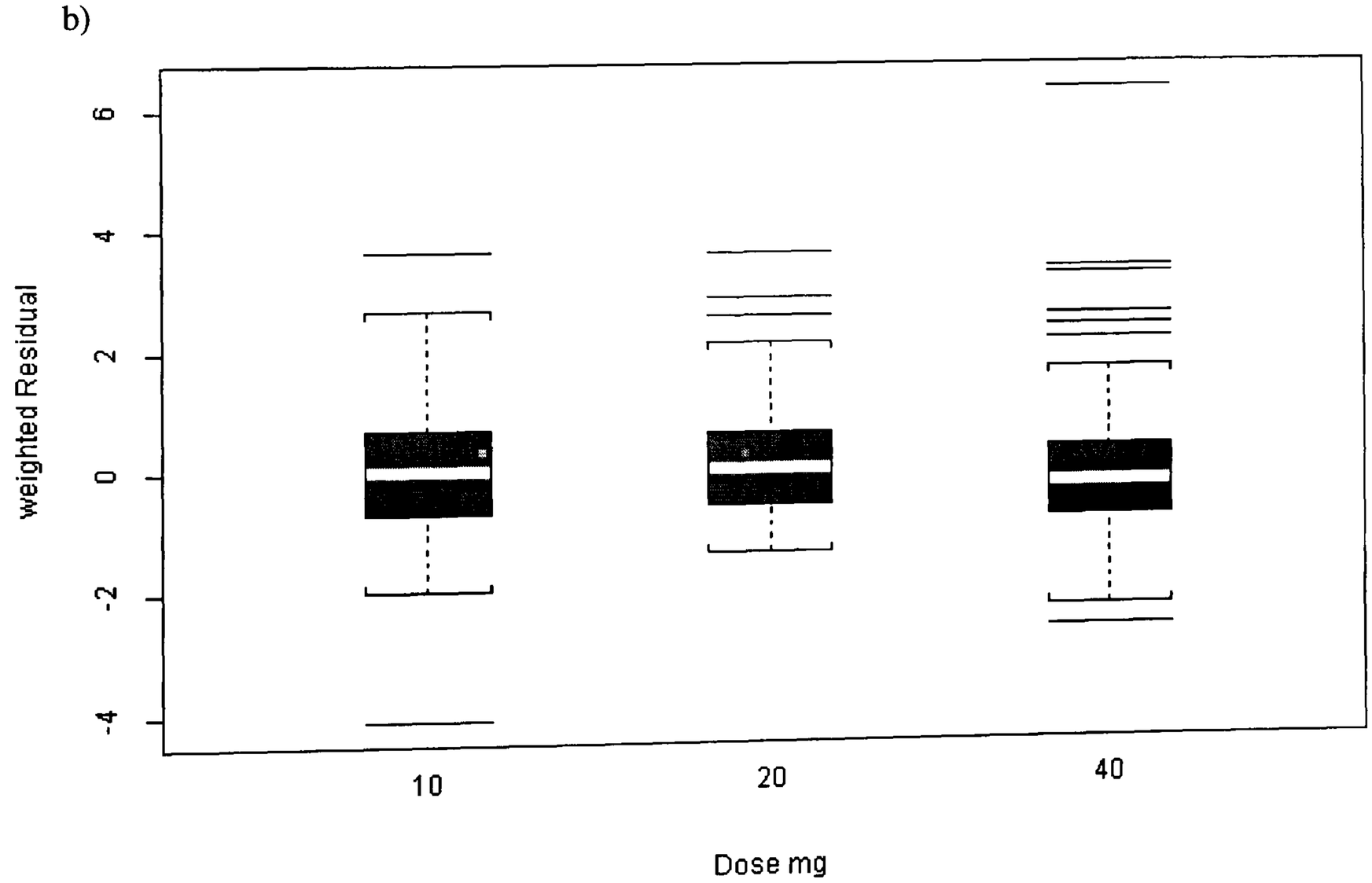
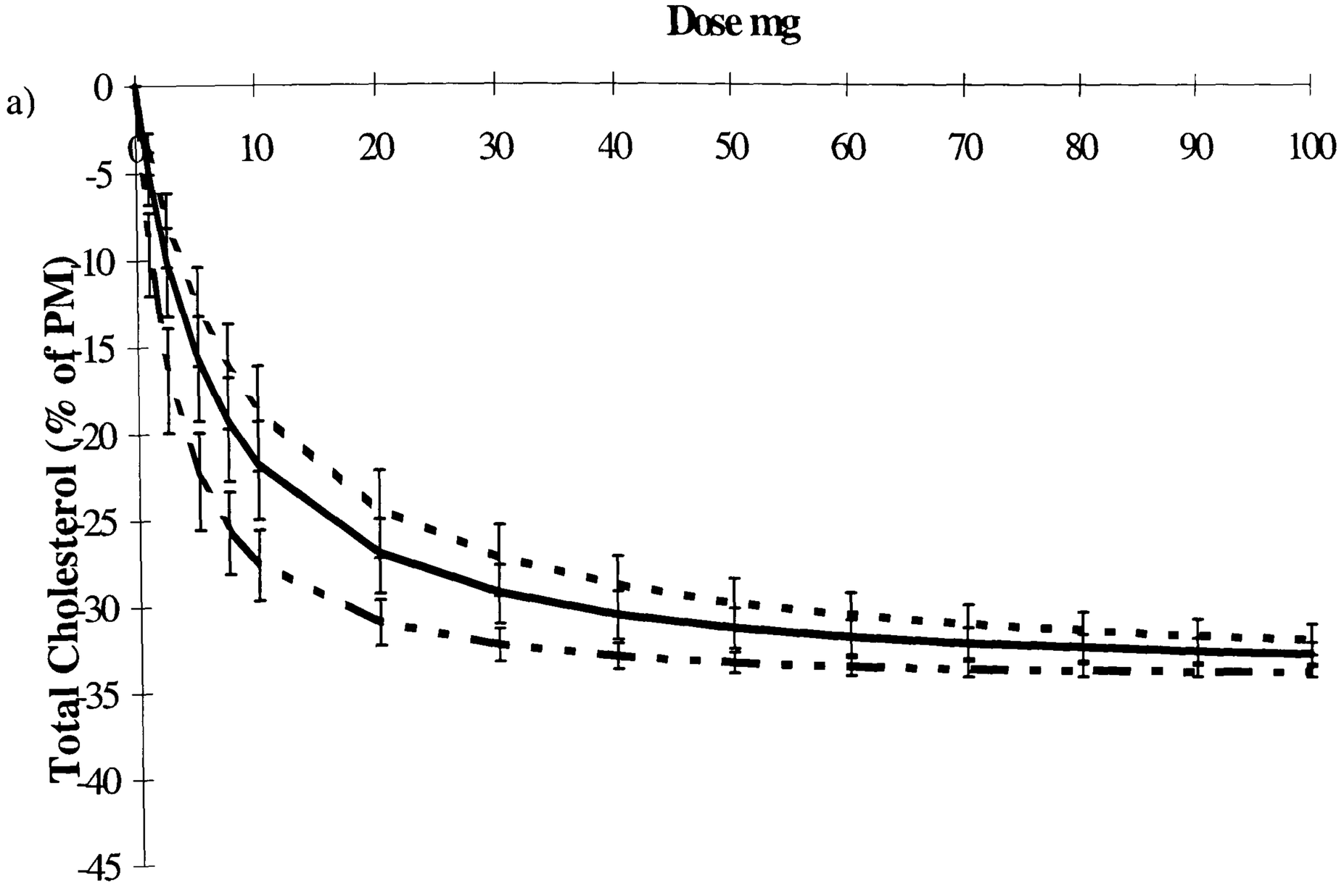
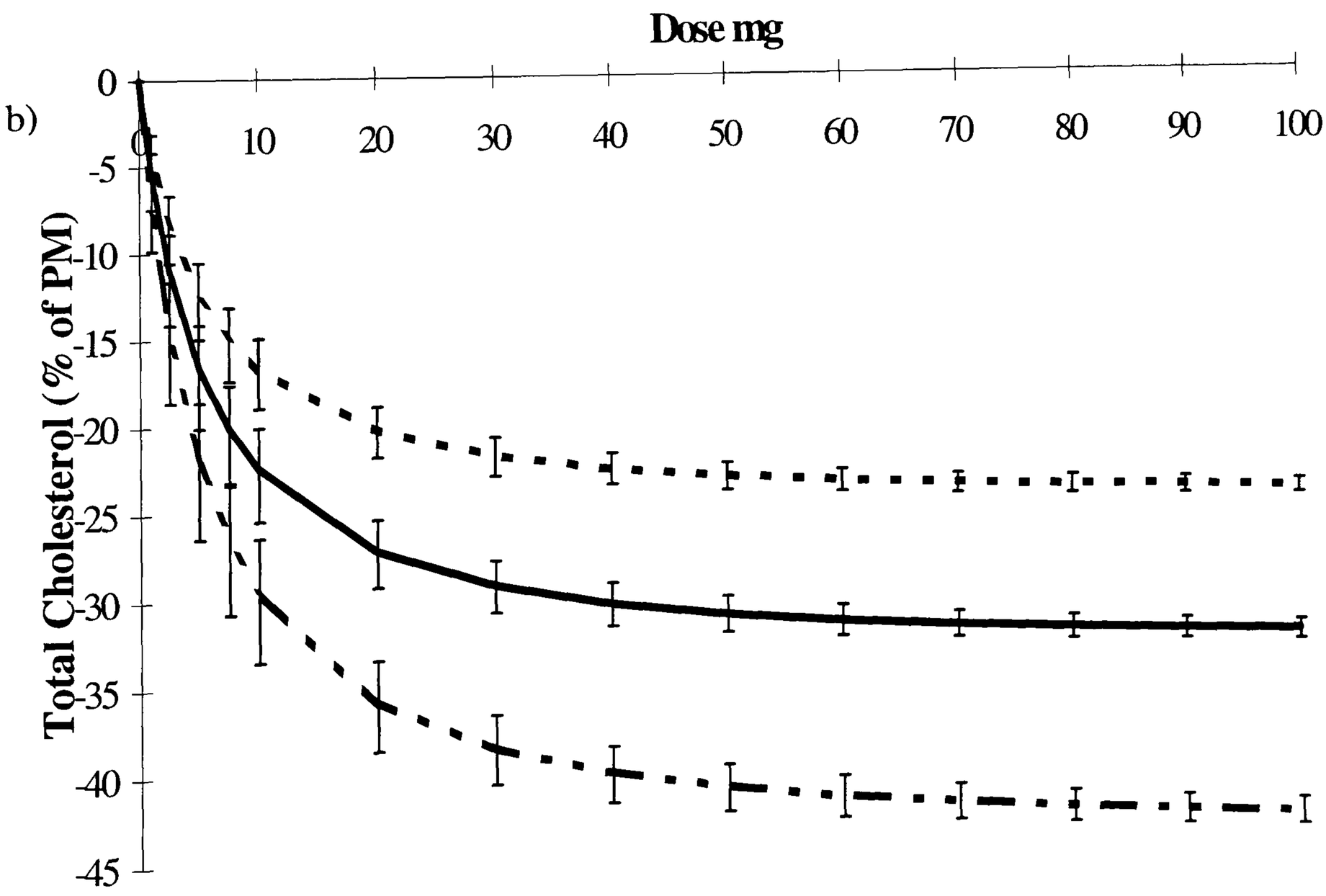


Figure 6.10 The predicted reduction in total cholesterol for a typical 70kg subject with a placebo measurement of 6 (dotted line), 7.8 (solid line) and 10 (dot-dashed line) mMol.L^{-1} in accordance with a)Run 45 Table 6.8 and b) Run 46 Table 6.8. The intervals around each line show the effect of body weight over the range of 50 (lower limit) to 100 (upper limit) kg on the predicted response





In contrast, the initial measurement did not affect the maximum possible percentage reduction when the covariate effect was related to D_{50} (Run 45). Body weight had the greatest influence on the predicted percentage reduction at doses around D_{50}. The effect decreased as the predicted percentage response approached Emax.

Model development and covariate effects for the LDL cholesterol response absolute reduction model

The development and covariate investigation for the LDL cholesterol using the absolute reduction model is shown in Table 6.9. The best structural model from the initial model development, Run 6 Table 6.4, is included for comparison. The additive component when a combined additive and proportional intraindividual variability model was tested, was estimated to be zero. Re-running with an additive instead of a proportional model increased the objective function (Run 6b), so the proportional model was used for the covariate investigation. Although using the FOCE method did not greatly alter the variability or precision in parameter estimates (Run 47), it was utilised in the covariate model development for consistency with the total cholesterol modelling. Testing covariance between the placebo measurement and the response (Run 48) or interindividual variability on both Emax and D_{50} (Run 49) did not significantly decrease the objective function. The addition of weight-corrected dose again reduced the objective function without increasing model complexity (Run 50). Including PM with Emax (Run 51) or D_{50} (Run 52) did not significantly decrease the objective function. Although including gender with either parameter did significantly decrease the objective function (Run 53 and 54), the parameter estimates for the difference were not significantly different from zero.

Table 6.9 Model development and covariate assessment for the absolute reduction in LDL cholesterol

Run No	Estimation Method	Covariate Model	OBJ. Fun	Model Compar-ison	$\bar{PM}$ (θ_1) $mMol.l^{-1}$ (SE)	Emax (θ_2) $mMol.l^{-1}$ (SE)	D_{50} (θ_3) $mg/mg.kg^{-1}$ (SE)	θ_4 (SE)	θ_5 (SE)	$\omega_{\bar{PM}}$ (SE)	ω_{Resp} (SE)	ω_{Emax} (SE)	ω_{D50} (SE)	ω_{cov}	σ
Run 6	FO	None	4.88		5.37 (2%)	2.60 (7%)	6.26 (22%)			8% (39%)	18% (63%)				13% (13%)
Run6b	FO	None	17.111		5.37 (2%)	2.57 (7%)	6.03 (23%)			9% (34%)	19% (61%)				0.49 mMol/L (16%)
Run 47	FOCE INTER	None	-6.153		5.35 (2%)	2.61 (7%)	6.32 (22%)			8% (39%)	17% (72%)				12% (13%)
Run 48	FOCE INTER	None	-6.276		5.34 (2%)	2.60 (6%)	6.36 (22%)			9% (73%)	19% (83%)			6% (344%	12% (15%)
Run 49	FOCE INTER	None	-6.166		5.35 (2%)	2.61 (6%)	6.35 (21%)			8% (39%)		16% (76%)	17% (821%		12% (15%)
Run 50	FOCE INTER	DOSE/WT	-8.257	vs 47 -2.1	5.35 (2%)	2.61 (6%)	0.09 (22%)			8% (38%)	16% (74%)				12% (13%)
Run 51	FOCE INTER	Emax=θ_1+(PM-5.37)* θ_4	-7.514	vs 47 P<0.30	5.35 (2%)	2.61 (6%)	6.48 (22%)	0.24 (90%)		10% (57%)	13% (125%				12% (15%)
Run 52	FOCE INTER	D_{50}=θ_3+(PM-5.37)* θ_5	-6.964	vs 47 p<0.4	5.34 (2%)	2.61 (7%)	6.46 (22%)		-1.17 (69%)	9% (39%)	14% (93%)				12% (14%)
Run 53	FOCE INTER	Emax=θ_1+θ_4 (if male)	-9.794	vs 47 p<0.05	5.35 (2%)	2.72 (7%)	6.18 (21%)	-0.46 (54%)		8% (34%)	14% (92%)				12% (13%)
Run 54	FOCE INTER	D_{50}=θ_3+θ_5 (if male)	-12.652	vs 47 P<0.025	5.35 (2%)	2.65 (6%)	5.27 (22%)		6.80 (59%)	9% (33%)	13% (93%)				12% (13%)
Run 54b	FOCE INTER	$\bar{PM}$=θ_1+θ_4(if male)	-7.344	vs 47 P<0.30	5.30 (3%)	2.62 (6%)	6.31 (21%)	0.22 (101%)		8% (37%)	15% (83%)				12% (13%)
Run 55	FOCE INTER	Emax=θ_1+θ_4 (if male) D_{50}=θ_3+ θ_5 (if male)	-13.021	vs 54 P<0.7	5.35 (2%)	2.65 (6%)	5.35 (22%)	6.78 (61%)	-0.73 (93%)	9% (33%)	11% (115%				12% (14%)
Run 56	FOCE INTER	D_{50}=θ_3+ θ_5 (if male) DOSE/WT	-12.546	vs 54 +0.1	5.35 (2%)	2.64 (6%)	0.08 (22%)		0.07 (73%)	9% (33%)	13% (91%)				12% (13%)

Including gender with the modelled $\overline{PM}$ (Run 54b) did not significantly decrease the objective function. Including gender on both Emax and D_{50} (Run 55) was not significantly different from the best one covariate model (Run 54), and correcting dose for body weight did not improve the parameter precision of the gender effect (Run 55).

Percentage reduction model

The development and covariate investigation for the LDL cholesterol model using the percentage reduction model is shown in Table 6.10. For the basic model, the population parameter estimates (SE) for Emax and D_{50} were 43.9% (±7%) and 6.02mg (±22%), respectively (Run 57 Table 6.9). The percentage reduction data was again shown to be most appropriately modelled using an additive intraindividual error model (Run 57b vs Run 57). There was no change in the parameter estimates upon using the FOCE method (Run 58) but it was again used for consistency. Estimating interindividual variability on both Emax and D_{50} significantly decreased the objective function (Run 59), so this variance structure was used for the covariate investigation. Estimating covariance between the parameters did not further decrease the objective function (Run 60). Using weight corrected dose was again shown to decrease the objective function (Run 61). In comparison to the covariate analysis using the absolute reduction model, including gender with Emax (Run 64) did not significantly decrease the objective function, yet including PM with Emax (Run 62) or D_{50} (Run 63) did significantly decrease the objective function. The parameter estimate for the gender effect on D_{50} was not significantly different from zero (Run 65). Out of the possible two factor covariate models (Run 66 to Run 69) only the model including gender and PM with D_{50} significantly further reduced the objective function, but, the confidence interval for the gender effect still included zero.

Table 6.10 Model development and covariate assessment for the percentage reduction in LDL cholesterol

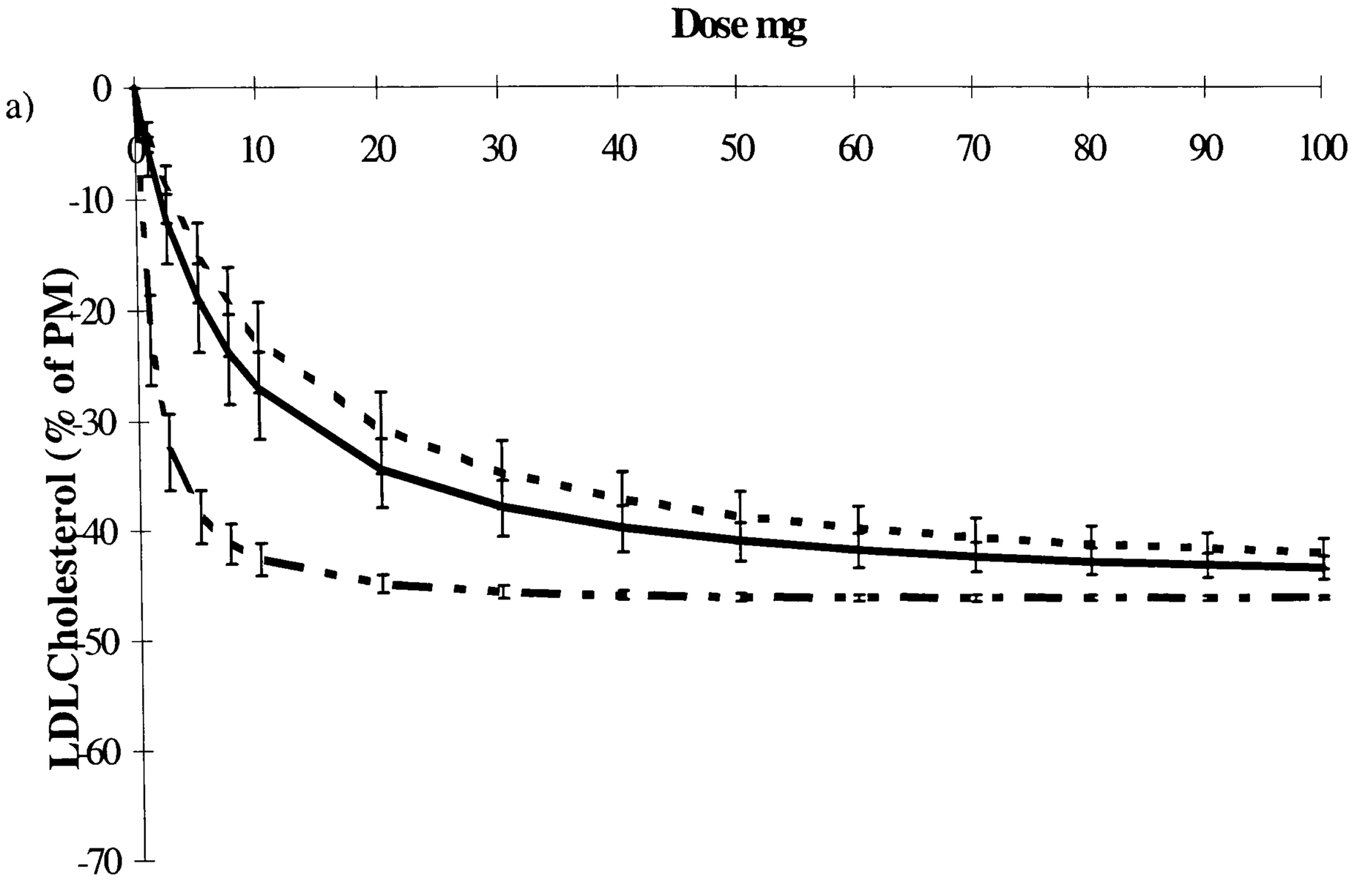
Run No	Estimation Method	Covariate Model	OBJ. Fun	Model Comparison	Emax (θ_1) % (SE)	D_{50} (θ_2) (SE)	θ_4 (SE)	θ_5 (SE)	ω_{Resp} (SE)	ω_{Emax} (SE)	ω_{D50} (SE)	ω_{cov} (SE)	σ (SE)
Run 57	FO	None	922.416		43.90 (7%)	6.02 (22%)			35% (37%)				10% (15%)
Run 57b	FO	None	893.741		45.70 (6%)	5.63 (23%)			33% (33%)				6.98 mMol/L (17%)
Run 58	FOCE INTER	None	893.741		45.70 (6%)	5.63 (23%)			33% (33%)				6.98 mMol/L (17%)
Run 59	FOCE INTER	None	888.564	vs 58 p<0.025	46.40 (6%)	6.61 (23%)				32% (38%)	61% (10%)		6.32 mMol/L (24%)
Run 60	FOCE INTER	None	888.343	vs 59 p<0.7	46.40 (6%)	6.51 (24%)				33% (38%)	65% (53%)	19% 235%	6.28 mMol/L (24%)
Run 61	FOCE INTER	DOSE/WT	886.593	vs 59 -2.0	46.60 (6%)	0.09 (24%)				31% (40%)	60% (55%)		6.37 mMol/L (26%)
Run 62	FOCE INTER	Emax=θ_1+(PM-5.37)* θ_4	883.123	vs59 P<0.025	46.00 (7%)	6.20 (24%)	6.71 (27%)			30% (41%)	54% (72%)		6.50 mMol/L (26%)
Run 63	FOCE INTER	D_{50}=θ_2+(PM-5.37)* θ_5	881.032	vs 59 P<0.01	47.10 (6%)	7.07 (22%)		-2.29 (30%)		30% (42%)	55% (63%)		6.37 mMol/L (25%)
Run 64	FOCE INTER	Emax=θ_1+θ_4 (if male)	886.257	vs 59 p<0.15	48.40 (6%)	6.50 (23%)	-8.42 (52%)			31% (38%)	59% (49%)		6.39 mMol/L (24%)
Run 65	FOCE INTER	D_{50}=θ_2+θ_5 (if male)	878.473	vs59 p<0.005	47.50 (6%)	5.11 (23%)		11.80 (66%)		29% 40%)	63% (42%)		6.15 mMol/L (25%)
Run 66	FOCE INTER	D_{50}=θ_2+θ_4 (if male) +(PM-5.37)* θ_5	874.463	vs 65 P<0.05	47.80 (6%)	5.61 (22%)	-1.62 (37%)	10.40 (84%)		28% (45%)	58% (49%)		6.24 mMol/L (25%)
Run 67	FOCE INTER	Emax=θ_1+(PM-5.37)* θ_4 D_{50}=θ_2+θ_5 (if male)	874.969	vs 65 P<0.075	46.80 (6%)	4.97 (23%)	5.41 (38%)	9.69 (71%)		27% (43%)	57% (51%)		6.31 mMol/L (25%)
Run 68	FOCE INTER	Emax=θ_1+θ_4 (if male) D_{50}=θ_2+θ_5 (if male)	878.511	vs 65 +0.04	47.10 (6%)	5.04 (23%)	2.34 (316%)	13.20 (80%)		29% (41%)	62% (41%)		6.16 mMol/L (25%)
Run 69	FOCE INTER	Emax=θ_1+(PM-5.37)* θ_4 D_{50}=θ_2+(PM-5.37)* θ_5	879.349	vs63 p<0.15	46.60 (6%)	6.73 (22%)	4.09 (53%)	-1.83 (44%)		29% (42%)	53% (74%)		6.41 mMol/L (26%)
Run 70	FOCE INTER	D_{50}=θ_2+(PM-5.37)* θ_5 DOSE/WT	877.847	vs63 -3.2	47.50 (6%)	0.10 (21%)		-0.03 (25%)		29% (44%)	51% (66%)		6.42 mMol/L (25%)
Run 71	FOCE INTER	Emax=θ_1+(PM-5.37)* θ_4 DOSE/WT	880.641	vs62 -2.5	46.10 (6%)	0.09 (23%)	6.86 (25%)			29% (43%)	48% (69%)		6.59 mMol/L (23%)

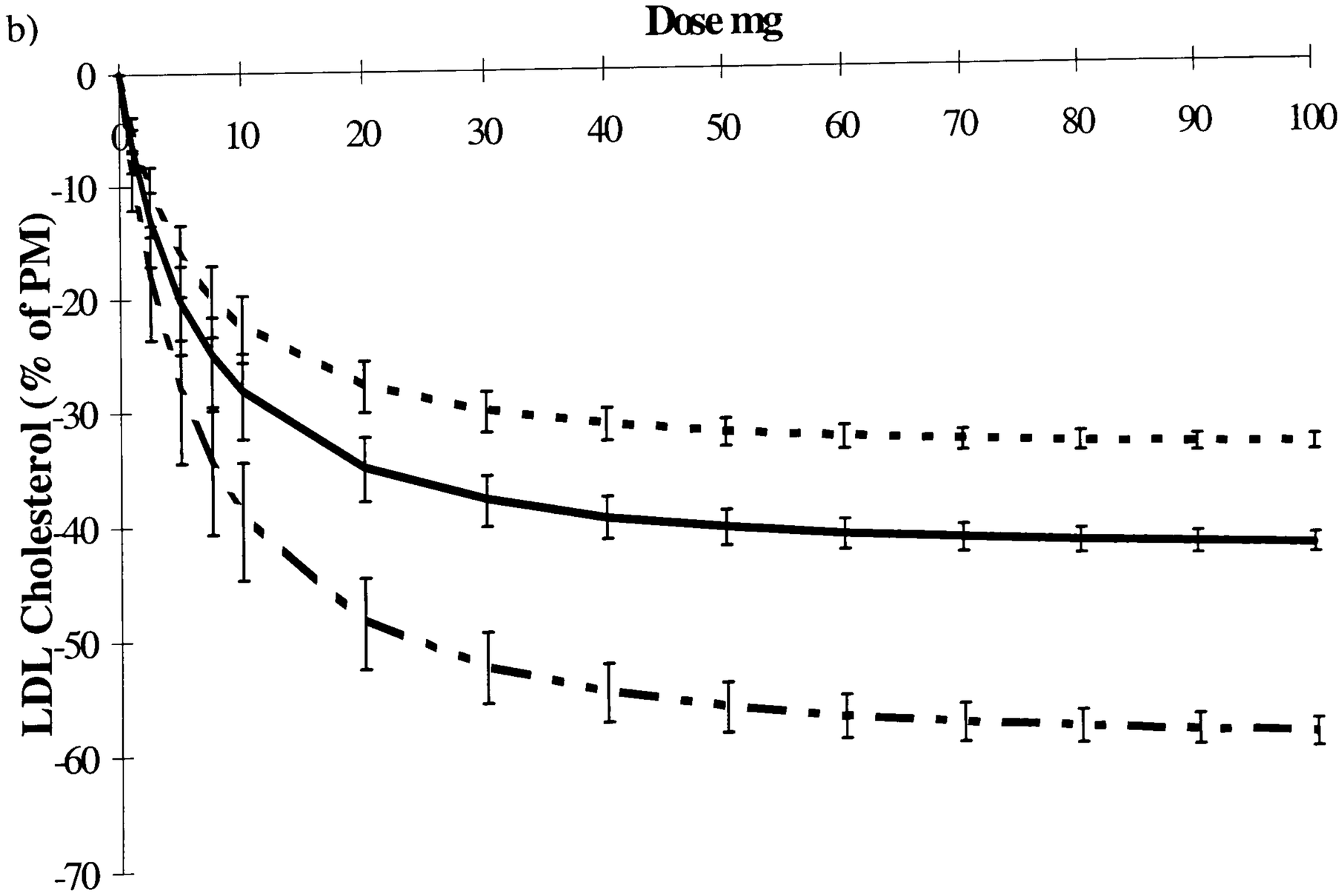
Therefore, although lower objective functions were obtained by including a gender effect, the only covariate model with sufficiently precise parameter estimates was PM related to D_{50} (Run 63). Again, the use of weight corrected dose further decreased the objective function and was included in the final model (Run 70). Run 71 shows the effect of PM on Emax with weight corrected dose for completeness.

The absolute reductions in ω_{D50} and ω_{EMAX}, between the basic (Run 59) and final model (Run 70), were 3% and 10%, respectfully. The absolute reductions correspond to percentage decreases in the interindividual variabilities (ω_{D50}^2 and ω_{EMAX}^2) of 18% and 30%, respectively.

Using the parameters from Run 70, the predicted D_{50} for a 75kg person, with initial measurements of 4, 5.4 and 8mMol.l^{-1} was 10.9, 7.5 and 1.2mg, respectively. Similarly, using the parameters from Run 71, the predicted maximum possible reduction would be 36.7, 46.3 and 64.1%, respectfully. These correspond to absolute maximum reductions of 1.5, 2.5 and 5.1mMol.L^{-1}, and lowest possible values of 2.5, 2.9, and 2.9mMol.l^{-1}, respectfully. Figure 6.11 shows the predicted percentage change for three different initial measurements for Run 70 (Figure 6.11a) and Run 71 (Figure 6.11b). As before, the lines show the predicted response for a 75kg person, and the upper and lower limits around each line correspond to the predictions for a 100 and 50kg person, respectfully. As with the total cholesterol predictions, the predictions for average weight (75kg) and average initial measurement (7.8mMol.L^{-1}) were very similar for the two models. Similarly, the differences in the predictions upon changing the initial measurements was again demonstrated.

Figure 6.11 The predicted reduction in LDL cholesterol for a typical 70kg subject with placebo measurements of 4 (dotted line), 5.4 (solid line) and 8 (dot-dashed line) mMol.L^{-1} in accordance with a)Run 70 Table 6.9 and b) Run 71 Table 6.9. The intervals around each line show the effect of body weight over the range of 50 (lower limit) to 100 (upper limit) kg on the predicted response





6.6.3 Simulation of responder rate

The distribution of total cholesterol after doses of 0, 2.5, 5, 7.5, 10, 20, 30, 40, 80, and 160 mg was simulated for a population of 1000 patients with a mean (SD) for PM and body weight of 8.0 (0.8)mMol.L^{-1} and 75 (13)kg, respectively. The simulation used the parameter estimates from the following Runs:

Run 45 - PM as a covariate of D_{50} and dose corrected for body weight

Run 46- PM as a covariate of Emax and dose corrected for body weight

In the simulations, the "placebo" response was assumed to be zero, and the pre-treatment baseline measurements were considered to be equivalent to the PM used in the model development (see Section 6.5.4).

Patients achieving a total cholesterol < 5.6 mMol.L^{-1} were considered to be completely controlled and defined as "responders". The responder rate was defined as the percentage of responders at each dose level.

The range in the simulated total cholesterol concentrations achieved at each dose level is shown on Figure 6.12. There was no obvious difference between the two models. The simulated percentage of responders by pre-treatment concentration are shown on Table 6.11 and Figure 6.13a. Predicted responder rates at doses of 10, 20 and 40mg were 22.6, 40.9, 54.5%, and 24, 41.9 and 56.2% for Run 45 and Run 46, respectively. In general, the predicted responder rate was 1 to 2% different over the whole dose range (0 to 160mg). The similarity between the responder rates is consistent with the similarity in the achieved concentrations (Figure 6.12). A doubling of dose from 10 to 20mg was predicted to increase the responder rate by ~80%, while a doubling from 20 to 40mg would only increase the responder rate by a further 34% (Figure 6.14 a).

Figure 6.12 Distribution of predicted total cholesterol concentrations across dose for a) Baseline on D_{50} model (Run 45) and b) Baseline on Emax model (Run 46). Horizontal line and boxes indicate median and interquartile range (Q1-Q3), respectively. The whiskers extend to the lowest and highest values that are still inside the region defined by Q1 - 1.5.(Q3 - Q1) to Q3 + 1.5.(Q3 - Q1), showing the range of the data. The other lines indicate values which lie outside this interval.

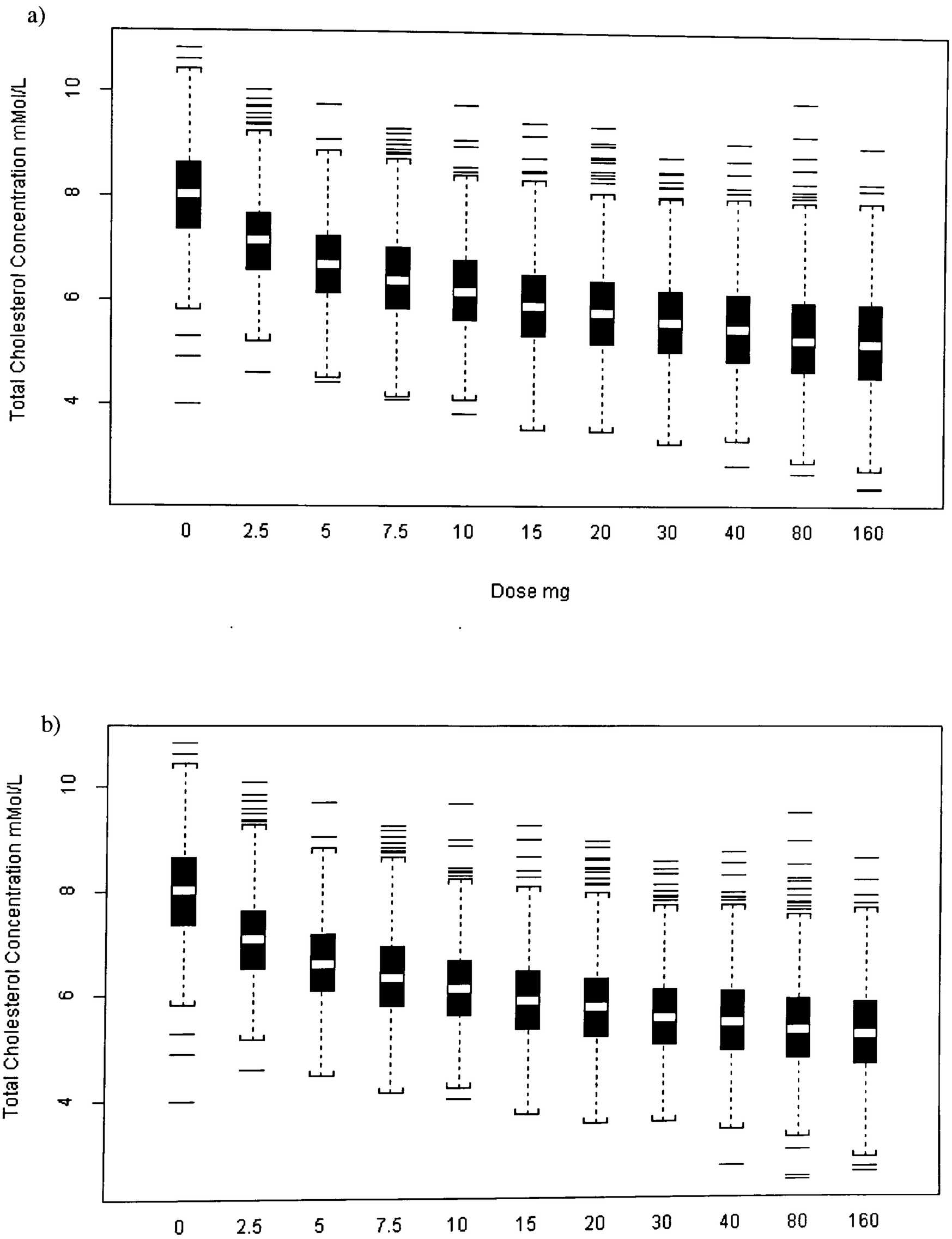


Table 6.11 Percentage responder rate in a simulated population of 1000 patients

Daily Dose		**0**	**2.5**	**5**	**7.5**	**10**	**15**	**20**	**30**	**40**	**80**	**160**
All patients n=1000												
Run45	*D_{50}	0.3%	2.9%	7.9%	16.7%	22.6%	35.5%	40.9%	49.4%	54.5%	61.5%	64.1%
Run46	*Emax	0.3%	3.1%	9.1%	17.6%	24.0%	36.3%	41.9%	51.2%	56.2%	63.3%	65.0%
All patients >8.8 mMol.L^{-1} n= 152												
Run45	*D_{50}	0.0%	0.0%	0.7%	2.6%	4.6%	11.2%	15.8%	18.4%	23.7%	31.6%	33.6%
Run46	*Emax	0.0%	0.0%	1.3%	2.6%	7.9%	19.1%	27.0%	32.9%	34.9%	45.4%	46.1%
All patients <7.2 mMol.L^{-1} n= 168												
Run45	*D_{50}	1.8%	15.5%	35.7%	53.0%	54.2%	73.2%	70.2%	80.4%	82.7%	88.1%	92.9%
Run46	*Emax	1.8%	14.9%	33.9%	55.4%	57.7%	80.4%	80.4%	88.7%	89.9%	94.0%	95.8%

* Parameter on which baseline is modelled as a covariate

Figure 6.13 Predicted percentage responding in a simulated population of 1000 patients, using the baseline on D_{50} model (Run 45) and the baseline on Emax model (Run 46). a) All baseline measurements b) Baseline measurements >8.8 $mMol.L^{-1}$ c) Baseline measurements <7.2 $mMol.L^{-1}$

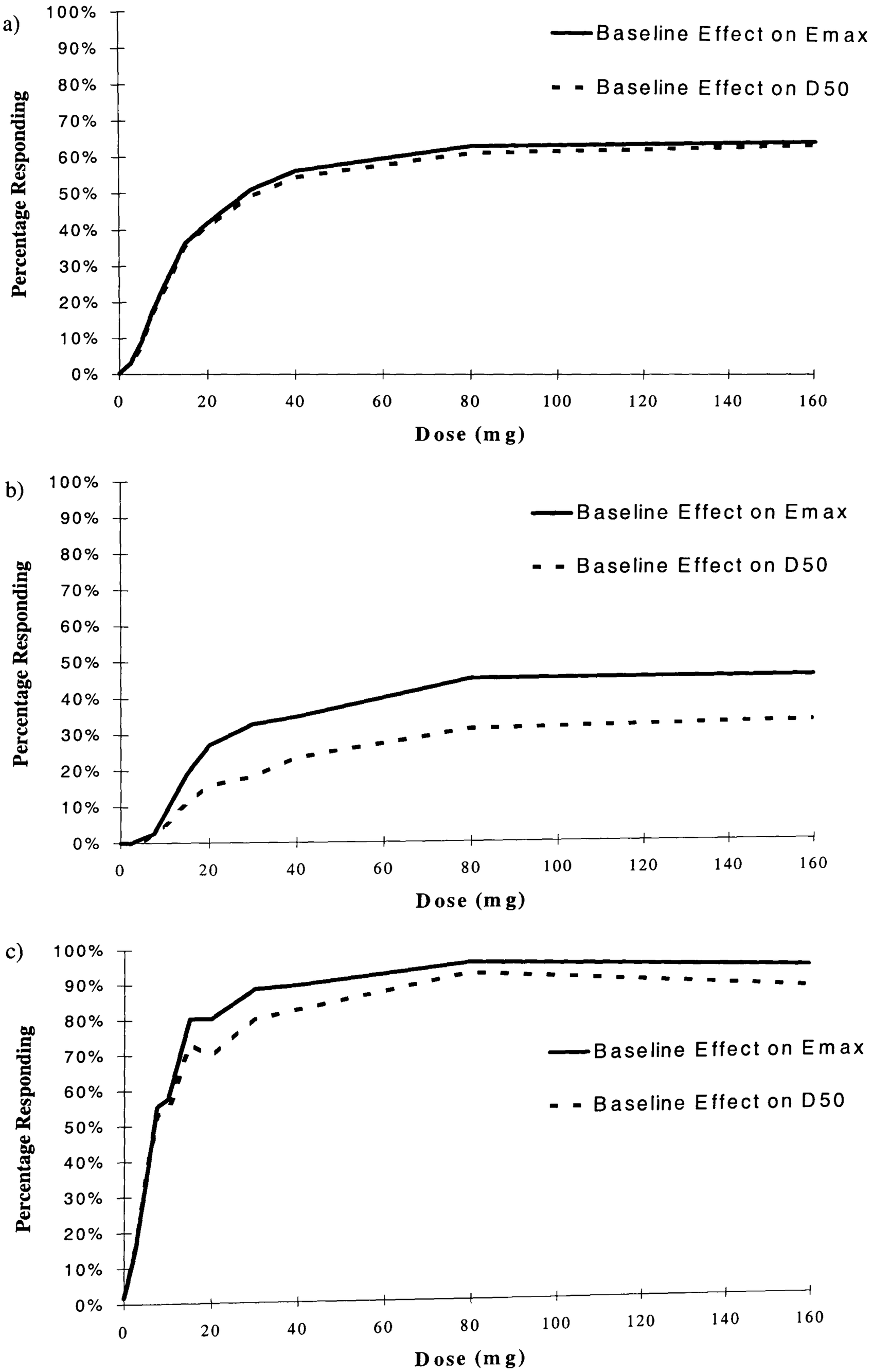
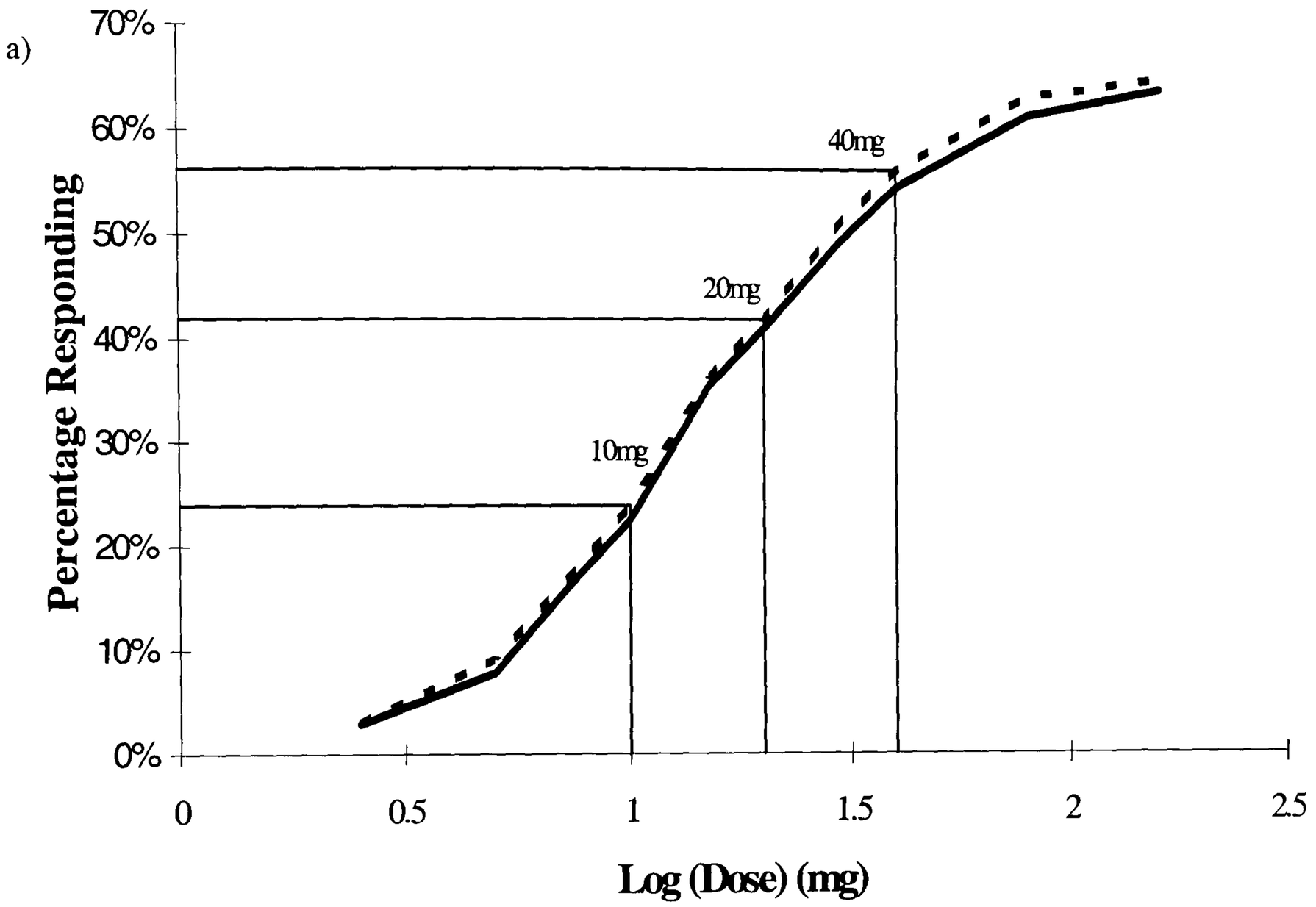
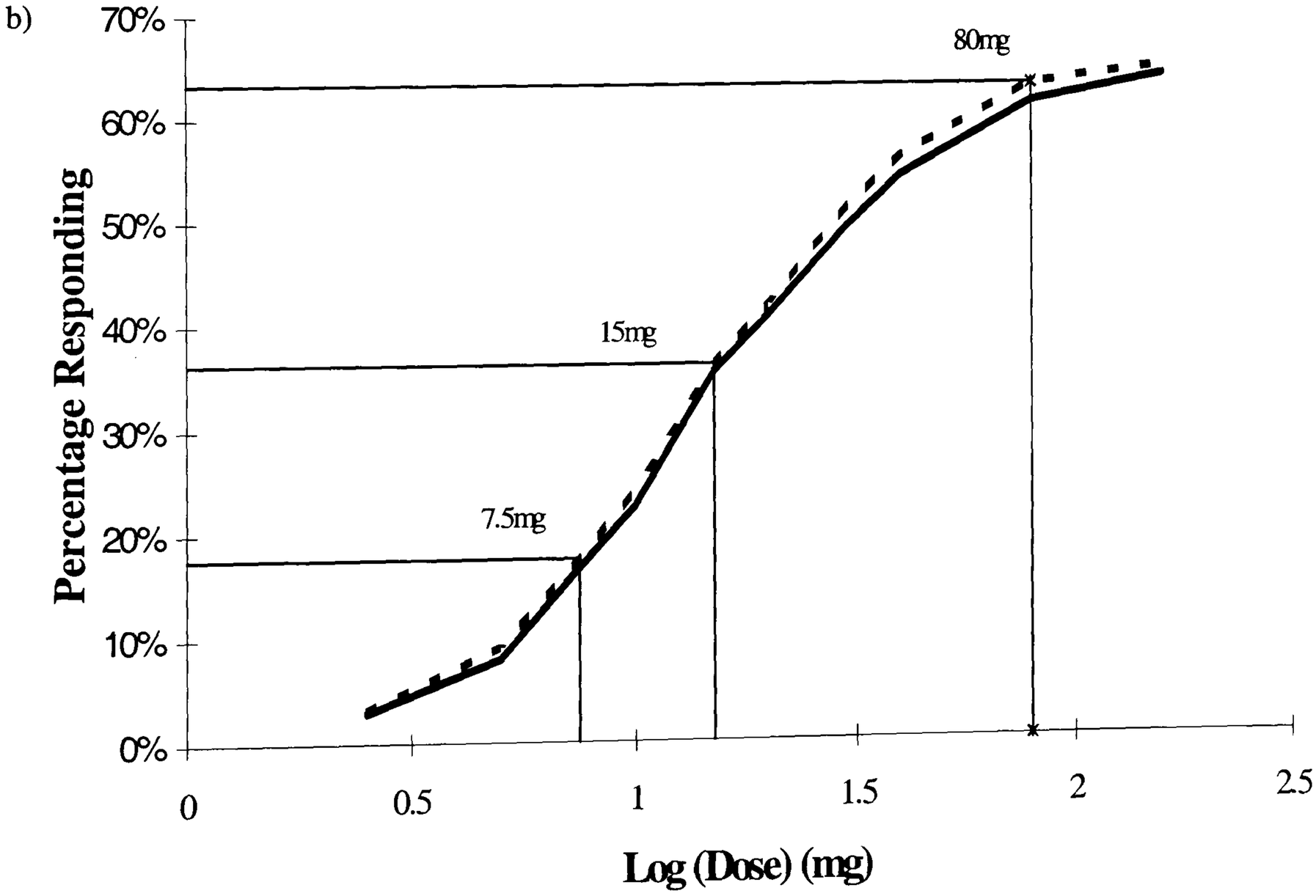


Figure 6.14 Predicted percentage responding in a simulated population of 1000 patients, using the baseline on D_{50} model (Run 45)-(dashed line) and the baseline on Emax model (Run 46)-(solid line). a) Response at 10, 20 & 40mg b) Response at 7.5, 15 & 80mg



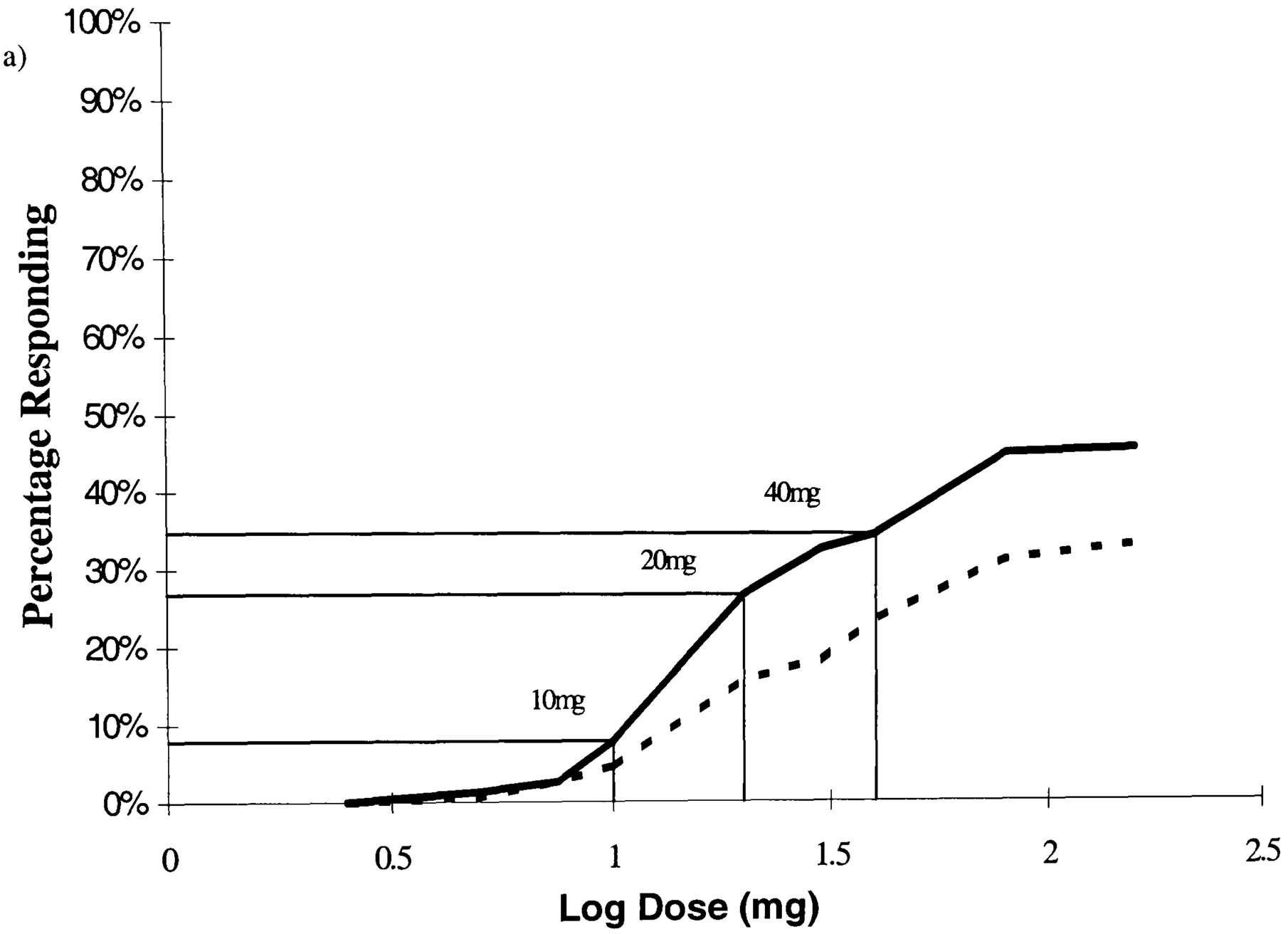


A larger than a doubling of dose would be required to double the number of responders with doses >20mg (Table 6.11). In comparison to the 10mg to 20mg to 40mg titration, an alternative titration of 7.5mg to 15mg to 80mg would increase the responder rate by at least 70% at each step (Figure 6.14b).

As expected, the responder rates were predicted to be markedly different between patients with high and low pre-treatment concentrations. For example, the responder rate in patients with a low pre-treatment concentration (<7.2 mMol/L^{-1}) taking 5mg daily was predicted to be similar to that in patients with a high pre-treatment (>8.8 mMol/L^{-1}) concentration taking 160mg daily (Table 6.11). In addition, when considering the tails of the pre-treatment concentration distribution (<7.22 or >8.8 mMol.L^{-1}), the predicted responder rate was different for the two models (Figure 6.13 b & c). For these individuals, the model from Run 46 (baseline as a covariate of Emax) predicted a significantly higher responder rates than the model from Run 45 (baseline as a covariate of D_{50}). This difference increased to a maximum over the dose range of 10 to 30mg. The maximum differences (14.5% and 18.3% for pre-treatment cholesterol of <7.2 mMol.L^{-1} and > 8.8 mMol.L^{-1}, respectively) decreased slightly as dose was further increased.

The potential dosage regimens for patients with high (>8.8 mMol.L^{-1}) and low (<7.2 mMol.L^{-1}) pre-treatment total cholesterol concentrations are compared in Figures 6.15 and 6.16, respectively.

Figure 6.15 Predicted percentage responding in a simulated population of 152 patients with pre-treatment cholesterol >8.8 mMol.L^{-1}, using the baseline on D_{50} model (Run 45)-(dashed line) and the baseline on Emax model (Run 46)-(solid line). a) Response at 10, 20 & 40mg b) Response at 7.5, 15 & 80mg



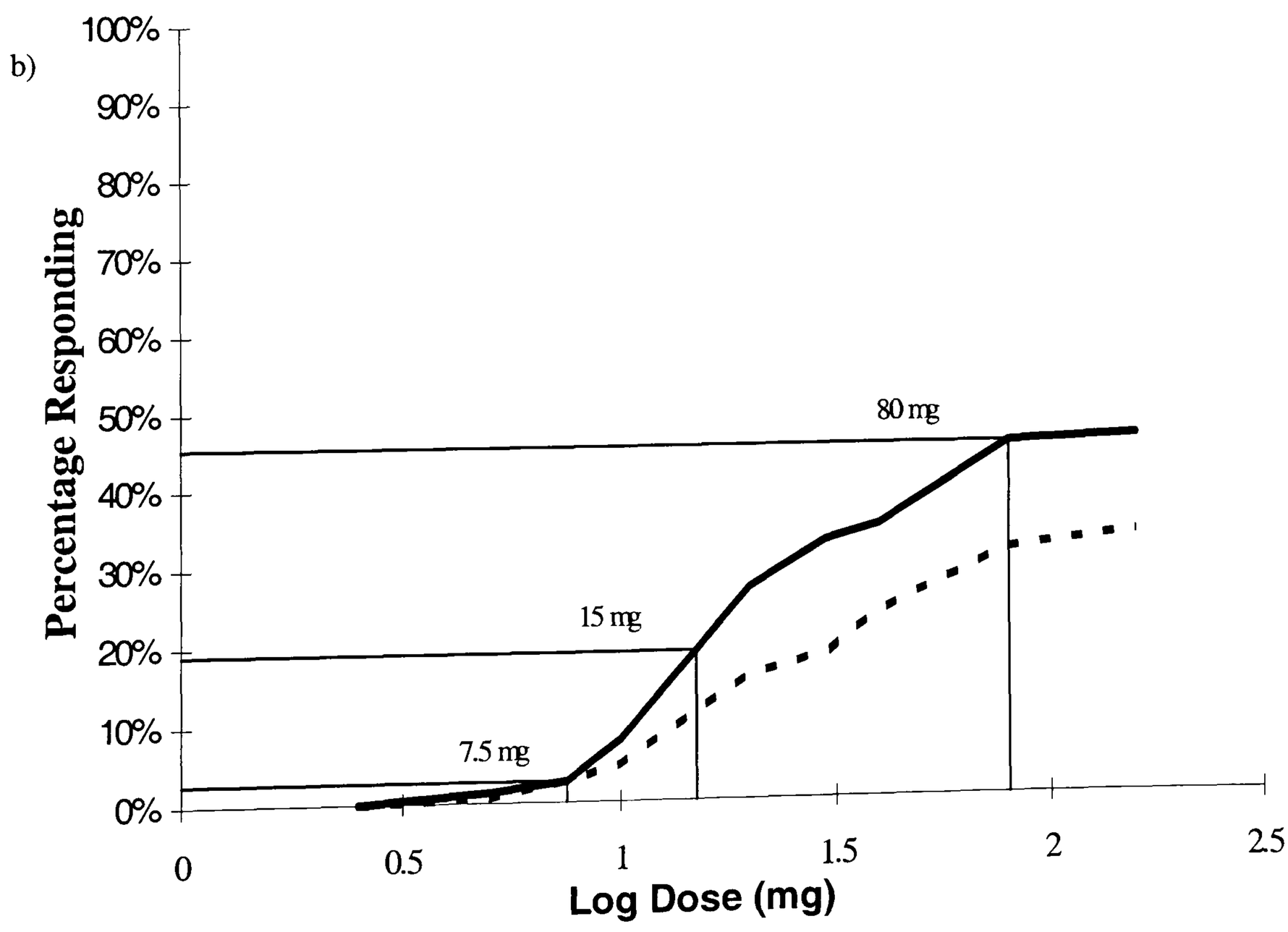
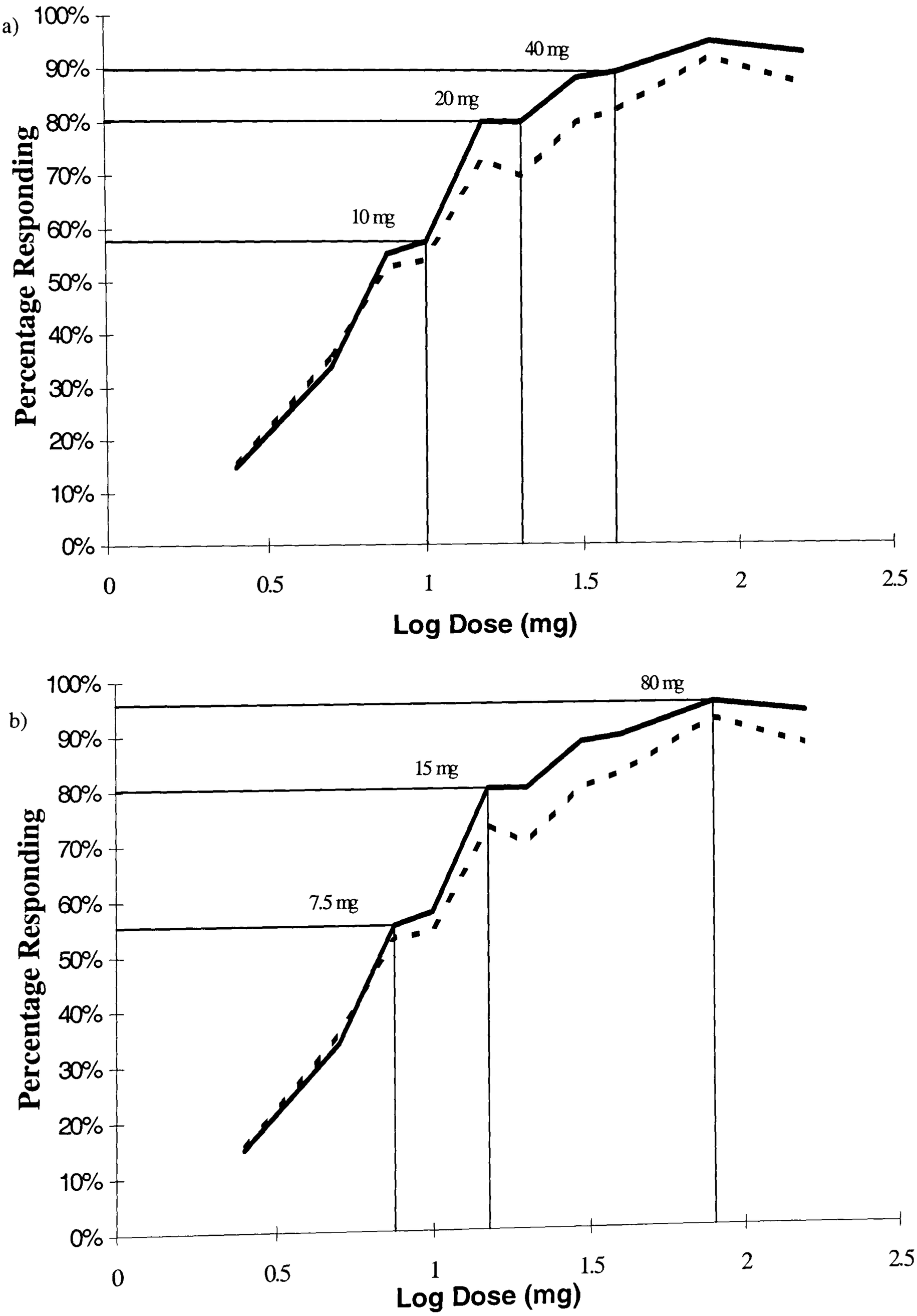


Figure 6.16 Predicted percentage responding in a simulated population of 168 patients with pre-treatment cholesterol <7.2 mMol.L^{-1}, using the baseline on D_{50} model (Run 45)-(dashed line) and the baseline on Emax model (Run 46)-(solid line). a) Response at 10, 20 & 40mg b) Response at 7.5, 15 & 80mg



6.7 Discussion

6.7.1 Mean reductions and analysis of variance

The mean total cholesterol pre-treatment (i.e. PM) was 7.8 ±1.1mMol.L^{-1} and therefore the average patient had at least a three fold increased risk of fatal CHD compared to subjects with a normal cholesterol i.e. < 5.2 mMol.L^{-1} (Study group,1998). In comparison, the mean total triglycerides pre-treatment(2.4 mMol.L^{-1}) were only marginally raised in comparison to the range of 2.3 to 5.6 mMol.L^{-1} for mild to moderate hypertriglyceridaemia. Treatment was therefore mainly required to lower the total cholesterol in general, and LDL cholesterol in particular.

The mean percentage reduction in total cholesterol after the 40mg dose i.e. 31%, was the same as that estimated in a previous investigation of subjects sampled from the same hypertensive, hypercholesterolaemic population (Macdonald et al., 1991). Changes in the lipid sub-fractions across the dose range were also consistent with previous dose ranging studies which utilised different patient populations (Table 6.3 vs Table 6.1). The average response to simvastatin would therefore appear to be consistent across various sub-groups of the potential target population. The equivalence of total cholesterol and total triglyceride responses after 6 and 12 weeks of dosing corresponds to the maximum response occurring with the first 6 weeks of treatment with a particular dose (Todd & Goa, 1990). Although the ANOVA and subsequent multiple comparisons indicate the significant treatment effects across the tested doses, extrapolation and prediction of responses to other doses and differences between potential future dosing strategies requires the utilisation of modelling techniques.

6.7.2 Population dose response relationships

Since there were no period effects, the lipid responses were modelled ignoring the dose administration sequence. The total triglycerides were only mildly raised, so it is not

surprising that the simplest step model was the most appropriate in this case. The effects on VLDL and HDL cholesterol were best described by the step-linear model which predicts that the change in HDL and LDL would increase linearly within the observed dose range. The mean parameter estimates for Emax and D_{50} were precisely estimated for the reduction in both total and LDL cholesterol. The parallel nature of the projected dose response curves (Figure 6.4), and the similarities in the estimated Emax and D_{50} values highlight that the principle effect of simvastatin is to reduce total cholesterol by removing circulating LDL cholesterol. The association between decreasing LDL cholesterol and increasing HDL cholesterol during treatment with simvastatin is consistent with previous studies (Tuomilehto et al., 1994). Although, the changes in the total:HDL cholesterol and LDL:HDL cholesterol ratios may be important to the assessment of the total benefit from treatment, reduction in CHD mortality has been more clearly related to changes in total or LDL cholesterol alone.

The LDL and total cholesterol responses over the 10 to 40 mg dose ranges were predicted to fall into the upper 70-90% of the projected dose response curve (Figure 6.4). However, the total and LDL cholesterol model extrapolations may be biased by the small portion of the dose response relationship covered by the study dose range. Although, formal model validation would require prospective evaluation over a wider dose range, the predicted responses were consistent with the results from the primary placebo controlled dose ranging studies detailed in Table 6.1 and shown in Figures 6.17 & 6.18. Nevertheless, the models did not extrapolate as well to the recent high dose studies (also shown in Figures 6.17 and 6.18), i.e. the predicted median responses for 160mg was less than that measured (Davidson et al., 1997). Whilst this is not surprising given that the observation was well outside the dose range used to develop the model, the lack of a placebo in the high dose study could also account for the difference.

Figure 6.17 Predicted percentage total cholesterol reduction versus the dose of simvastatin (mg): Comparison with dose ranging studies

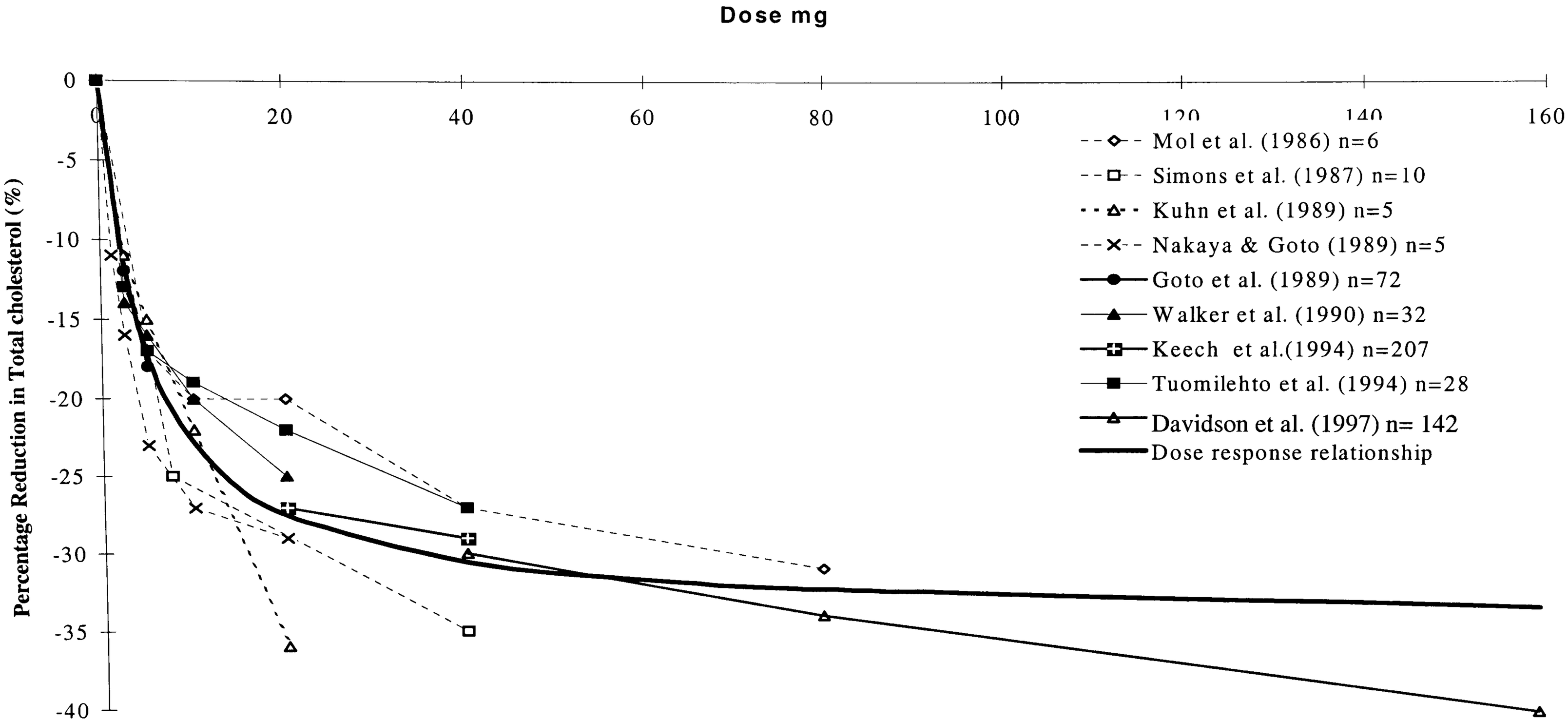
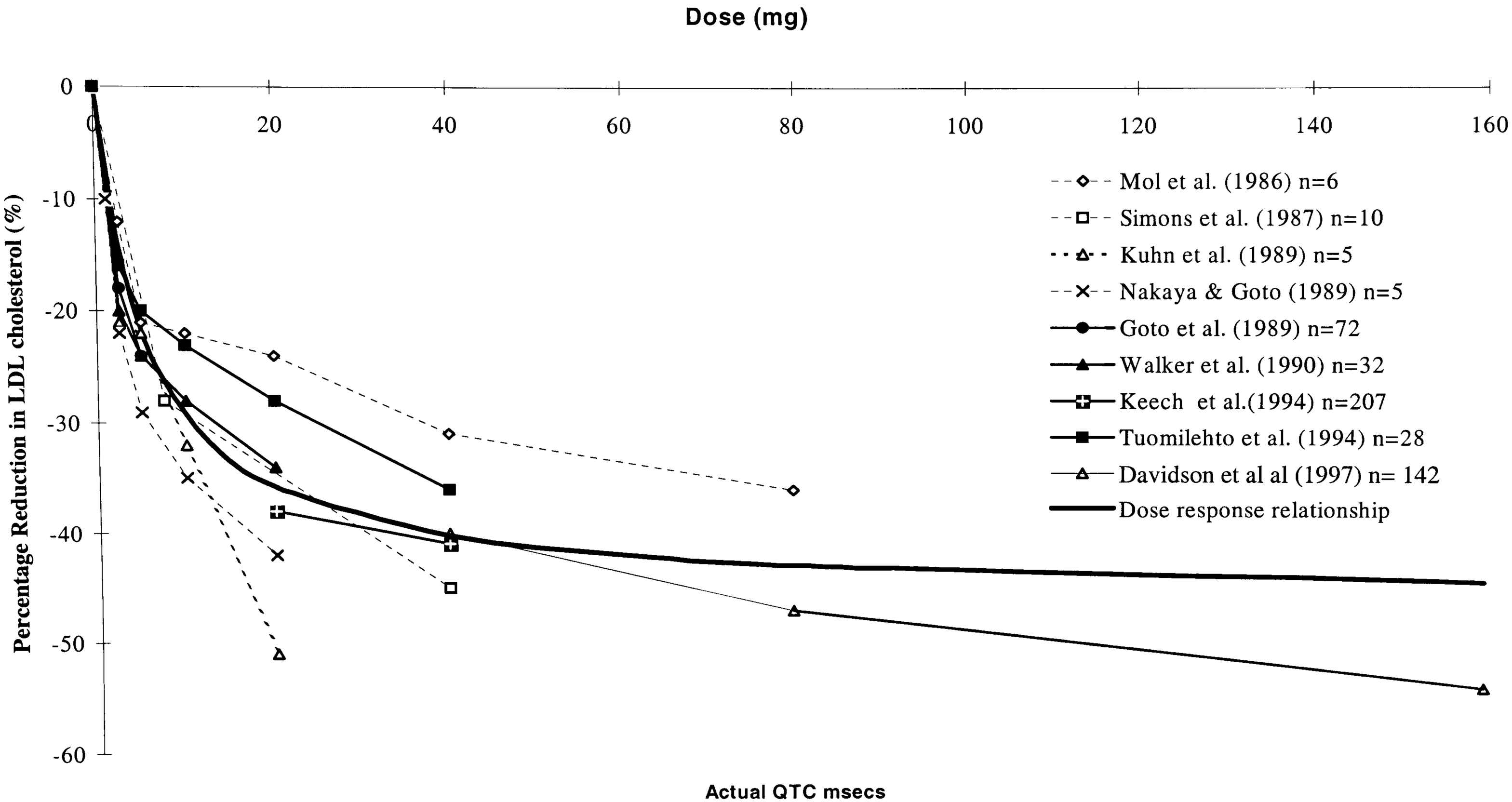


Figure 6.18 Predicted percentage LDL cholesterol reduction versus the dose of simvastatin (mg): Comparison with dose ranging studies



However, other emerging work appears to support the notion that the HMG-CoA reductase inhibitors have a response curve which is still log-linear at doses 3 to 4 times the current maximum dose (Bradford et al., 1991; Pedersen & Tobert, 1996), and data in this range is needed to support the models derived in this analysis .

6.7.3 Covariate analysis

Previously, pre-treatment concentration and gender have been shown to be predictors of the total and LDL response to simvastatin (Miserez et al., 1994). In this analysis, attempts were made to determine which aspect of the dose response relationship each covariate most influenced. Initially, an absolute reduction model was used, however, percentage reduction from baseline is most often reported when comparing response of lipid lowering therapies. Utilising the percentage reduction models reduced the number of interindividual variablity terms required in the basic model. The transformation of the data led to an additive intraindividual variability model being more appropriate than the proportional error model used with the absolute reduction model. This change highlights the importance of rechecking the modelling assumptions.

In contrast to the absolute reduction model for total cholesterol, which determined that PM was best included as a covariate of Emax, changing to the percentage reduction model indicated that PM could equally be a covariate of D_{50}. While the high degree of correlation between Emax and D_{50} will always be a confounding factor, larger numbers and a wider coverage of the dose response relationship may allow the effect of PM on each or both parameters to be more accurately determined. For the LDL cholesterol, utilising the percentage reduction model allowed estimation of interindividual varibility on both Emax and D_{50}, and determination of a statistically significant effect of PM on Emax and D_{50}. Therefore, although the percentage reduction model helped uncover more potential covariate relationships it did not help to clarify their relative importance.

Gender was a potential covariate for both the total and LDL cholesterol responses, and gender differences in both the pharmacokinetics (Cheng et al., 1992) and pharmacodymanics of simvastatin have been previously demonstrated (Clifton et al., 1994; Miserez et al., 1994). However, estimates of the relevant parameter were not significantly different from zero. Confounding with PM and the female preponderance in the study population possibly prevented precise estimation of gender differences.

The relationship between PM and response is consistent with the action of simvastatin on the number of liver LDL receptors. A shift in the equilibrium between production and removal of LDL particles towards a higher rate of removal should theoretically produce a more profound reduction when the circulating levels are high. However, in this analysis it was not possible to determine whether the effect would result in a steeper decline, a greater overall reduction or both.

Nevertheless, the importance of the pre-treatment level in comparison of responses was highlighted. On this basis, a difference between baseline levels could also account for the disparity between the predicted and observed response to the 160mg dose (Figure 6.17 and 6.18).

The association between body weight and the response to simvastatin is more difficult to justify since the use of an additional parameter to account for the effect did not have a large affect on the fit. Furthermore, although weight reduction in association with a lipid lowering diet is reported to decrease plasma cholesterol by up to 25% (Study Group, 1988), the average weight did not change over the study duration, and subjects from this study population have previously failed to respond to dietary measures (Farish et al., 1990). Nevertheless, a weight corrected dose may be justified pharmacokinetically. Liver size is related to body weight, so the efficiency with which the active metabolites are cleared from the liver, via biliary excretion, may increase with increasing weight. This explanation may also theoretically account for gender effects found in previous studies.

While the detected covariate relationships are consistent with previously postulated covariate effects, the large majority of the interindividual variability remained unexplained. Further work, in a larger population, may allow the covariate relationships so far identified to be fully characterised and other covariates, which may help to explain more of the interindividual variability, to be determined.

6.7.4 Simulation of responder rate

For hypercholesterolaemia as an isolated risk factor, it is recommended that total cholesterol should be maintained below 5.6 $mMol.L^{-1}$. For simplicity, the responder rate was predicted using this concentration. However, if the incidence of multiple risk factors within the population was known, a range of target concentrations could be used to more accurately predict the overall responder rate.

The accuracy of simulations are conditional on the adequacy of the models, so the simulations used parameters from both Run 45 and Run 46 to test for differences. A larger responder rate was predicted for the model with PM related to Emax (Run 46), and the magnitude of this difference was predicted to increase with both increasing dose and the increasing value for PM.

The change in responder rate with increasing dose is consistent with the Emax model used in the simulations i.e. on approach to the asymptotic part of the Emax curve a larger and larger dose is required to produce the same increase in response and, therefore responder rate.

Although, it would be possible to individualise dose based on the pre-treatment cholesterol measurements, the range of commercially available tablet strengths limit the dose selection. The current available strengths in the UK (10 and 20mg) allow for titration from 10mg in steps of 10 mg. However, this would seem to only allow for titration in the "flatest"

portion of the dose response curve. A smaller tablet strength of 5 to 7.5 mg would allow smaller titrations starting from the D_{50} and therefore the steeper portion of the dose response curve. However, rationalisation of treatment, based on achievement of a particular "target" cholesterol level, could lead to a completely different set of dose strengths. Importantly, the choice of doses has to take into account the relationship between dose and adverse effects. Fortunately, the toleration to all doses including 80mg, was good in the primary dose ranging studies, and any side-effects have usually been mild (Section 6.1.4). However, for a prophylactic treatment even mild side-effects may be sufficient to reduce compliance, so it is prudent to dose patients with the lowest possible dose required to achieve the desired effect. With this in mind, the choice of 7.5, 15 and 80 mg doses may for several reasons be more advantageous. Firstly, an average responder rate similar to that for the 20mg dose could be achieved with an initial dose of 15 mg (Table 6.11), and the lower dose may lead to a reduced incidence of side-effects. A subsequent titration to 80mg would provide a pronounced increase in responder rate for resistant patients. Secondly, for patients with a low pre-treatment measurement (i.e. <7.2 mMol.L^{-1}) 7.5mg may be a more prudent starting dose, since the responder rate is predicted to be very similar to that for the 10 mg (53 to 55.% vs 54.2 to 57.7%, Table 6.11), and yet the smaller dose may again be sufficient to decrease the incidence of adverse effects. Furthermore, a subsequent titration to 15mg would provide a responder rate of between 73 to 80%, which is substantial given that another three fold increase in dose would be required to increase responder rate by another 10%. Lastly, since the responder rate is much lower in patients with a high pre-treatment measurement (i.e.>8.8 mMol.L^{-1}) a starting dose of 80mg may be appropriate since the risk of developing CHD could outweigh the risks of any potential adverse events.

Patients not adequately responding to this dose, may only achieve a greater reduction with the addition of a second agent such as a bile acid sequestrant (Da Col et al., 1990, 1993;

Desager et al., 1991) or a fibrate (Deslypere, 1992; Feussner et al., 1992). However, the increased potential for more serious adverse effects i.e. myopathy and rhabdomyolysis must be considered if a combination with a fibrate is to be advocated (Plosker & McTavish, 1995).

6.7.5 Study design

The dearth of published knowledge on the dose response relationship for simvastatin, appears, despite the number of dose ranging studies, to be due to the acceptance of the poor information provided by current practices in the design and analysis of dose ranging studies. Specifically, use of the parallel design has not provided individual dose response information and, therefore, only a conservative exploration of the dose response relationship.

The present study design has only explored doses over a narrow range at the upper end of dose response relationship, and the identification of both the structural and covariate models was restricted by this limitation. In particular, it was not possible to determine whether the correlation between PM and response was most appropriately modelled by including it with D_{50} or Emax. Escalation of subjects over a wider range of doses would have allowed better characterisation of the dose response relationship and the covariate effects. This approach would seem particularly appropriate for simvastatin given the low incidence of adverse effects. The lag time of six weeks until the maximal effect is achieved could restrict the number of doses used in any future escalation design. However, the number of subjects achieving the desired total cholesterol concentration could be increased by altering the starting dose and titration steps based on the pre-treatment value.

6.7.6 Future clinical practice

The potential shortcomings of the present range of doses must be balanced against the therapeutic success of simvastatin. However, with the link between control of

hypercholesterolaemia and reduction in cardiovascular morbidity and mortality becoming established, the need to rationalise treatment may now be more important. Effective clinical management of an individual patient's response requires that information is provided to help rationalise between dose escalation and the addition of other drug therapy. Covariate effects (i.e. pre-treatment level and gender) may alter the dose response relationship and influence this decision.

Previously, the comparisons of HMG CoA inhibitor potencies has been based on mean dose response relationships (Illingworth et al., 1994). The results of this investigation have shown how these may lack accuracy and would benefit from the estimation and comparison of population dose response relationships (Marshall et al., 1994) as developed by Sambol and Sheiner (1991).

6.8 Conclusions

The analysis has identified structural models for each of the lipid responses to simvastatin. Despite the restrictions of the study design, an Emax relationship for the reduction in total and LDL cholesterol was established as the most appropriate model. Average predictions for responses outwith the studied dose range were consistent with observations made in previous dose ranging studies.

In the covariate analysis it was not possible to determine whether the correlation between PM and response was best modelled by including it with Emax or D_{50} or both. The estimation of the effect of gender on the reduction in LDL cholesterol was confounded by the majority of females having a high PM. Modelling the response as a function of dose per kg body weight further decreased the objective function without increasing the model complexity.

Simulations predicted that the percentage of responders (patients attaining a cholesterol <5.2 mMol.L^{-1} would increase from 24% on 10mg to 56% on 40mg. However, based on

this analysis a more rationale set of doses may be 7.5, 15 and 80 mg, and it was predicted that these would offer greater flexibility since more of the dose response range could be covered. In particular, it was predicted that this would allow patients to be prudently titrated in accordance with pre-treatment measurements.

It may be hypothesised from this analysis that a more appropriate design and interpretation of the primary dose ranging studies would have resulted in different doses of simvastatin being used in phase III trials. Furthermore, a better understanding of the dose response relationship and possible importance of covariates at an early stage in the clinical development programme may have helped to provide a definitive recommended dose range before the drug was established in clinical practice.

CHAPTER 7

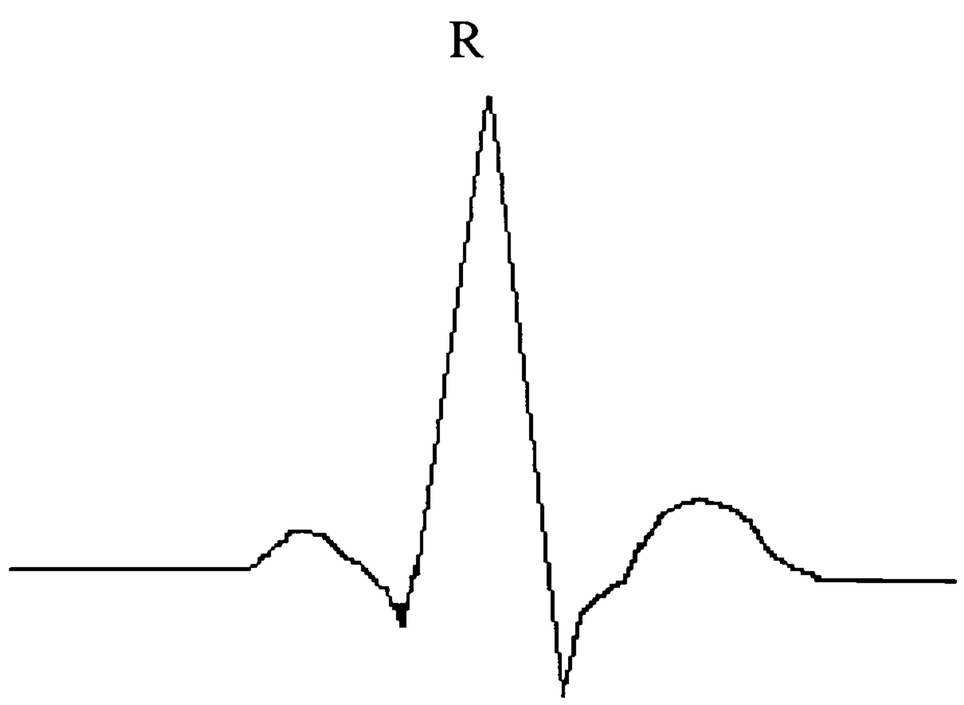
DOSE/ CONCENTRATION/ RESPONSE RELATIONSHIP FOR A NOVEL CLASS III ANTI-ARRHYTHMIC DOFETILIDE: COMPARISON BETWEEN PATIENTS AND HEALTHY VOLUNTEERS

In this chapter mixed effects modelling is used to establish the dose concentration response relationship for a novel class III anti-arrhythmic drug under development for the treatment of re-entrant cardiac arrhythmias. Effect compartment methodology is used to account for the temporal delay between peak plasma concentration and maximum increase in the QTc interval, a surrogate marker of Class III anti-arrhythmic activity. A comparison between healthy volunteers and patients with ischeamic heart disease is made by testing for differences in the parameters of resulting PK/PD models. Covariates which could help explain the variability in the PK and PD components of the model are investigated. To aid in the design of future studies, fixed dose and dose per kilogram body weight short term infusions are compared using simulation. The potential safety implications for each regimen are discussed. Similarly, the steady state concentrations which give rise to QTc prolongations associated with an increased risk of Torsades de Pointes (TdP) were also predicted.

7.1 Introduction

7.1.1 Re-enterant cardiac arrhythmias

Abnormal heart rhythms known as cardiac arrhythmias can be characterised by electro-cardiographic (ECG) techniques. The effectiveness of drug treatment and other procedures in the correction or the prevention of rhythm abnormalities can be detected and measured by analysing ECG patterns. The PQRST waveform describes one cardiac cycle.



Various alterations in the time and shape of this waveform correspond to known rhythm disturbances and can be attributed to abnormal electrical conduction in particular areas of the myocardium. Both increased automaticity of pace maker cells which initiate the electrical wave fronts, and cyclical re-activation of the myocardium by wavefronts are common causes of cardiac arrhythmias. Re-activation results from temporal and spatial inhomogeneities in conduction and refractoriness within the myocardium; which allow advancing wavefronts to fractionate into more independently circulating activation wavelets. Re-excitation of previously refractory myocardium produces a re-entrant arrhythmia which can result in symptomatic tachycardias, atrial fibrillation and life-threatening episodes of ventricular fibrillation (VF).

7.1.2 Class III antiarrhythmics

Agents which lengthen the refractory period form an important class of drugs for the treatment and prevention of re-entrant arrhythmias (Singh, 1993). Amiodarone and sotalol are effective agents in the control of various types of arrhythmia, and show class III activity along with other pharmacological actions (Beckers & Kulbertus, 1987; Singh & Nademanee, 1987; Nora & Zipes, 1993). Although a recent study has shown evidence to the contrary (Kuck, 1996), amiodarone is generally considered to be effective in maintaining sinus rhythm (Middlekauff et al. 1993) and improving the survival of patients with life threatening arrhythmias (Herre et al. 1989; Ceremuzynski, 1993; Greene, 1993). However, it has an exceptionally long terminal half-life (20 to 60 days), so steady state conditions are not achieved until several months after the start of therapy (Rodden, 1993).

The slow peripheral accumulation is highly variable due to extensive plasma protein binding (>98%) and the incidence of adverse events i.e. impairment of thyroid function and pulmonary toxicity have not been found to correlate with plasma concentrations (Ulrik et al. 1992; Greene, 1993). The bradycardic side-effects of sotalol have been largely removed by resolving the racemic mixture and administering the d-isomer which has no beta-blocking activity (Johnston et al. 1985). However, control of ventricular rate through antagonism of the beta-adrenoreceptor may be important, since the mortality rate with the single isomer has been shown to be greater than with placebo (Waldo et al. 1996). Nevertheless, the predictable pharmacology of a pure class III antiarrhythmic may offer the advantage of predictable efficacy and the potential of a safer treatment option for both the termination and prevention of cardiac arrhythmias.

7.1.3 Dofetilide

Dofetilide (UK-68,798) (N-(4-(2-(2-(4-(methane sulphonamido) phenoxy) N-methyethylamino) ethyl) phenyl) methane is a pure class III antiarrhythmic, as stratified by the Vaughan Williams classification for antiarrhythmics. Accordingly, it selectively inhibits the rapid component of the delayed rectifier potassium current IKr, prolongs the period that the cardiac tissue is refractory to further excitation, but does not affect the velocity of myocardial conduction (Gwilt et al. 1991; Carmeliet, 1992). The prolongation of the effective refractory period (ERP) and, therefore, the action potential duration (APD), prevents the propagation of cycling waves of reactivation (Gwilt et al. 1991; Knilans et al. 1991). Dofetilide has been shown to be effective in treatment and prevention of arrhythmias with a re-entrant mechanism i.e. atrial fibrillation (AF) (Suttorp et al. 1992) , paroxysmal supraventricular tachyarrhythmias (pSVT) (Connelly et al. 1992; Wong et al. 1992) and ventricular tachycardia and fibrillation (VT/VF) (Echt et al. 1991; Fananapazir & Cropp, 1992; Thomsen et al. 1992).

7.1.4 Pharmacokinetics of dofetilide

Previous studies have shown a linear relationship between dose and both AUC and Peak concentration (Sedgwick et al. 1991; Rasmussen et al. 1992). Elimination from the plasma following intravenous infusion has been shown to be biexponential with a terminal half-life of 7.5 to 9.0 hours (Rasmussen et al. 1992; Smith et al. 1992; Funck-Brentano, 1993).

Over 70% of the parent drug appears to be excreted unchanged in the urine while the remainder is inactivated by metabolism. The potential for metabolic interactions is therefore limited and in addition dofetilide is only moderately bound to plasma proteins (64%).

7.1.5 Pharmacodynamics of dofetilide

The QT interval is the time between the depolarisation and repolarisation of the ventricular myocardium, and corresponds to the ventricular refractory period. It is measured by standard ECG methods and is used in this chapter as an non-invasive surrogate marker of class III antiarrhythmic activity and safety.

Although QT prolongation has been successfully modelled (Whiting et al. 1980; Holford et al. 1981) , the reliability of QT as a general marker for antiarrhythmic effect has previously been questioned (Vaughan-Williams, 1985). However, its relevance when considering class III specific agents has more recently been highlighted (Podrid, 1992).

The length of the QT interval is inversely related to heart rate, so antiarrhythmics with a negative inotrophic effect can prolong the QT interval.

Development of individual regression equations has been shown to produce the least biased heart rate corrected QT (QTc) measurements (Kelman et al. 1981). However, the population regression models are the most commonly employed i.e. (Bazett, 1920). When heart rate changes are small within individuals the two approaches are comparable.

Dofetilide has no effect on heart rate, but correction is still required to allow comparison of the QT intervals measured within and between study days.

7.1.6 Torsades de Pointes (TdP)

All antiarrhythmic drugs have a relatively narrow therapeutic window. The maximum therapeutic concentrations are limited by the risk of proarrhythmia. A major concern with the class III antiarrhythmics is Torsade de Pointes (TdP), a form of polymorphic VT which can degenerate into ventricular fibrillation. The incidence of TdP is associated with many factors and a wide variety of clinical settings (Morganroth, 1987; Keren & Tzivoni, 1991). However, the most frequently reported cause of this arrhythmia is exposure to drugs known to delay repolarisation (Stratmann & Kennedy, 1987). The overall incidence of TdP is low even for the class III drugs (Mattioni et al. 1989), though prevention is important since episodes can be life-threatening (Sclarovsky et al. 1983; Kuck et al. 1984; Brown et al. 1986; Dancey et al. 1997). High plasma drug levels lead to excessive prolongation of QTc, so overdose is a common cause of TdP (Neuvonen et al. 1979; Belton et al. 1982; Kuck et al. 1984). Prolongation of QTc is the single most common precursor to TdP (Zehender et al. 1991). Stratmann and Kennedy reviewed 197 cases of TdP 49 % had a predose QTc > 420 msecs, in comparison the normal range for QTc interval in healthy volunteers is 380 to 400 msecs (Stratmann & Kennedy, 1987). In 89% of the cases investigated the pre-TdP QTc was > 560 msecs. Similarly, in 79% of the cases pre-TdP QTc had been prolonged by > 25%. While the degree of prolongation has not as yet been shown to correlate with efficacy (Surawicz & Knoebel, 1984), it may be possible to identify what is sufficient for efficacy but not associated with an increased risk of TdP. The QTc interval may therefore be a particularly useful surrogate in the determination of therapeutic regimens for class III antiarrhythmics.

7.2 Background to analysis

Early pharmacodynamic data for dofetilide suggested that the minimum dose to cause a measurable change in QTc was 5mcg.kg^{-1} and 1.5mcg.kg^{-1} in healthy volunteers and patients with ischaemic heart disease (IHD), respectively. This difference suggested that the dose concentration response relationships may be different between patients and volunteers. Since it is known that disease processes can alter the efficacy of many drugs, the presence of ischaemic heart disease may increase the sensitivity of the myocardium to dofetilide. Alternatively, the attenuation of effect could simply be the result of an increase in target tissue concentration as a result of a disease related reduction in clearance or plasma protein binding. Moreover, a combination of both these effects could play a role in disease related modification of efficacy.

In drug development, comparative studies of matched groups of patients and volunteers are used to determine the potential for disease related changes to influence the dose response relationship. The data from such a study is used in this chapter to develop a PK/PD model for dofetilide.

7.3 Aims

The primary aims in this chapter were :-

1) To develop a PK/PD model to describe the effect of dofetilide on QTc interval

2) To compare the response to dofetilide in patients with ischaemic heart disease with that in healthy subjects.

3) To estimate the variability in the pharmacokinetic and pharmacodynamic parameters and to identify and explore any covariate relationships which help explain the variability.

4) To compare the variability in pharmacokinetics and pharmacodynamics following fixed dose and dose per kilogram regimens

5) Use the PK/PD relationship to predict the safety of various dosage regimens with regard to the incidence of Torsade de Pointes, and thus to help in rational design of future studies.

7.4 Study design

Two groups of patients with ischaemic heart disease (IHD) were given increasing doses of 1, 2, 4 and 4, 6, 8mcg.kg^{-1} of dofetilide, respectively. (A third group was to receive 6, 8, 10mcg.kg^{-1}, but this arm of the study was closed due to lack of recruitment). The healthy volunteers received 1, 6, 10μg.kg^{-1} of dofetilide.

Individuals were given three escalating doses and a randomly inserted placebo dose, on a single blind basis. Each dose was administered via a syringe driver, as an infusion over 30 minutes. A one week washout period was allowed between each study day.

7.5 Data

Eight patients were recruited into each of the two patient groups and the healthy volunteer group. Three females were recruited into the study (two were healthy volunteers). A total of 1594 plasma samples and 1416 measures of QTc were available for analysis. Patient 10 only received one dose and placebo, being lost to follow up after the after 4mcg/kg dose.

Measurements for Patient no 11 at the highest dose of 8mcg/kg were unobtainable, for the same reason .

Table 7.1 summarises the demographic, biochemical and the pre-dose QT/QTc data for the healthy volunteers and IHD patients. While the two subgroups were matched for height and age, the IHD patients were significantly heavier (mean difference14.4kg) than the healthy volunteers (Table 7.1). In terms of clinical biochemistry the two groups were also well matched. However the mean creatinine clearance (CLcr) for the healthy volunteers ($68ml.min^{-1}$) was significantly lower ($P<0.005$) than that estimated for the IHD patients ($92ml.min^{-1}$) Table 7.1. There was no difference between the mean predose baseline QT/QTc values.

Table 7.1 Demographics and biochemistry

	AGE	WT	HT	BIL	AST	ALT	SCRT	Clcr	Baseline QT	Baseline QTc
	years	kg	cm	umol.l^{-1}	IU.l^{-1}	IU.l^{-1}	umol.l^{-1}	ml.min^{-1}	msecs	msecs
EHD										
Mean	56.3	*82.2	171.6	7.1	24.0	25.0	92.3	**91.9	396	421
SD	7.4	9.8	7.4	2.7	6.0	15.0	11.0	15.7	31	24
Median	56.0	81.0	174.0	7.0	24.0	21.0	92.3	91.9	397	420
VOL										
Mean	59.1	*67.8	168.0	9.0	24.0	28.0	95.1	**68.4	395	413
SD	10.1	16.6	10.3	6.7	7.0	13.0	15.2	17.5	29	32
Median	61.0	65.0	170.0	9.0	24.0	26.0	96.7	62.2	393	409
Total										
Mean	57.2	77.9	170.4	7.8	24.0	27.0	93.2	84.1	395	418
SD	8.3	13.9	8.4	4.4	7.0	14.0	12.5	19.8	30	27
Median	57.0	77.5	173.0	7.0	24.0	26.0	93.6	86.9	395	418

Significant difference between means (* P<0.025 and **P<0.005)

7.6 Methods

Modelling was implemented using both the first order (FO) and first order conditional estimation (FOCE) methods (Chapter 3)

7.6.1 Pharmacokinetics

Intravenous infusion models with up to three compartments were compared. Models were parameterised using micro rate constants, with a central compartment volume of distribution also being estimated in each case.

7.6.2 Pharmacodynamics

The pharmacodynamic measure QT was corrected for heart rate (HR) to give QTc (the corrected QT interval) using Bazett's formula (Bazett, 1920)

$$QTc = \frac{QT}{\sqrt{RR}}$$

where RR is the RR interval from the eletrocardiogram.

The placebo response was smoothed using a three point moving average, and the QTc measurements were corrected by subtracting the smoothed placebo response. Missing placebo values were replaced by the average of the values on either side. After placebo correction the QTc, measurement of dofetilide pharmacodynamic response was modelled using two approaches. In the first instance the post-dose QTc measurements were corrected by subtracting the predose measurements. Change in QTc was modelled as function of concentration (C).

i.e. Δ QTc = f (C)

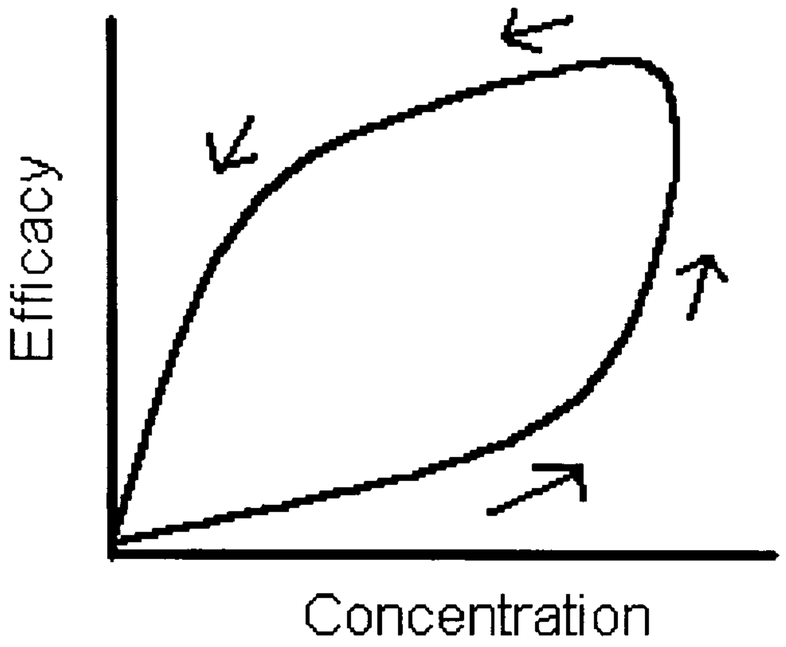
In a second set of models the baseline was included as an additional parameter

i.e. Δ QTc = f (C) + Baseline

A linear and an Emax model were used as functional forms for f(C)

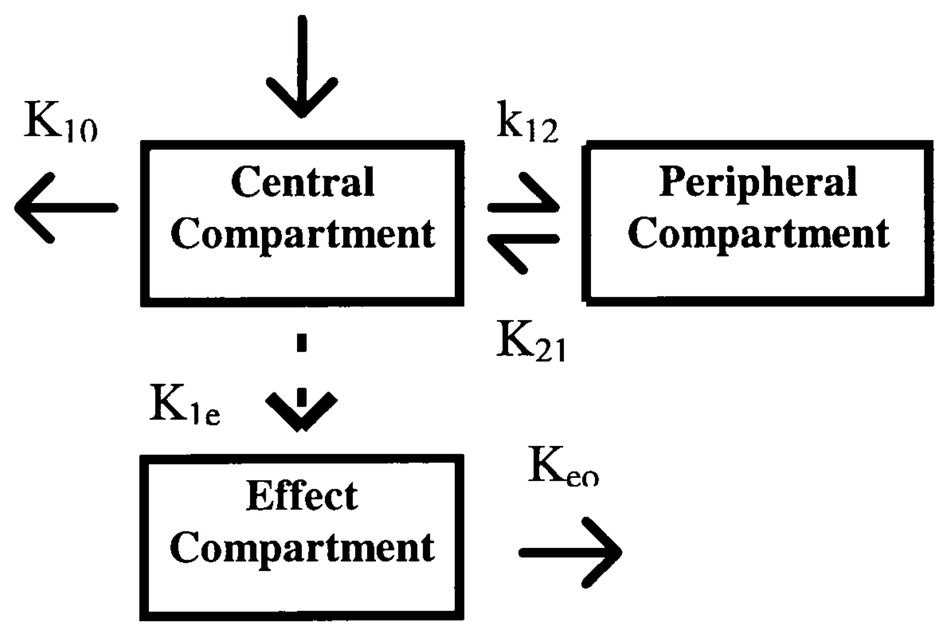
7.6.3 PK/PD modelling

A direct relationships between compartmental concentrations and QTc was initially considered. However, a temporal discrepancy between concentration and effect i.e. hysteresis (shown in adjacent diagram) was evident in the QTc vs plasma concentration plots



The relationship was therefore modelled using the standard effect compartment methodology described previously in Chapter 1.2.3 and outlined in more detail below.

By considering drug transfer between the central compartment and the effect compartment to be governed by first order processes, the change in the amount of drug in the effect compartment (Ae) can be related to the amount in the central compartment (A_1)



$$\frac{dAe}{dt} = A_1 . K_{1e} - Ae . Keo \qquad \text{Eq 7.1}$$

where K_{1e} and Keo are first order rate constants.

Since Ae cannot be directly measured, it is more useful to relate drug effect to the concentration in the plasma compartment. The compartmental amounts can be replaced by the corresponding volume and concentration terms

$$Ve . \frac{dCe}{dt} = V_1 . K_{1e} . C - Ve . Keo . Ce \qquad \text{Eq 7.2}$$

where V_1 and Ve are the volume of distribution terms for the central and effect compartments, respectively. The corresponding drug concentrations for the drug in each compartment are given by C and Ce, respectively.

At steady state there is no net transfer of drug between the plasma and the effect compartment so $\frac{dCe}{dt} = 0$ and the right side of Eq 7.2 must therefore be equal to zero. If we arbitrarily define $Ce_{ss} = C_{ss}$ (Eq 7.3) then to satisfy Eq 7.2

$$Ve.Keo.Ce_{ss} = V_1.K_{1e}.C_{ss} \qquad \text{Eq 7.4}$$

The volume of distribution for the effect compartment under this assumption of equal steady state concentrations can therefore be derived

$$Ve = V_1.\frac{K_{1e}}{Keo} \qquad \text{Eq 7.5}$$

Thus Eq 7.2 may be written as

$$\frac{dCe}{dt} = Keo(C - Ce) \qquad \text{Eq 7.6}$$

The rate constant Keo represents the loss from the effect compartment and characterises the dis-equilibrium between C and the measured effect. The half-life for the equilibrium is therefore 0.693/Keo.

The differential equation Eq 7.6 could be used to simultaneously fit the pharmacokinetic and pharmacodynamic data. Differential equation solvers are available within NONMEM, but runtimes can be vastly increased and the modelling can become cumbersome. In this chapter, the PK data was modelled in a separate step to the estimation of Keo and the PD model. The need for differential equations was avoided by fixing K_{1e} to be very small in comparison to the other rate constants (i.e. 0.01 * K_{10}) and treating the effect compartment as an extra pharmacokinetic compartment. The assumption $Ce_{ss} = C_{ss}$ was preserved by utilising Eq 7.5 in the NMTRAN code (Appendix 1.4).

7.6.4 Covariate modelling

Potential differences between healthy volunteers and IHD patients were investigated by estimating separate parameters for each sub group. Relationships between the various biochemical or demographic covariates and the individual PK/PD parameter estimates were investigated (Chapter 3). Both weight and ideal body weight were considered. Ideal body weight (IBW) was calculated as follows-

IBW(men) (kg)= 50 +0.91. (height(cm) - 152)

IBW(men) (kg)= 45 +0.91. (height(cm) - 152) (Synder et al. 1975)

Renal function was assessed by calculating the serum creatinine clearance (CLcr) using the Cockcroft Gault equation (Rowland & Tozer, 1989a).

$$CLcr = \frac{(140 - Age) \times Weight}{\text{serum creatinine}} \times [1.23(male); or 1.04(female)]$$

where age is in years , weight in kg and serum creatinine in $\mu mol.l^{-1}$.

7.7 Results

7.7.1 Noncompartmental pharmacokinetic estimates

Both $AUC_{0\text{-}inf}$ and Cmax increased in proportion to the administered dose (Figure 7.1a & b). The slopes for the two linear relationships were mean (SE) $0.064 ng.hr.ml^{-1}.mcg^{-1}$ (2%) and $0.023 ng.ml^{-1}.mcg^{-1}$ (7%), respectively. A small negative relationship between both dose corrected $AUC_{0\text{-}inf}$ and Cmax and increasing body weight could be shown (Figure 7.2 a & b). The slopes for these relationships were $-4\ hr.ml^{-1}.kg^{-1}x10^{-7}$ (SE 42%) and $-3\ ml^{-1}.kg^{-1}x10^{-7}$ (SE 16%), respectively.

Figure 7.1 Relationships between dose and a) $AUC_{0\text{-}inf}$ and b) Cmax

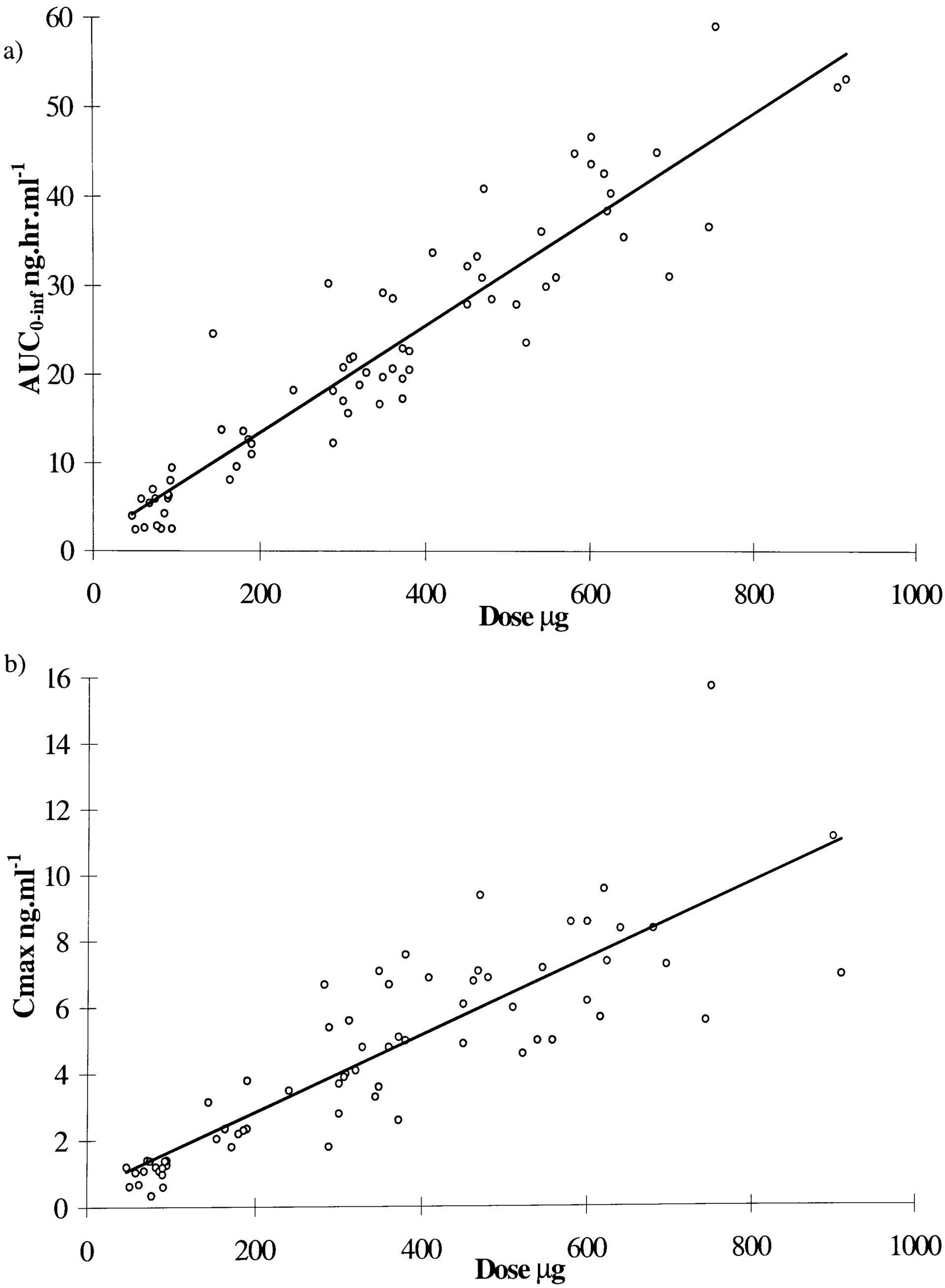
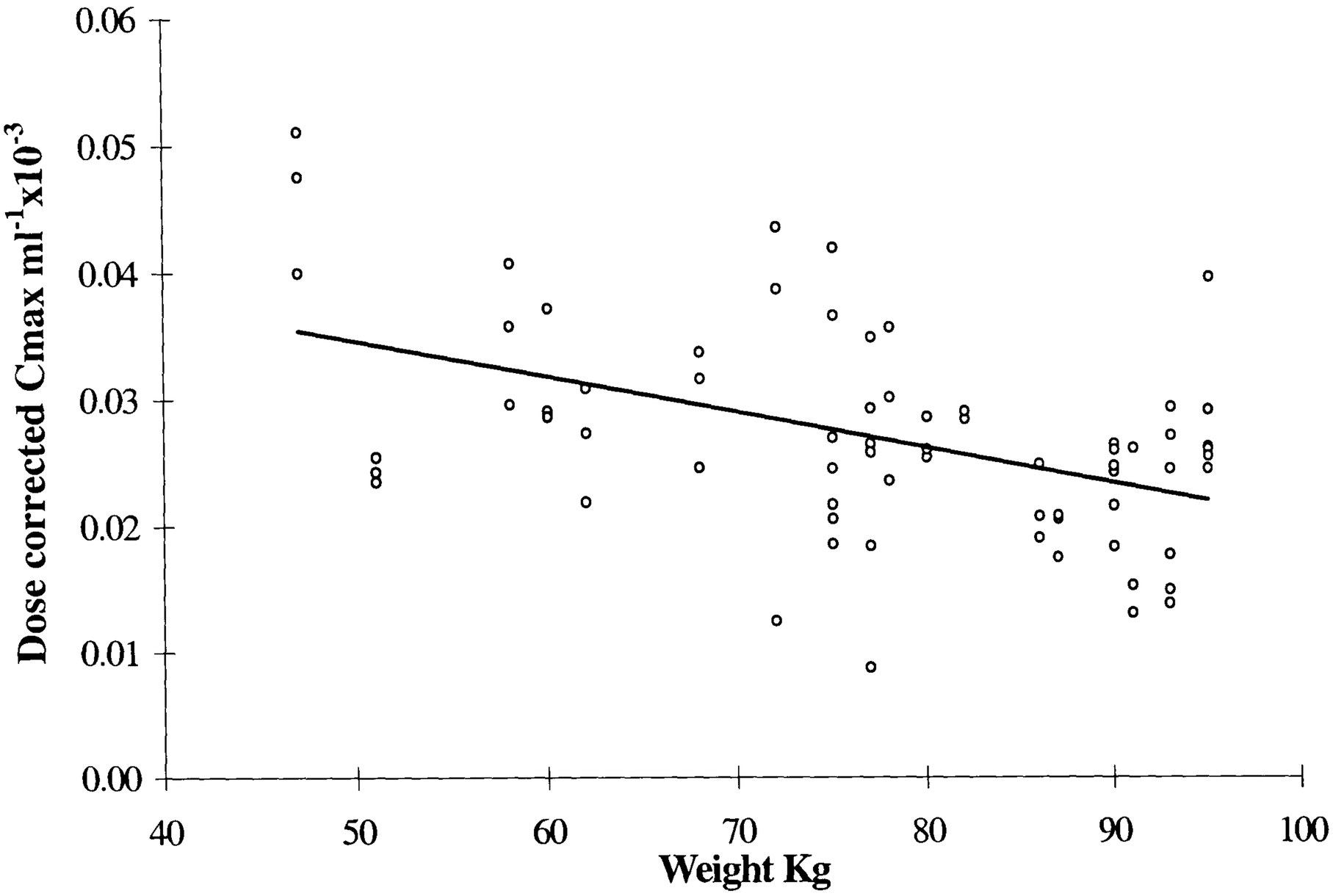
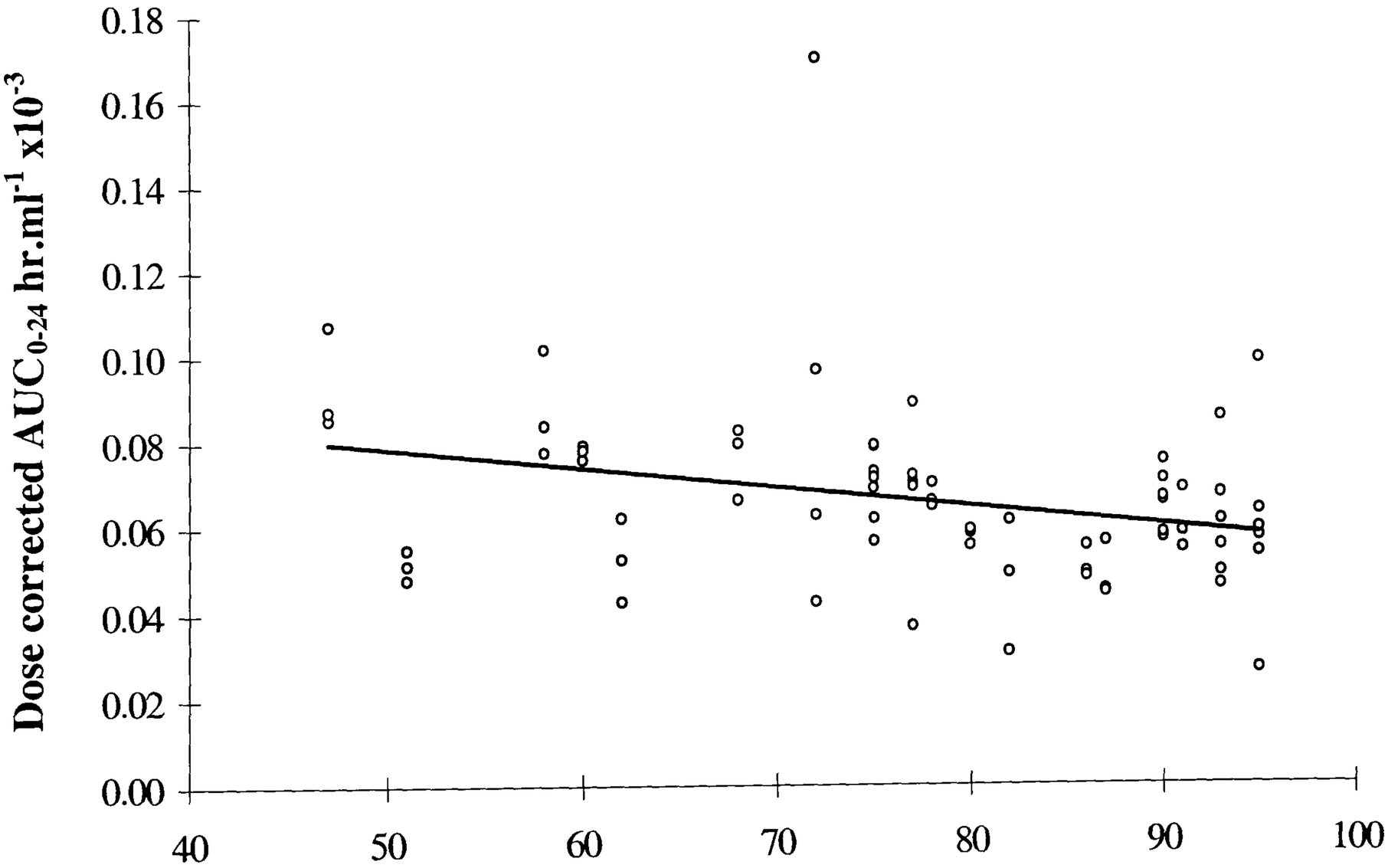


Figure 7.2 Relationships between body weight and a) Dose corrected Cmax and b) Dose corrected $AUC_{0\text{-}inf}$

a)



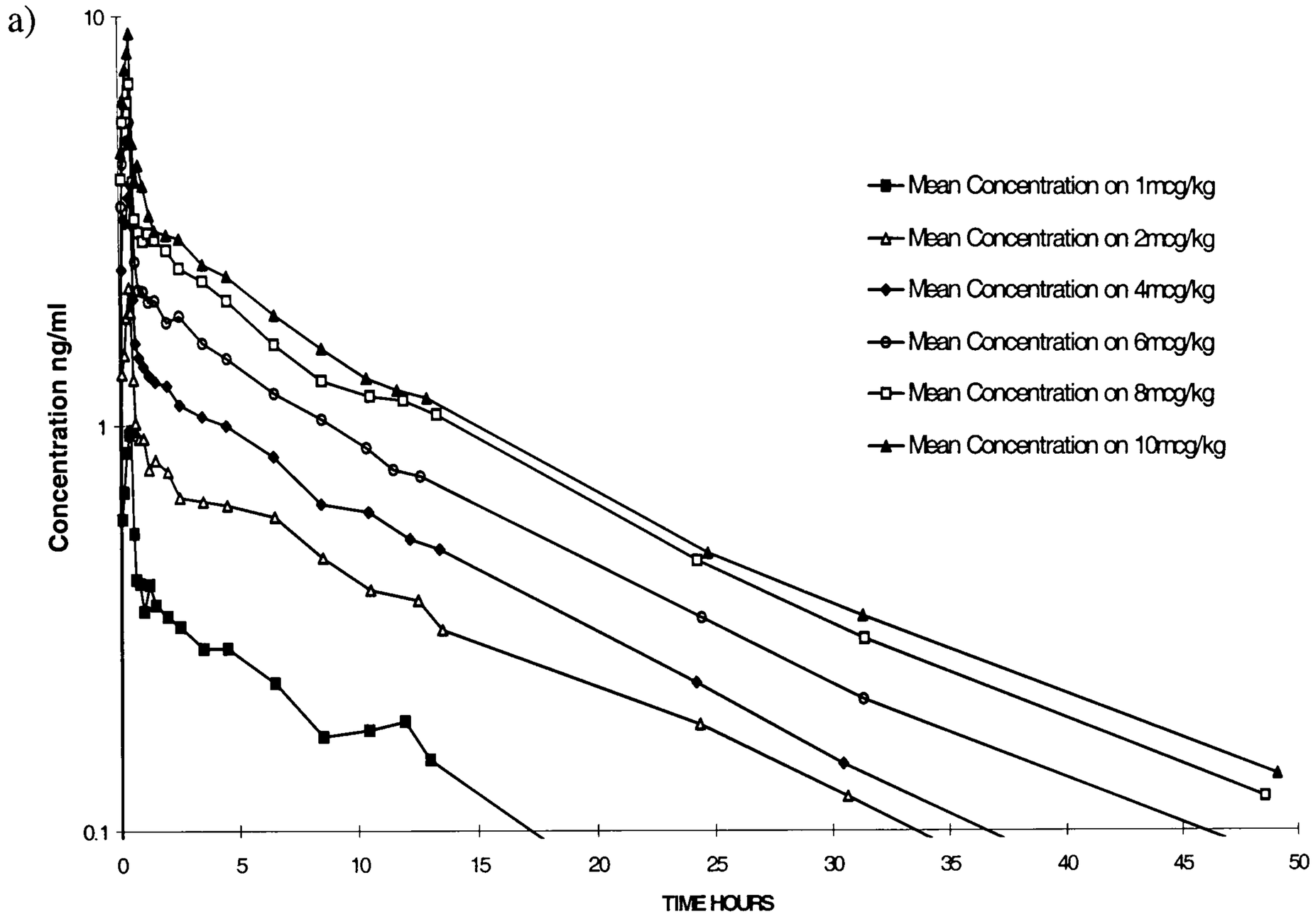
b)



7.7.2 Pharmacokinetics

In accordance with previous studies (section 7.1.4), the post infusion distribution was shown to be at least biexponential (Figure 7.3). Development of a two compartment population pharmacokinetic model is shown in Table 7.2. In run 1, the intraindividual variability component was modelled using the exponential expression (Chapter 3), and the parameters were estimated using the FO method. In run 2, the combined expression for intraindividual variability was utilised, but the parameters were still estimated using the FO method. The large decrease in the value of the objective function, combined with the greater degree of precision in the parameter estimates, especially the interindividual variability, indicated that the second error structure was more appropriate. However, using the model described in run 2, there was a significant bias in the weighted residuals as shown in Figure 7.4 a and b. In run 3, this bias was removed by using the FOCE method to estimate the population parameters as shown in Figure 7.5 a and b. A decrease in variability and increase in the precision of the parameter estimates was associated with the improvement in fit. There was no indication in the residuals that a more complex model was required (Figure 7.5b). As expected, using a three compartment model did not further improve fit, so the two compartment model was deemed to be the most appropriate model. In run 4, the FOCE method was retained but the additive expression from the intraindividual variability component was removed. The large increase in objective function indicated that although small ($0.05 ng.ml^{-1}$) and imprecisely estimated, the additive component was important to the model fit. In run 5, the precision of the interindividual variability estimates for K_{10} and V_1 were improved by removing the interindividual variability term from the estimation of K_{12}. However, the large increase in objective function indicated that the reduced model complexity resulted in a compromised fit.

Figure 7.3 Mean log concentration over a) 50 hours and b) 5) hours



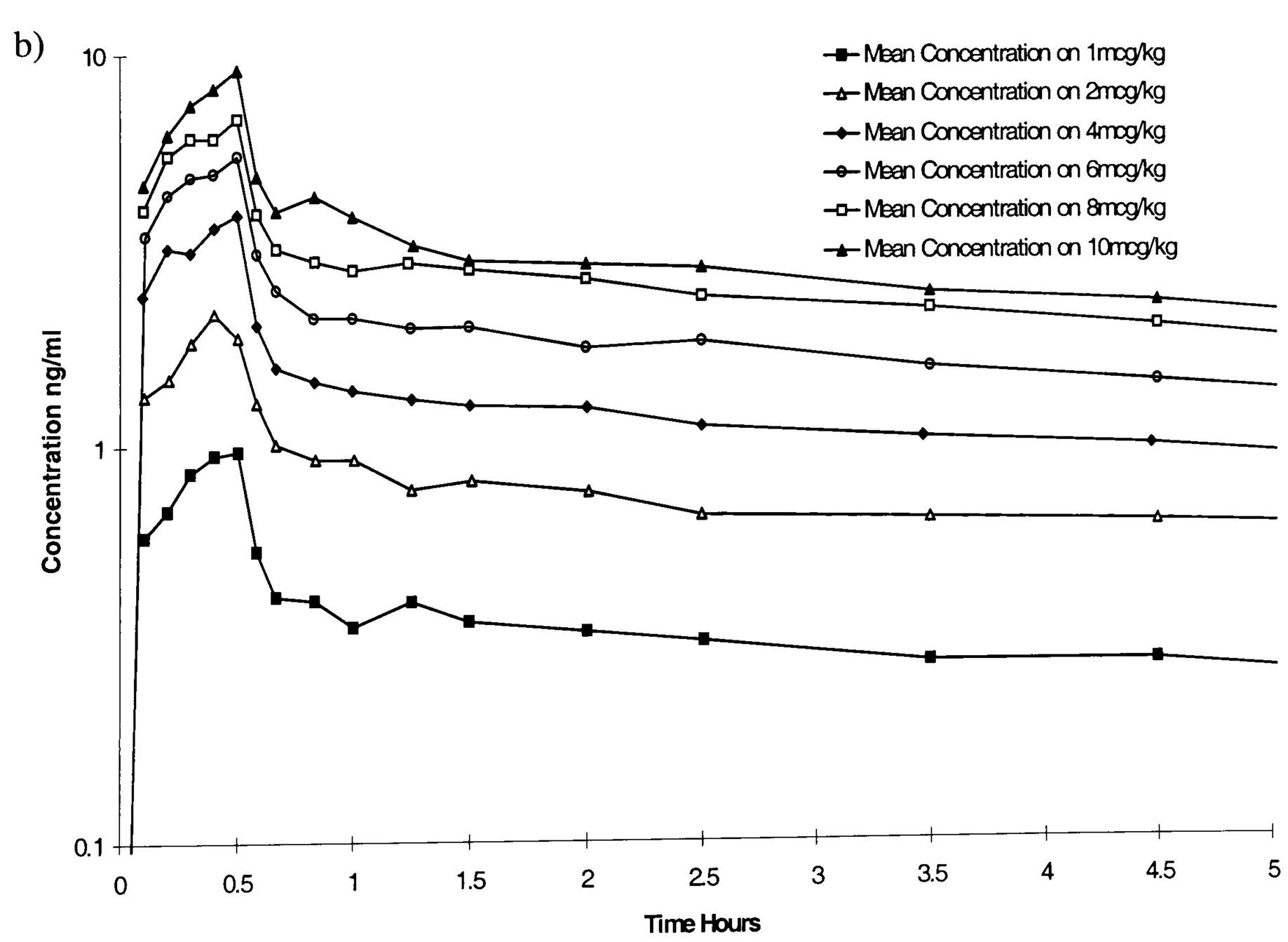


Table 7.2 Population Pharmacokinetic model development for a two compartment IV infusion model

Run	Method	Objective Function	K_{10} hr^{-1} (SE%)	K_{12} hr^{-1} (SE%)	K_{21} hr^{-1} (SE%)	V_1 L (SE%)	ω_{K10} %CV (SE%)	ω_{K12} %CV (SE%)	ω_{K21} %CV (SE%)	ω_{V1} %CV (SE%)	σ_{EXP} %CV (SE%)	σ_{ADD} $ng.ml^{-1}$ (SE%)
1	**FO**	**-2204.49**	**1.40**	**27.40**	**1.04**	**23.90**	**282**	**355**	**58**	**241**	**85**	
			(37)	(32)	(45)	(21)	(434)	(406)	(155)	(25)	(213)	
2	**FO**	**-2670.91**	**3.31**	**22.10**	**0.64**	**14.60**	**233**	**91**	**158**	**77**	**55**	**0.07**
			(41)	(31)	(29)	(22)	(168)	(247)	(142)	(222)	(111)	(40)
3	**FOCE**	**-3000.353**	**1.13**	**14.7**	**1.16**	**13.5**	**45**	**43**	**17**	**46**	**20**	**0.05**
	Interaction		(12)	(12)	(4)	(13)	(109)	(120)	(32)	(106)	(12)	(44)
4	**FOCE**	**-2382.01**	**0.98**	**14.10**	**1.08**	**14.50**	**38**	**33**	**18**	**39**	**30**	
	Interaction		(1)	(12)	(4)	(12)	(123)	(159)	(27)	(119)	(11)	
5	**FOCE**	**-2894.173**	**1.23**	**16.3**	**1.16**	**12.3**	**27**		**28**	**32**	**21**	**0.04**
	Interaction		(23)	(25)	(5)	(24)	(50)		(55)	(43)	(15)	(40)
6	**FOCE**	**-2846.457**	**1.42**	**13.9**	**0.915**	**13.4**	**71**	**65**	**31**	**69**	**22**	**0.06**
	No Interaction		(18)	(20)	(38)	(14)	(92)	(101)	(220)	(97)	(23)	(45)

Figure 7.4 Goodness of fit plots for a two compartment model fit using the FO estimation method (run 2)

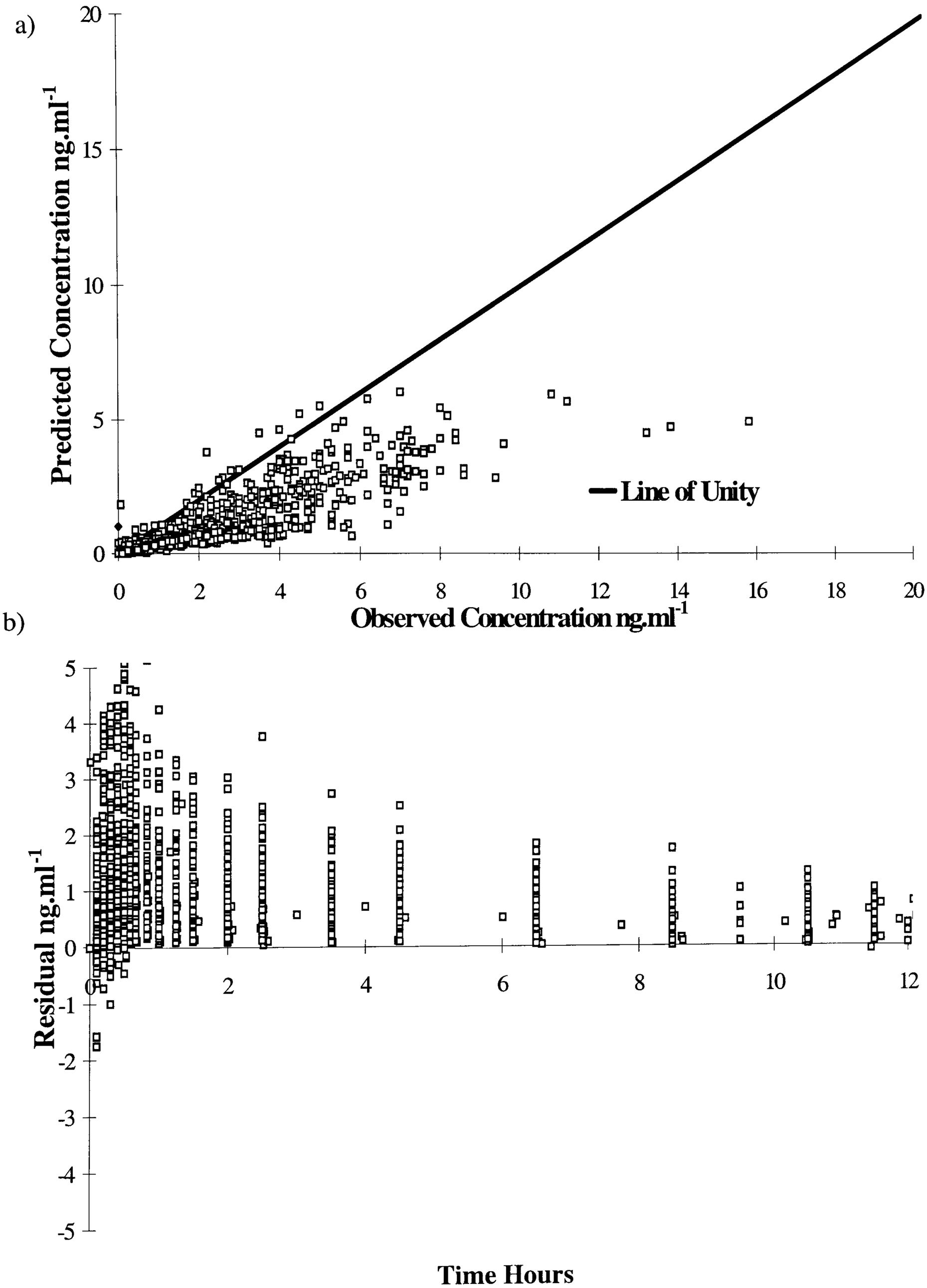
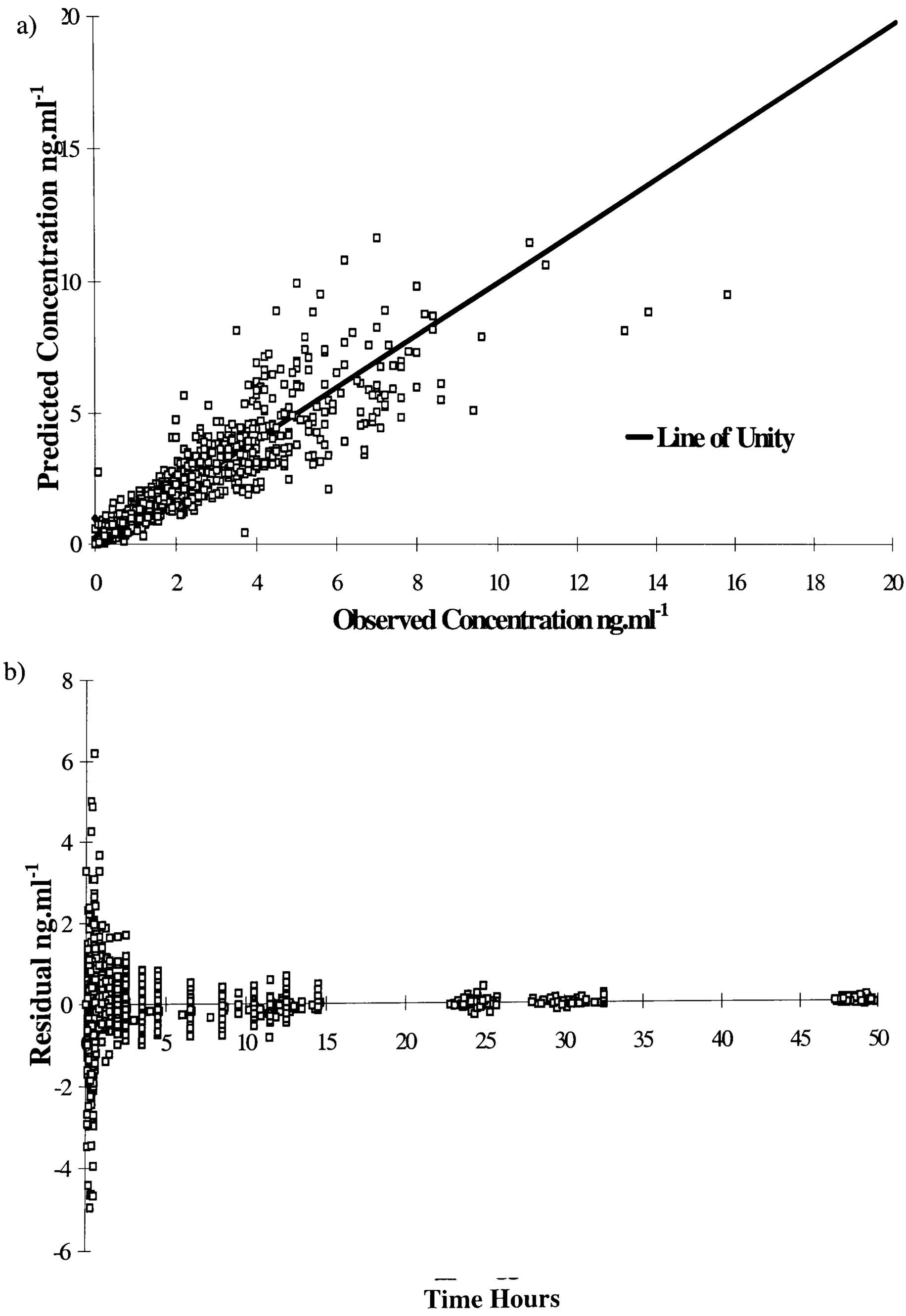


Figure 7.5 Goodness of fit plots for a two compartment model fit using the FOCE interaction method (run 3).



7.7.3 Interaction vs no interaction with FOCE method

The population parameters estimated using the "no interaction" option were different to those estimated using the "interaction" option (Table 7.2 run 6 vs run 3). This was most easily demonstrated by comparing CL , Q, Vss, $T_{1/2}$ α and $T_{1/2}$ β obtained for each method (table 7.3). The derived population terminal and distributional half-life estimates were very similar. However, the clearance (CL), inter compartmental clearance (Q) and volume of distribution at steady state (Vss) estimates were different for the two methods (Table7.3).

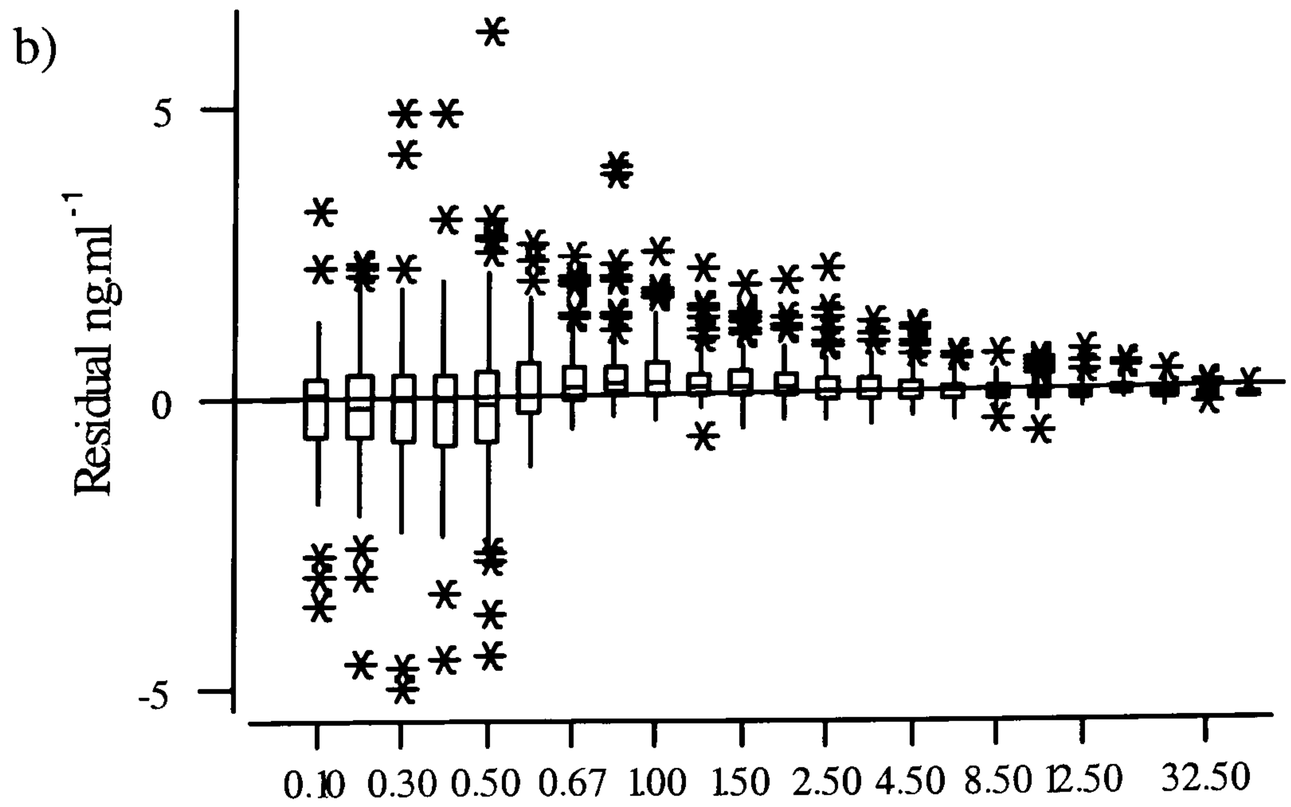
Table 7.3 Derived CL, Q,Vss, T1/2α and T1/2β for the FOCE interaction (run3) and FOCE nointeraction (run 6) methods

Run	Method	CL $L.hr^{-1}$	Q $L.hr^{-1}$	Vss L	$T_{1/2}$ α min^{-1}	$T_{1/2}$ β hr^{-1}
3	FOCE *interaction*	15.2	198	185	2.5	8. 9
6	FOCE *nointeraction*	19.0	186	217	2.2	9.0

As previously discussed, the objective functions are estimated differently, so method comparison has to be based on goodness of fit plots (Chapter 3). Figure 7.6 shows the residual plots for each method grouped by sample times. The "no interaction" method showed a slight positive bias at the 0.67, 0.83, 1.0, 1.25 and 1.5 time points i.e during the first hour post infusion. The SE's estimated by this method were also larger than those estimated using the "interaction" method (Table 7.2). So both model fit and parameter precision were better when the "interaction" method was used.

Therefore, a two compartment model, incorporating both additive and exponential components for intraindividual variability, and using the FOCE with "interaction" estimation method was considered to best describe the pharmacokinetics (run 3 Table 7.2).

Figure 7.6 Residuals versus time for the a) Interaction (run 3) and b) No interaction (run 6) FOCE methods used for the two compartment pharmacokinetic model.
Horizontal line and boxes indicate median and interquartile range (Q1-Q3), respectively. The whiskers extend to the lowest and highest values that are still inside the region defined by Q1 - 1.5.(Q3 - Q1) to Q3 + 1.5.(Q3 - Q1). The *'s indicate values which lie outside this interval.



7.7.4 Healthy volunteer vs IHD patients

While the pharmacostatistical model can be developed using any parameterisation, prospective application of covariate relationships is more intuitive when they are related to parameters that have a physiological basis (Chapter 3). Further modelling was undertaken using models parameterised in terms of volume of distribution and clearance using standard relationships within NMTRAN. The individual parameter estimates from run 3 Table 7.2 are shown by subject group in Table 7.4. The average estimates of V_1 and Vss were respectively lower and higher in the IHD patients, however, these differences were not found to be statistically significant (run 7 to 10 Table 7.5).

7.7.5 Covariate analysis

Graphical analysis identified four possible covariate relationships: Vss with both body weight and ideal body weight (Figure 7.7 a and b) and CL with both CLcr and body weight (Figure 7.8 a and b). The importance of these relationships was tested using the series of models described in Table 7.6. In accordance with the relationship between dose corrected Cmax and body weight (section 7.7.1), relating Vss to body weight significantly decreased the objective function (run 11 Table 7.6). The comparison between run 12 and run 13 demonstrates that the relationship was dependent on the association between body weight and the volume of distribution of the peripheral (V_2) rather than the central compartment (V_1). The difference in the relationships is shown graphically in Figure 7.9. The decrease in the %CV (ω_{VSS}) of 3.5% (run 11 v run 3, Table 7.6) after inclusion of body weight is equivalent to a 33% reduction in the variability in Vss (ω_{VSS}^2). The variability was not further reduced by modelling Vss as a function of ideal body weight (run 14 Table 7.6).

Table 7.4 Summary of the individual distribution half-life, terminal half-life and volume parameters (V_1 and Vss) split by healthy volunteers and IHD patients

IHD Group					Volunteer Group				
ID	**$T_{1/2}$ α (mins)**	**$T_{1/2}$ β (hrs)**	**Vss (L)**	**V_1 (L)**	**ID**	**$T_{1/2}$ α (mins)**	**$T_{1/2}$ β (hrs)**	**Vss (L)**	**V_1 (L)**
1	2.4	8.9	215	11.4	**17**	2.5	9.2	236	16.5
2	2.4	10.5	204	11.6	**18**	3.2	8.8	151	13.3
3	2.7	8.8	231	14.7	**19**	2.2	9.7	227	16.4
4	3.0	8.0	160	14.6	**20**	3.1	10.9	137	12.2
5	2.8	9.2	181	15.9	**21**	2.4	9.8	210	16.1
6	2.5	8.3	222	18.9	**22**	2.6	9.3	163	13.7
7	2.6	11.3	171	13.2	**23**	4.0	8.1	112	11.8
8	1.9	9.2	194	11.9	**24**	3.0	8.2	196	16.5
9	3.7	9.4	257	31.4					
10	2.9	6.8	169	17.0					
11	2.1	7.7	220	15.7					
12	2.3	8.7	139	10.5					
13	2.4	10.0	192	14.0					
14	0.5	9.8	188	2.2					
15	2.7	8.8	205	13.3					
16	2.6	8.8	179	18.4					
Median	2.5	8.9	194.0	14.0		2.6	9.2	179.2	16.1
Mean	2.4	9.0	196.6	14.4		2.8	9.2	179.1	15.0
SD	0.7	1.1	30.4	6.0		0.6	0.9	42.1	2.3

Table 7.5 Comparison between the population pharmacokinetic parameters for healthy volunteers and IHD patients using the FOCE with interaction estimation method

Run [1]	Parameter Tested	Run3 Parameter Estimate	Parameter Estimate for the IHD Group	Parameter Estimate for the Volunteer Group	Obj Fun	Δ Obj Fun [2]	LRT [3]
7	CL ($L.hr^{-1}$)	15.2	16	13.9	-3002.58	-2.23	P<0.15
	(SE%)	(4)	(5)	(8)			
8	V_1 (L)	13.5	12.4	15.1	-3000.86	-0.51	P<0.5
	(SE%)	(14)	(20)	(4)			
9	Vss (L)	185	193	174	-3001.90	-1.55	P<0.3
	(SE%)	(4)	(4)	(9)			
10	Q ($L.hr^{-1}$)	198	200	195	-3000.41	-0.11	P<0.8
	(SE%)	(5)	(6)	(9)			

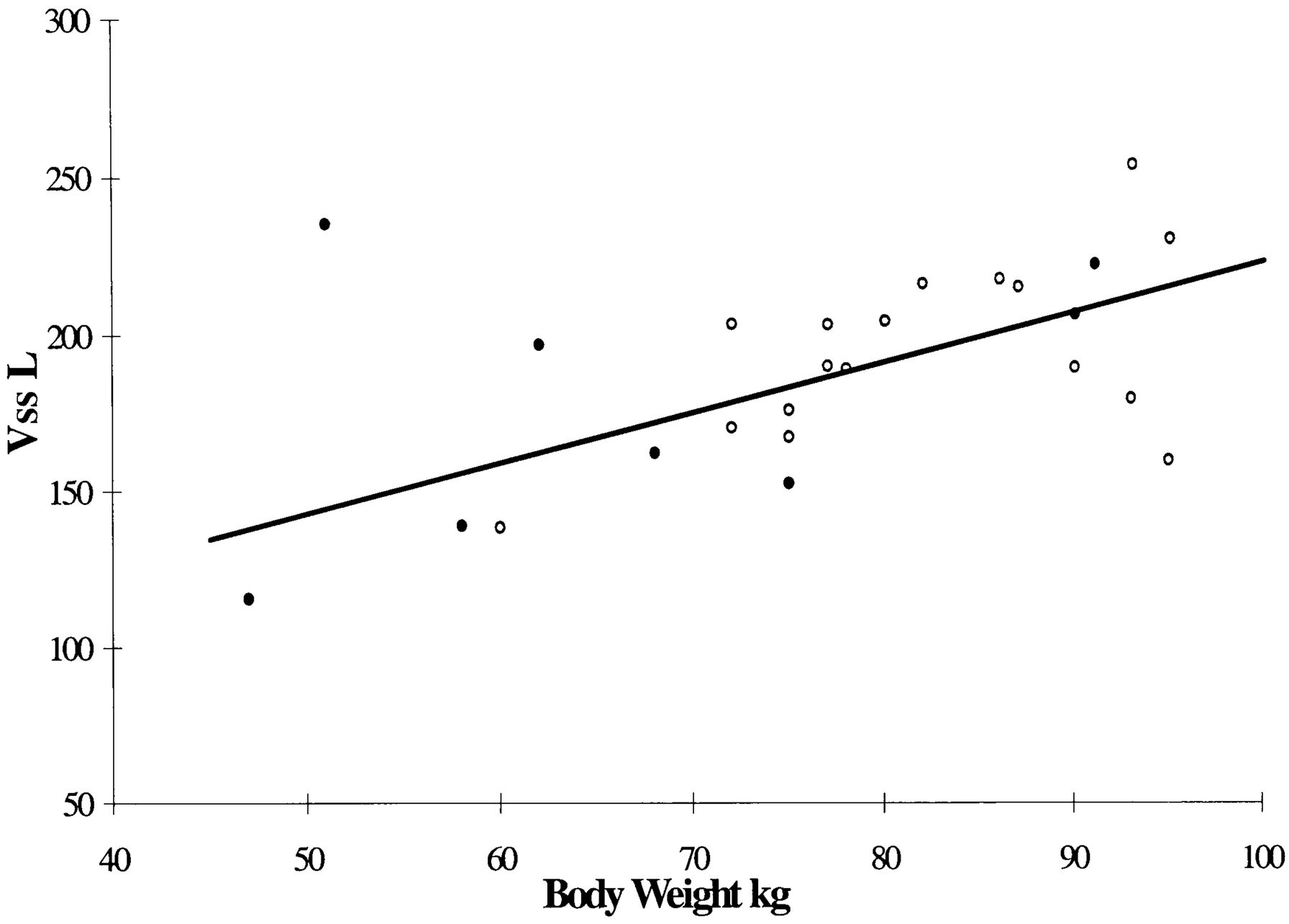
1) Differences in the parameter estimates between the groups are tested individually in runs 7 - 10 by comparing them to run 3.

2) Comparison with objective function from. run 3 Table7.2

3) LRT = Likelihood Ratio Test

Figure 7.7 Covariate relationships between Vss and a) Body weight and b) Ideal body weight. The closed circles represent the healthy volunteers and the open circles the IHD patients.

a)



b)

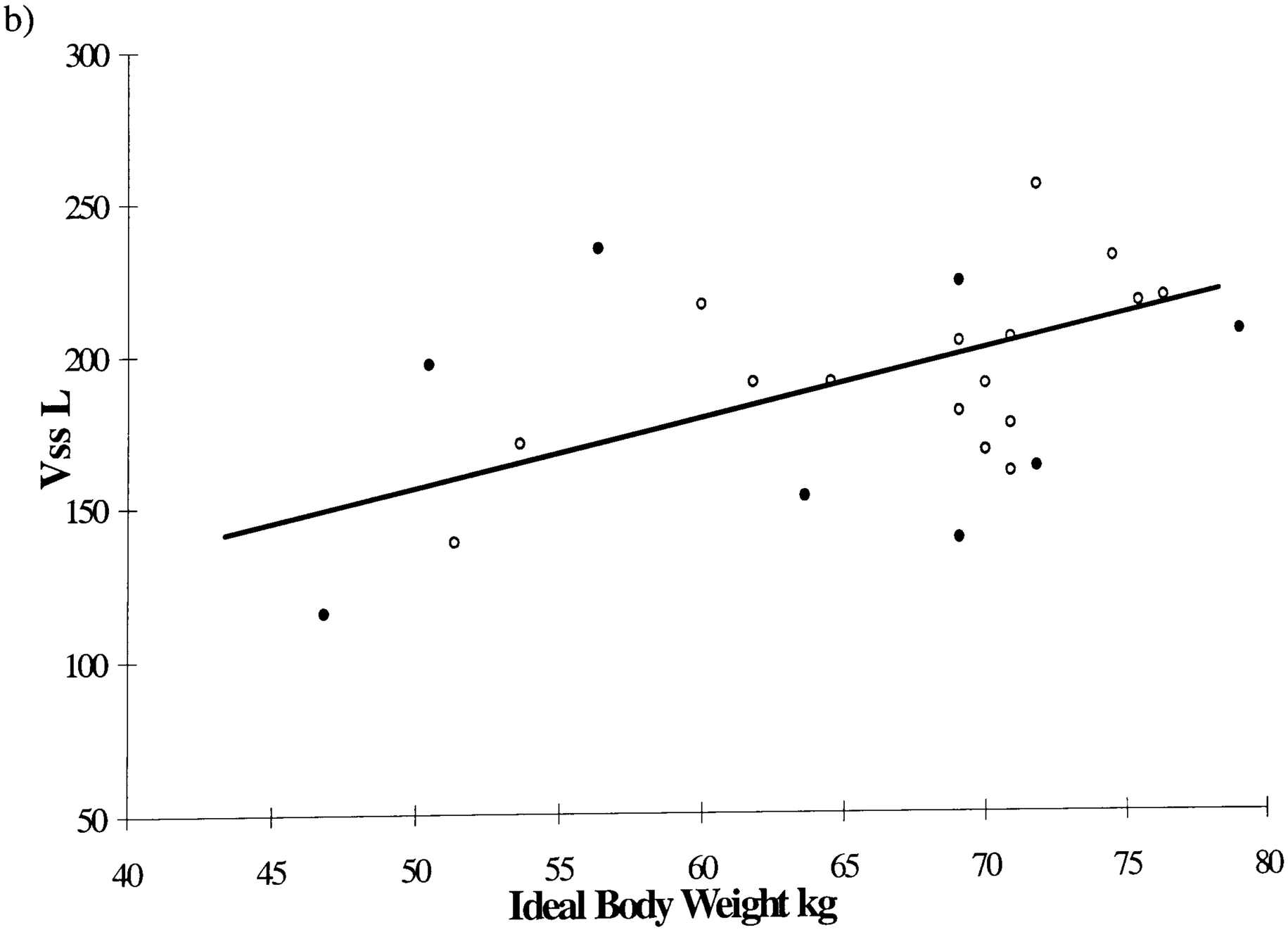
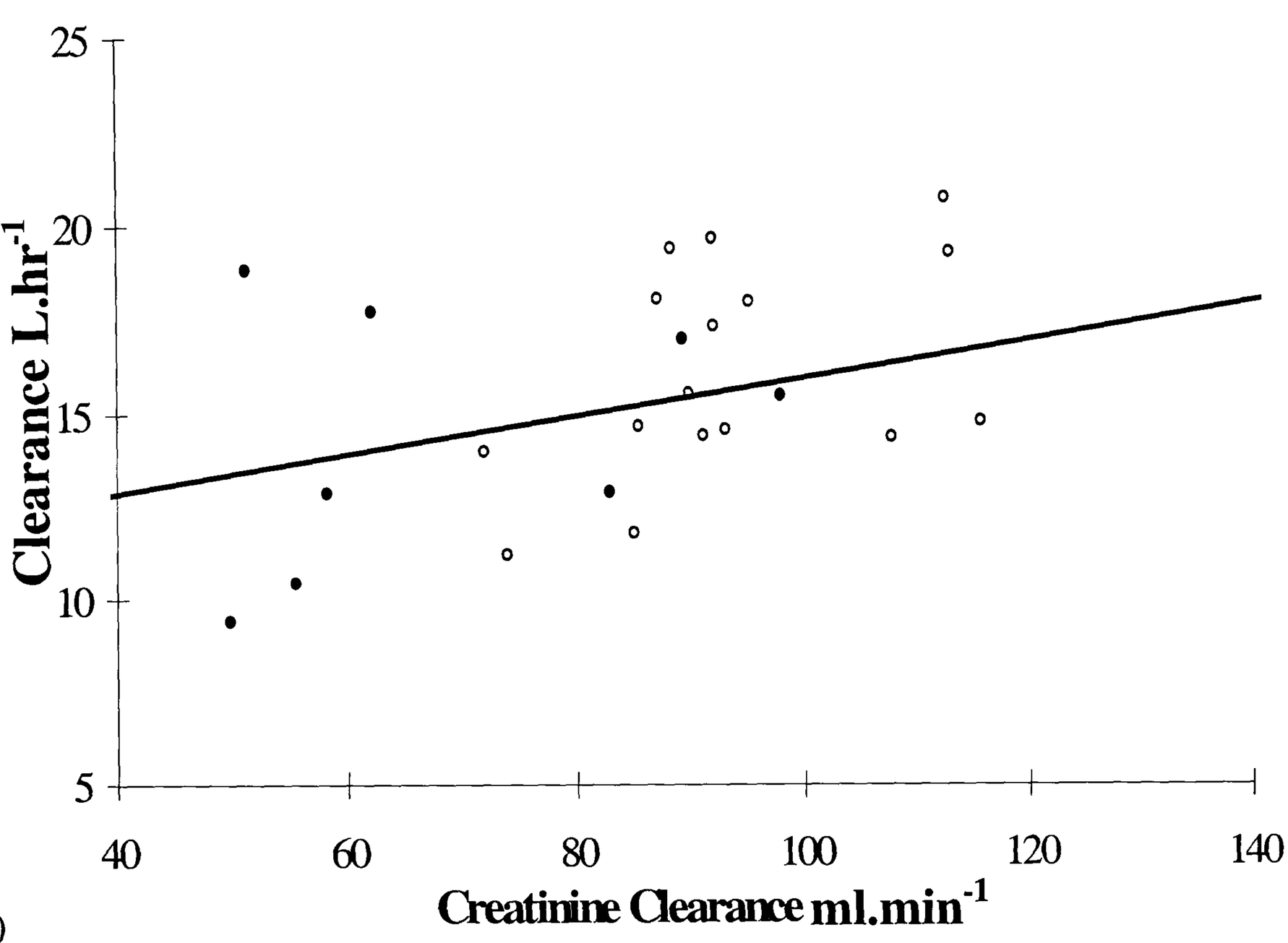


Figure 7.8 Covariate relationships between dofetilide clearance and a) Creatinine clearance, b) Body weight. The closed circles represent the healthy volunteers and the open circles the IHD patients.

a)



b)

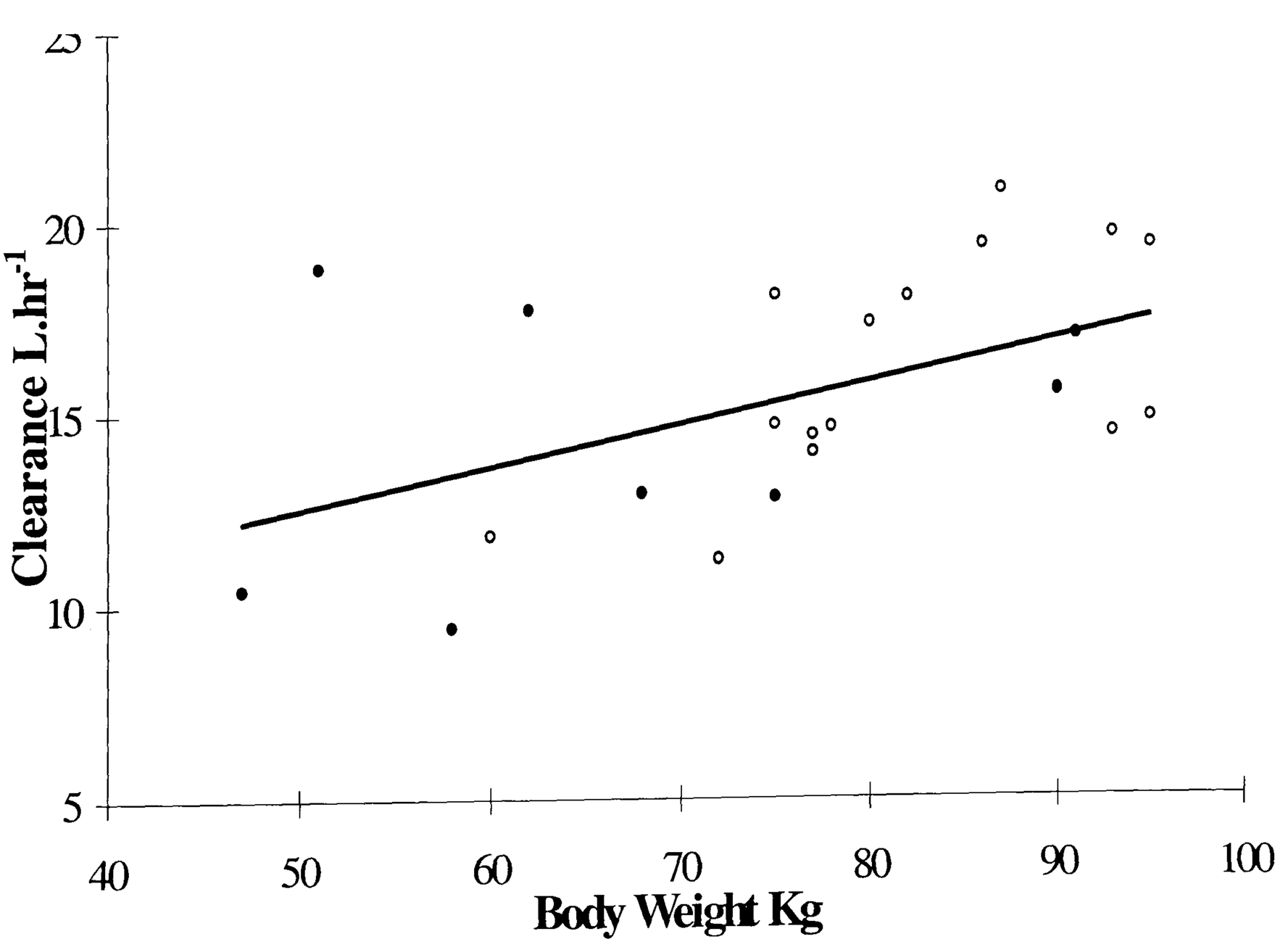
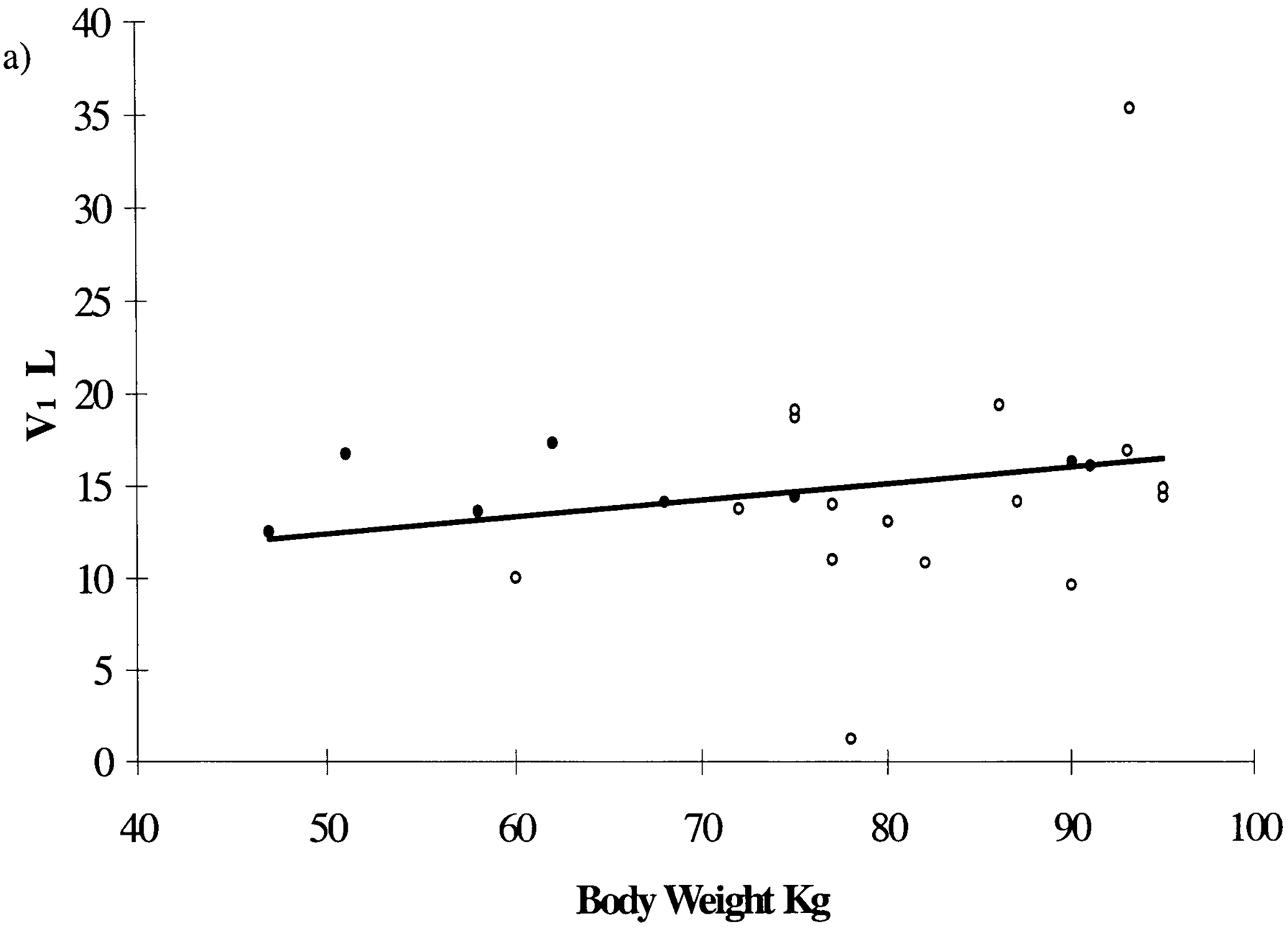


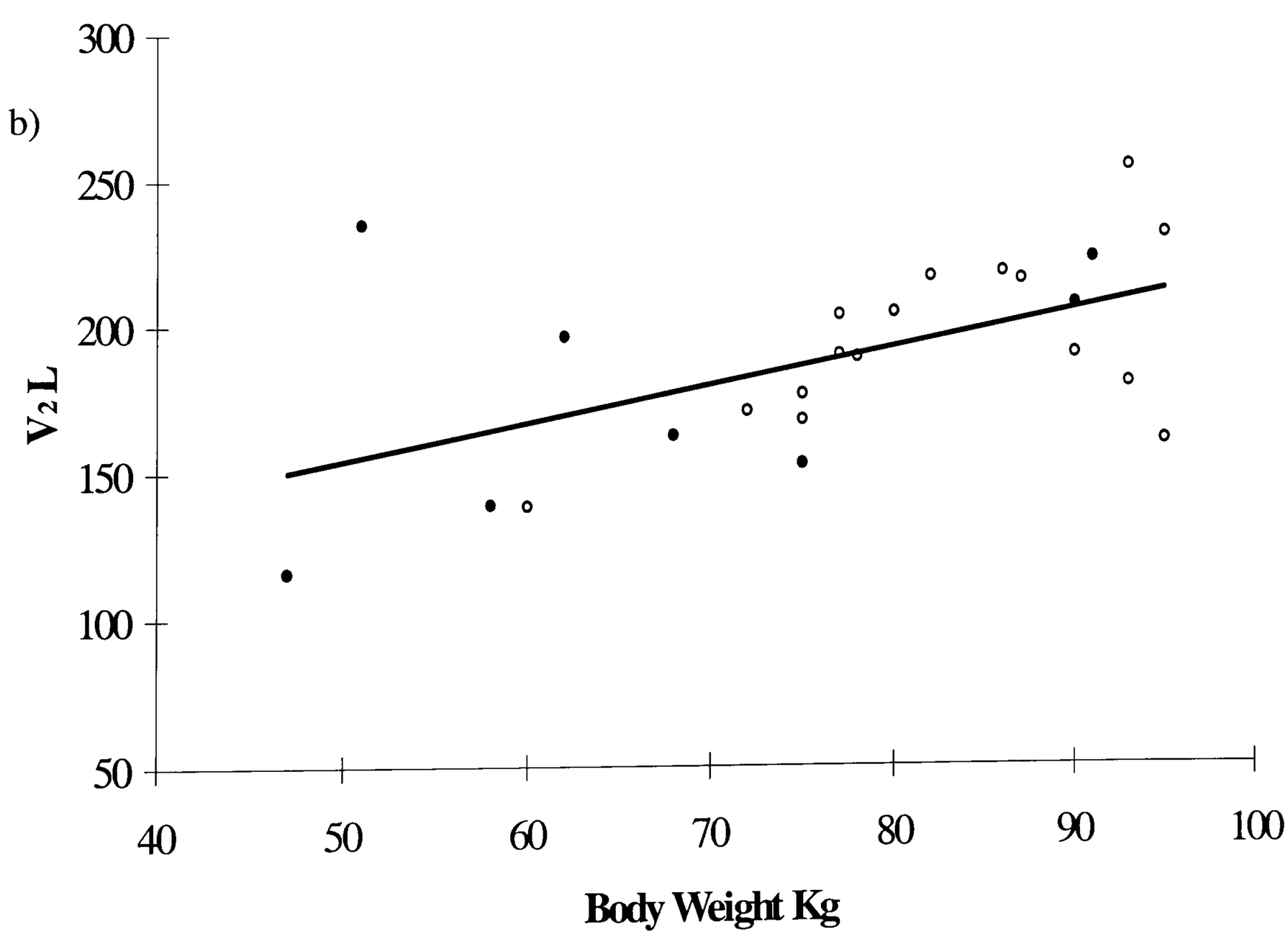
Table 7.6 Covariate model development for the pharmacokinetics

	Model	**Obj. Fun.**	**Model Comparison**	**P-Value**	θ_5 &/or θ_6	**SE (%)**	ω_{CL}	ω_{Vss}	ω_Q	ω_{Vc}
3	Basic model from table 3 Run 3 Table 7.2	-3000.35					21.3	19.2	25.4	59.5
11	$Vss = \theta_1 + \theta_5$ x (WT-77.5)	-3011.17	11 vs 3	P<0.005	1.53	40	21.4	15.7	25.4	59.4
12	$V_1 = \theta_2 + \theta_5$ x (WT-77.5)	-3002.19	12 vs 3	ns	0.0557	105	21.4	19.2	25.4	59.0
13	$V_2 = \theta_2 + \theta_5$ x (WT-77.5)	-3010.81	13 vs 3	P<0.005	1.46	41	21.4	16.4	25.2	59.7
14	$Vss = \theta_1 + \theta_5$x (IWT-75)	-3005.88	14 vs 3	P<0.025	1.9	45	21.3	17.0	25.4	59.5
15	$CL = \theta_2 + \theta_5 / SCRT^1$	-3000.35	15 vs 3	ns	2.05	3400	21.4	19.2	25.4	59.3
16	$CL = \theta_2 + \theta_5$ x (WT-77.5)	-3007.14	16 vs 3	P<0.01	0.1160	44	17.9	19.2	25.4	59.4
17	$CL = \theta_2 + \theta_5$ x (CRCL-86.9)	-3004.51	17 vs 3	P<0.05	0.0435	86	19.0	19.2	25.4	59.4
18	$Vss = \theta_1 + \theta_5$ x (WT-77.5) $CL = \theta_2 + \theta_6$ x (WT-77.5)	-3017.7	18 vs 11	P<0.025	1.5200 0.114	40 44	18.0	15.7	25.3	59.5

Parameters θ_1 and θ_2 were estimated to be similar across all runs (including run 3) since they represent the parameter estimates for the median patient.
SCRT = serum creatinine and ns=not signficant

Figure 7.9 Covariate relationships between a) V_1 and body weight , b)V_2 and body weight. The closed circles represent the healthy volunteers and the open circles the IHD patients.





While modelling CL as a function of 1/SCRT did not decrease the objective function (run 15 Table 7.6) there was a significant reduction when CL was modelled as a function of body weight (run 16 Table 7.6). An absolute reduction in ω_{CL} of 3.4% indicated that 30% of the variability in CL (ω_{CL}^2) could be explained by body weight. Inclusion of CLcr in the CL model significantly reduced the objective function (run17 Table 7.6) but the slope of the resultant model was not significantly different from zero (SE >50%CV). The relationship between body weight and CL is consistent with the association between $AUC_{0\text{-inf}}$ and body weight (section 7.7.1).

When both Vss and CL were related to body weight, a further significant reduction in the objective function was observed (run 18 vs run 11 Table 7.6), and the values of θ_5 , θ_6 , ω_{CL} and ω_{VSS} were consistent with the previous estimates (run 18 vs run 11 & run 17 Table 7.6). Allowing for a covariance between CL and Vss did not alter the selection of body weight as a significant covariate of each. Run 18 was therefore the final covariate model for the pharmacokinetics of dofetilide. The relationships between Vss and CL,and body weight are equivalent to $1.5L.kg^{-1} + 68L$ and $0.114L.hr^{-1}.\ kg^{-1} + 6.4L.hr^{-1}$, respectively.

7.7.6 Pharmacodynamic analysis

The mean QTc time profile shown in Figure 7.10 was consistent with that for the individual subjects. The maximum measured QTc interval increased in proportional to the administered dose (Figure 7.11a). Although the variability in Tmax for the QTc interval decreased with increasing dose, there was no indication of a relationship between the mean Tmax and dose (figure 7.11b), indicating that the lag between concentration and QTc response (Figure 7.12a) is likely to be due to a delay in the distribution of dofetilide to the site of drug effect. Since the plot of QTc versus the population predicted peripheral compartment concentration showed a proteresis, distribution into the peripheral pharmacokinetic compartment occurs at a slower rate than the accumulation at the site of

drug effect (Figure 7.12b). Therefore, the small temporal delay between the observed concentration and QTc response requires an effect compartment model and the half-life of the delay between plasma concentration and effect should be between the distribution and terminal phase half-lives (2.5 minutes to 8.9 hours).

Figure 7.10 Mean QTc versus Time. Vertical line indicates the planned time for the end of the infusion.

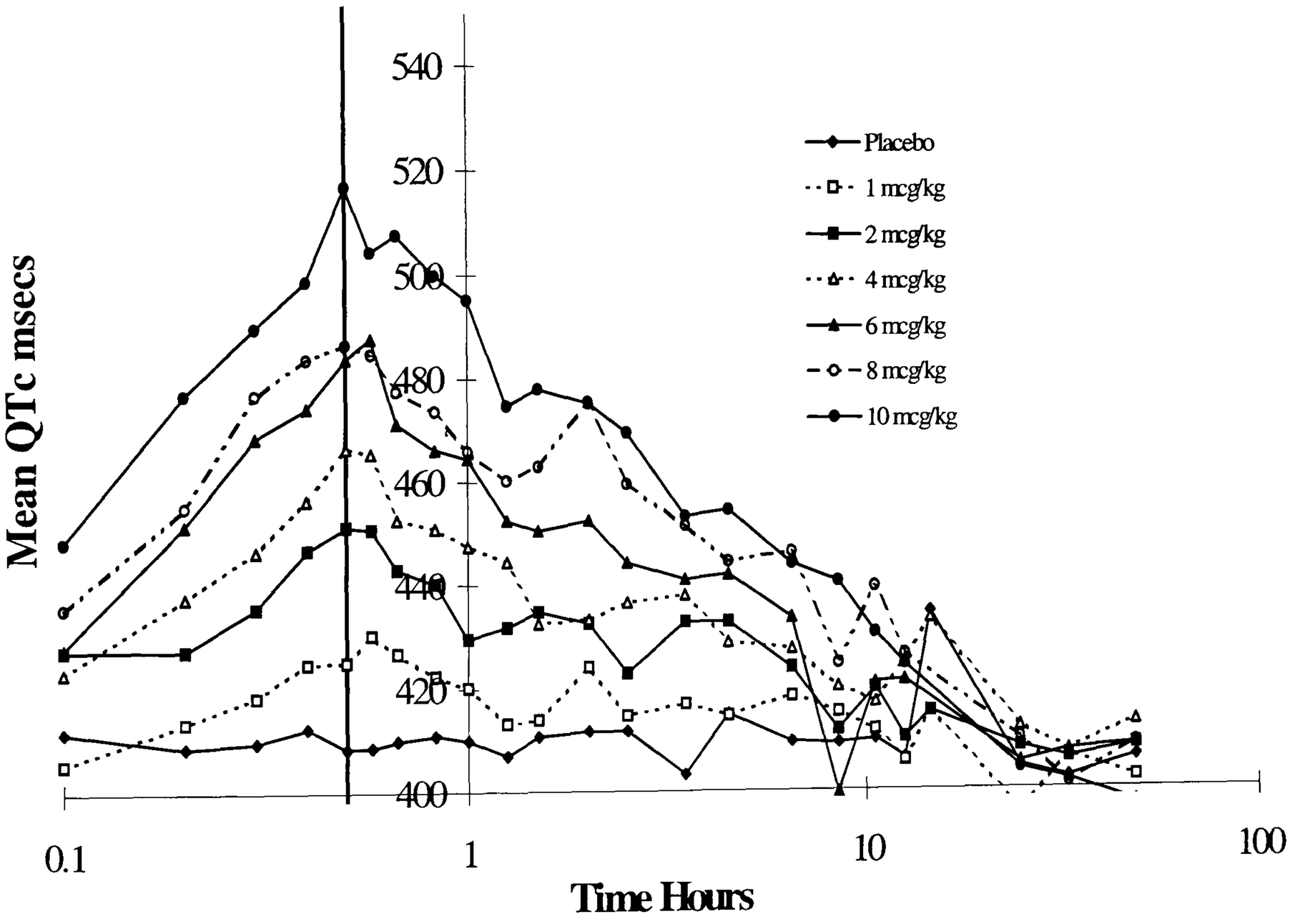


Figure 7.11 a) Maximum achieved QTc interval versus administered dose (slope of linear regression was 0.12 msecs. mcg^{-1}) b) Time for the maximum achieved QTc interval versus administered dose

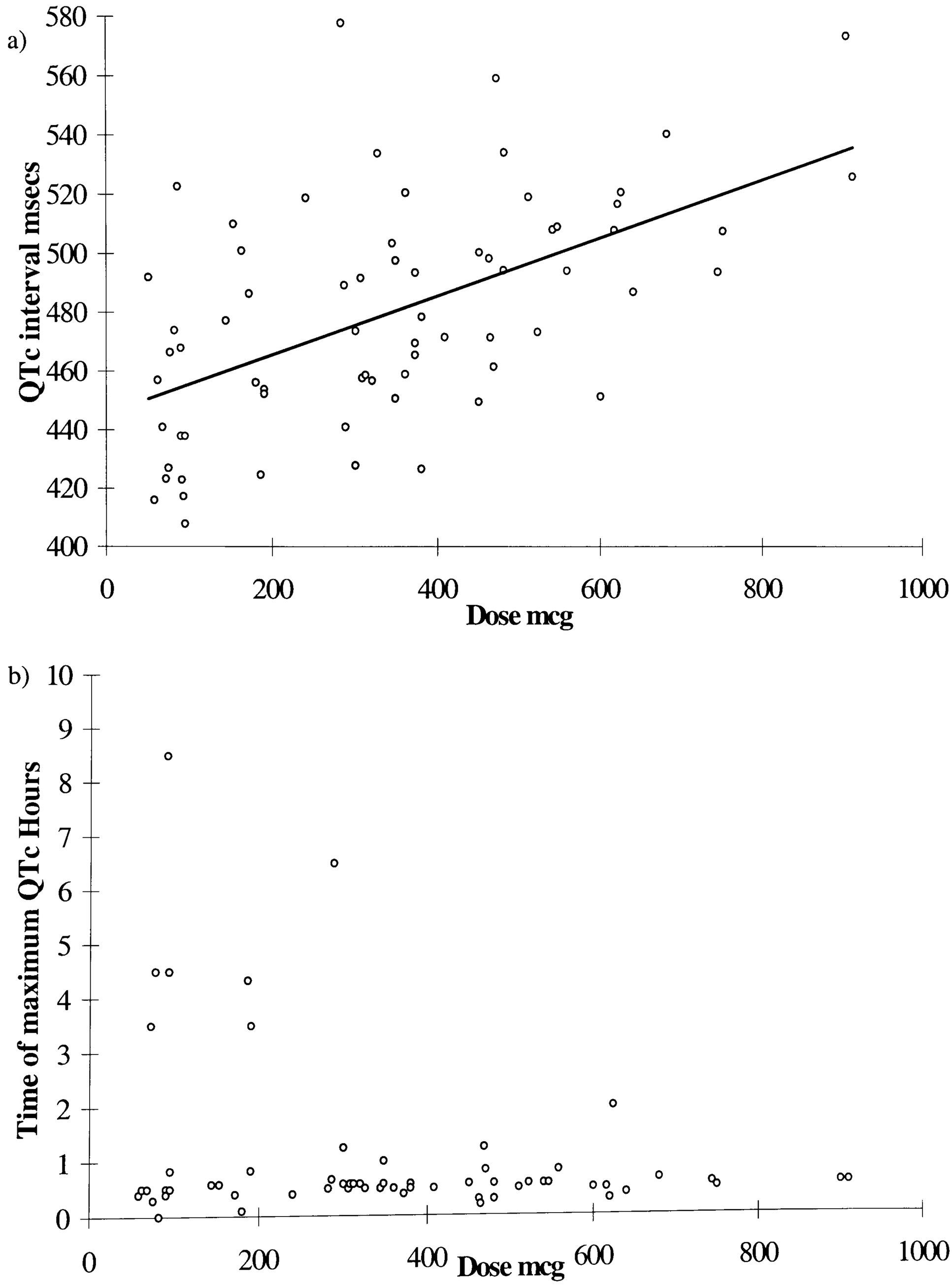
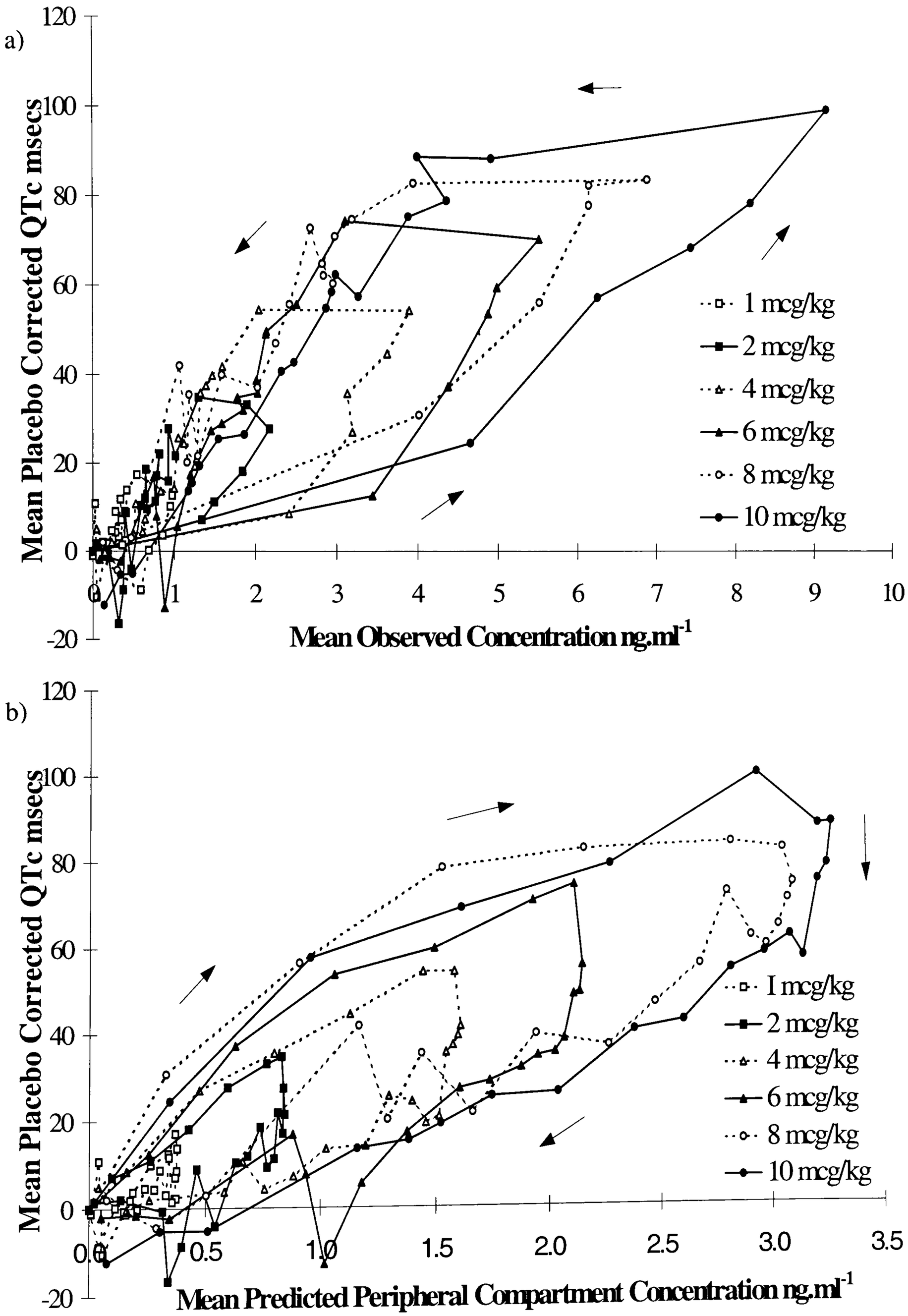


Figure 7.12 Mean placebo corrected QTc versus a) The mean measured concentration b) The population predicted peripheral compartment concentration. The arrows show the time sequence for the measurements



7.7.7 PK/PD analysis

Development of the population pharmacodynamic model is shown in Table 7.7. In Run 19, the relationship between baseline corrected QTc and the concentration in the effect compartment (Ce) was described using a linear relationship, the intraindividual variability component was described using a combined error model, and the parameters were estimated using the FOCE with "interaction" method (Chapter 3). The estimates of 42% and 22msecs for the exponential and additive variability, respectively, and are equivalent to 40msecs at predicted QTc prolongation of 50msecs. In Run 20, the introduction of an additional parameter to model the placebo corrected baseline QTc significantly decreased the intraindividual variability and the objective function (Δ-655). The estimates for the exponential and additive components were 12% and 20msecs, respectively, are equivalent to 26 msecs at predicted QTc prolongation of 50 msecs. While the parameter estimate for the slope of the concentration response relationship was unchanged, the Keo estimate was increased from 1.29hr^{-1} to 3.85 hr^{-1} and the half-life of the hysteresis was decreased from 0.5 to 0.2 hours. Therefore, the variability in baseline has more affect on the temporal displacement estimate than the slope estimate, which characterises the sensitivity of the myocardium to dofetilide.

The standard error for the exponential component was large (SE% > 100%) indicating that the estimate was not significantly different from zero. Run 21 was a repeat of run 20 with intraindividual variability being described using the additive expression only. Since the increase in the objective function was not significant, the simpler form was retained and the specification of "interaction" or "no interaction" was no longer required.

In run 22, an Emax relationship was modelled using the same intraindividual variability model. A further reduction in objective function was achieved but the Emax and Ce_{50} were estimated with less precision than the slope parameter of the linear model.

Table 7.7 Pharmacodynamic model development

Run	Model	FOCE with	Objective Function	Keo hr^{-1} (SE)	Base-line msecs (SE)	Slope msecs. $ml.ng^{-1}$ (SE)	Emax msecs (SE)	Ce_{50} ng ml^{-1} (SE)	ω_{Keo} %CV (SE)	$\omega_{Base-line}$ %CV (SE)	ω_{Slope} %CV (SE)	ω_{Emax} %CV (SE)	ω_{Ce50} %CV (SE)	σ_{EXP} %CV (SE)	σ_{ADI} ng.ml (SE)
19	QTc = Slope x Ce.	**Interaction**	**10241.52**	**1.29** *9*		**17.5** *18*			**27** *151*	**72** *81*				**42** *61*	**22** *27*
20	QTc = Slope x Ce + Baseline	**Interaction**	**9586.35**	**3.85** *12*	**1.35** *91*	**17.1** *7*			**30** *169*	**157** *37*	**24** *45*			**12** *126*	**20** *21*
21	QTc = Slope x Ce + Baseline	**NA**	**9587.84**	**3.79** *14*	**1.61** *88*	**16.9** *7*			**28** *251*	**154** *55*	**24** *44*				**21** *18*
22	QTc = (Ce x Emax) / (Ce + Ce_{50}) + Baseline	**NA**	**9538.65**	**4.52** *12*	**-5.16** *47*		**193** *24*	**6.35** *39*	**32** *128*	**84** *55*		**38** *104*	**65** *82*		**20** *20*

NA Not applicable: The use of interaction requires that the intraindividual variability model has an exponential or proportional component.

The individual parameter estimates for linear and Emax models are shown in Table 7.8. Notably, the Keo and the slope (at Ce_{50} i.e.Emax/Ce_{50}) were largest for the Emax. The reduced precision with the Emax model is most likely due to the largest predicted Ce for the majority of individuals being much lower than the estimated Ce_{50} Figures 7.13 -7.15 show that the Emax model is essentially linear over the observed Ce range for most individuals (individuals 43 and 44 are possible exceptions). The slope of the Emax model at Ce_{50} is therefore not comparable to the slope of the linear model. The linear model for the prolongation of QTc is therefore the most appropriate for the majority of individuals over the dose range investigated.

Table 7.8 Individual parameter estimates for QTc prolongation

	Emax Model					Linear Model		
ID	**Keo hr^{-1}**	**Baseline msecs**	**Emax msecs**	**Ce_{50} ng ml^{-1}**	**Emax/ Ce_{50} msecs.ml. ng^{-1}**	**Keo hr^{-1}**	**Baseline msecs**	**Linear msecs.ml .ng^{-1}**
IHD								
1	4.09	-7.01	217.36	6.08	35.74	3.89	1.02	21.07
2	4.64	-4.81	166.13	10.15	16.36	3.90	0.71	12.49
3	4.27	-3.06	146.50	8.69	16.85	3.49	1.26	12.54
4	3.72	-10.77	186.72	7.14	26.15	3.41	0.66	14.43
5	2.80	-8.40	225.76	6.14	36.75	2.94	0.99	21.09
6	5.27	-4.77	210.83	4.98	42.33	4.65	2.75	24.60
7	4.75	-13.53	153.85	7.28	21.13	3.70	0.47	10.76
8	4.50	-4.39	160.88	5.82	27.66	3.73	1.63	16.72
13	4.55	-3.90	172.58	3.86	44.75	4.05	16.62	17.73
15	4.27	-4 00	186.88	5 00	37.39	3.78	11.84	17.52
16	4.84	-7.80	188.66	5.99	31.49	4.25	1.39	18.03
17	7.78	-11.13	184.33	5.97	30.87	6.07	1.74	15.17
18	4.82	-16 00	215.68	6.62	32.58	4.18	0.95	16.18
19	3.33	-6.24	191.48	8.77	21.84	2.90	1.18	13.76
20	4.03	-8.44	202.96	5.13	39.55	3.45	5.32	19.36
21	4.28	-5.80	188.22	5.73	32.82	3.52	3.51	18.54
Median	4.39	-6.63	187.55	6.04	32.03	3.75	1.33	17.12
Mean	4.50	-7.50	187.43	6.46	30.89	3.87	3.25	16.87
SD	1.07	3.73	23.33	1.63	8.65	0.74	4.55	3.66
VOL								
37	8.22	-2.44	156.88	8.87	17.69	5.87	3.13	11.91
38	4.51	-4.90	209.05	6.26	33.40	3.71	7.38	15.71
39	4.87	-9.07	442.42	14.04	31.50	4.49	0.80	21.80
40	4.22	-3.99	142.27	4.94	28.82	3.02	4.21	14.83
41	3.94	-3.78	232.38	7.93	29.31	3.29	3.60	17.72
42	3.68	-6.11	421.83	14.60	28.90	3.41	0.92	21.11
43	4.39	-37.58	189.88	2.12	89.74	3.27	0.91	19.58
44	4.91	-3.01	121.63	1.13	107.61	3.44	30.70	16.71
Median	4.45	-4.45	199.47	7.09	30.41	3.43	3.36	17.21
Mean	4.84	-8.86	239.54	7.49	45.87	3.81	6.46	17.42
SD	1.43	11.79	124.23	4.97	33.26	0.94	10.04	3.34
All								
Median	4.44	-5.96	188.44	6.11	31.49	3.71	1.51	17.12
Mean	4.61	-7.96	204.80	6.80	35.88	3.85	4.32	17.06
Sd	1.18	7.20	75.38	3.08	20.92	0.79	6.82	3.49

Figure 7.13 Individual placebo corrected QTc prolongation and predicted Emax response versus effect compartment concentration for the IHD patient group recieving doses 1, 2, 4 mcg.kg^{-1}

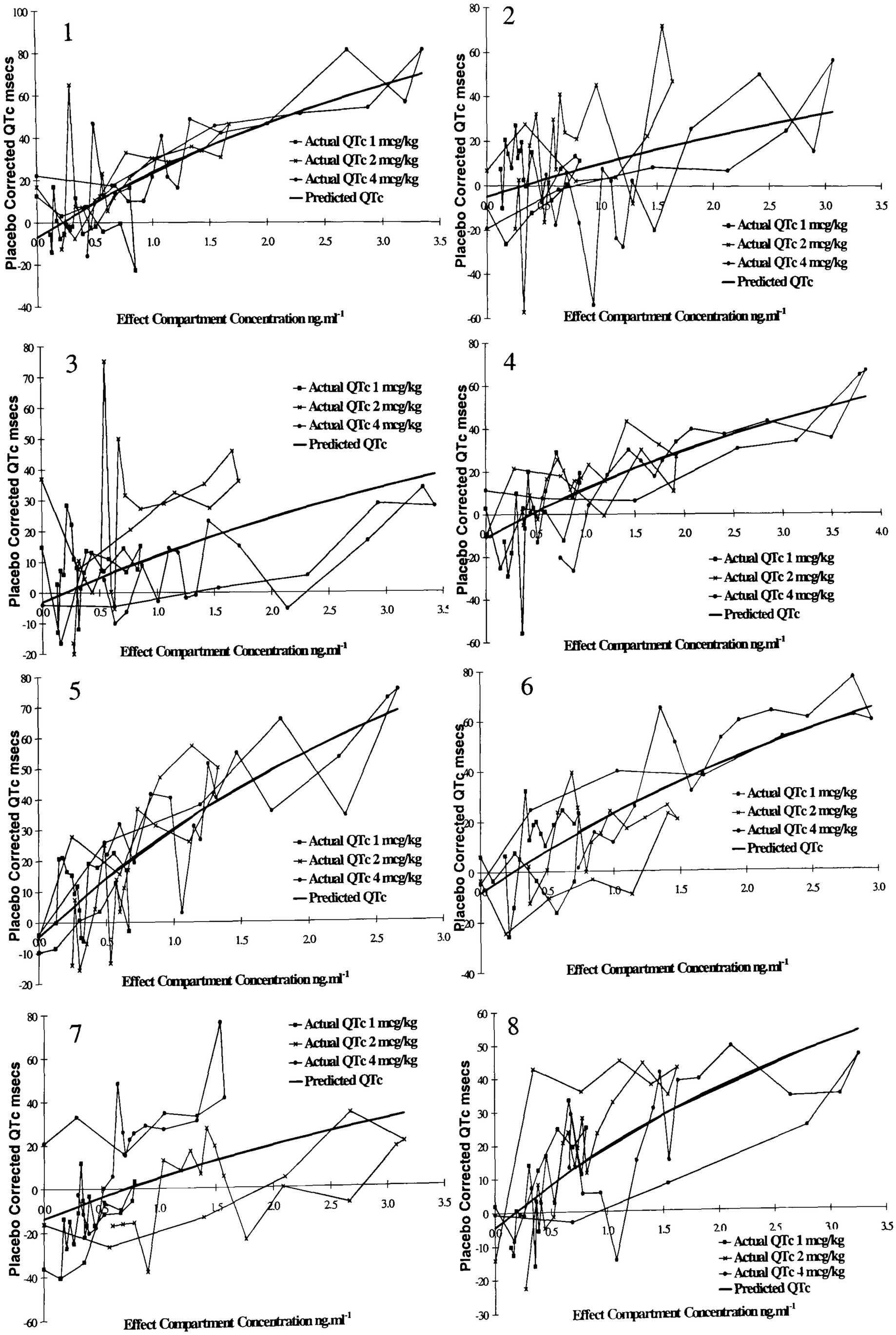


Figure 7.14 Individual placebo corrected QTc prolongation and predicted Emax response versus effect compartment concentration for the IHD patient group recieving doses 4, 6, 8 mcg.kg^{-1}

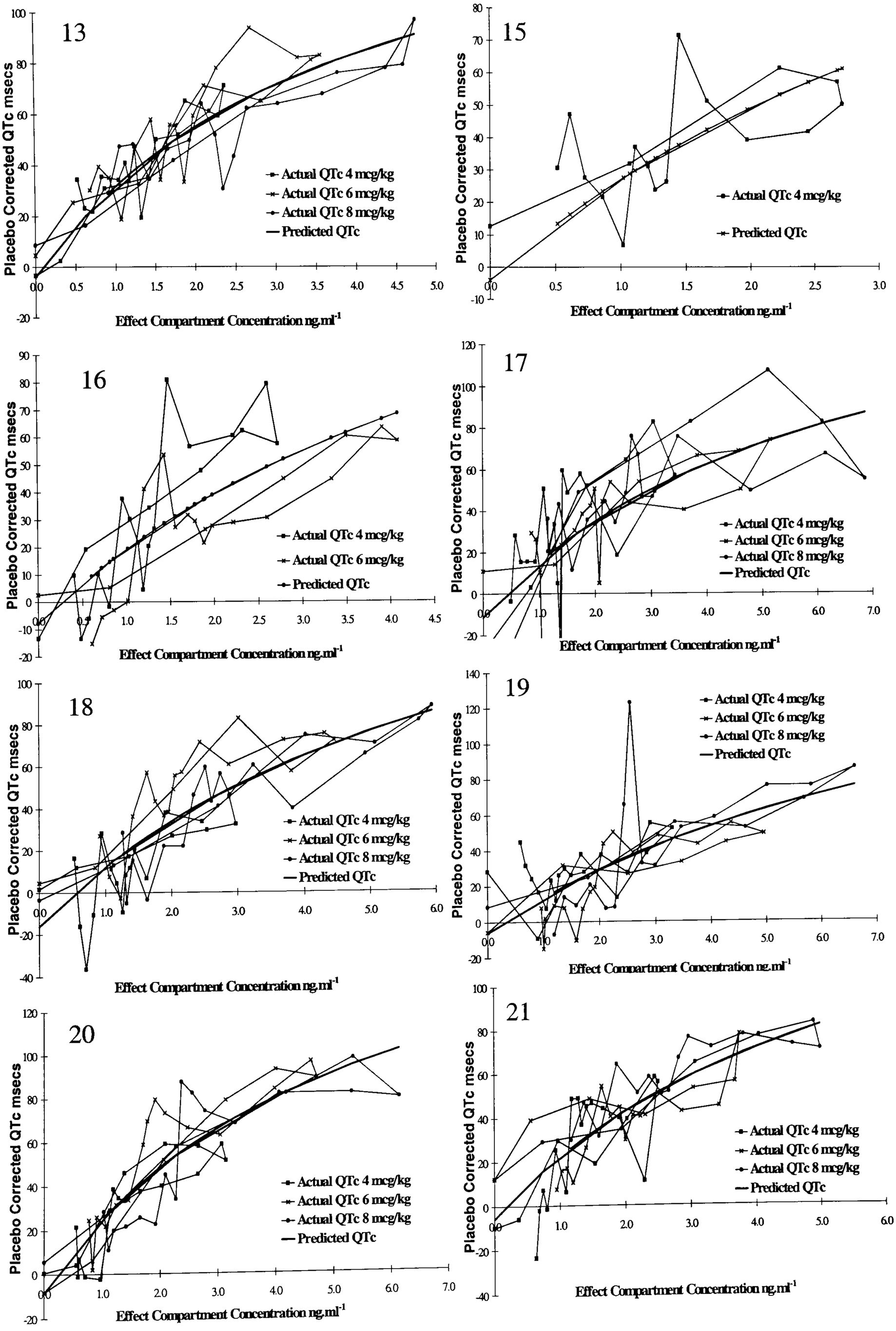
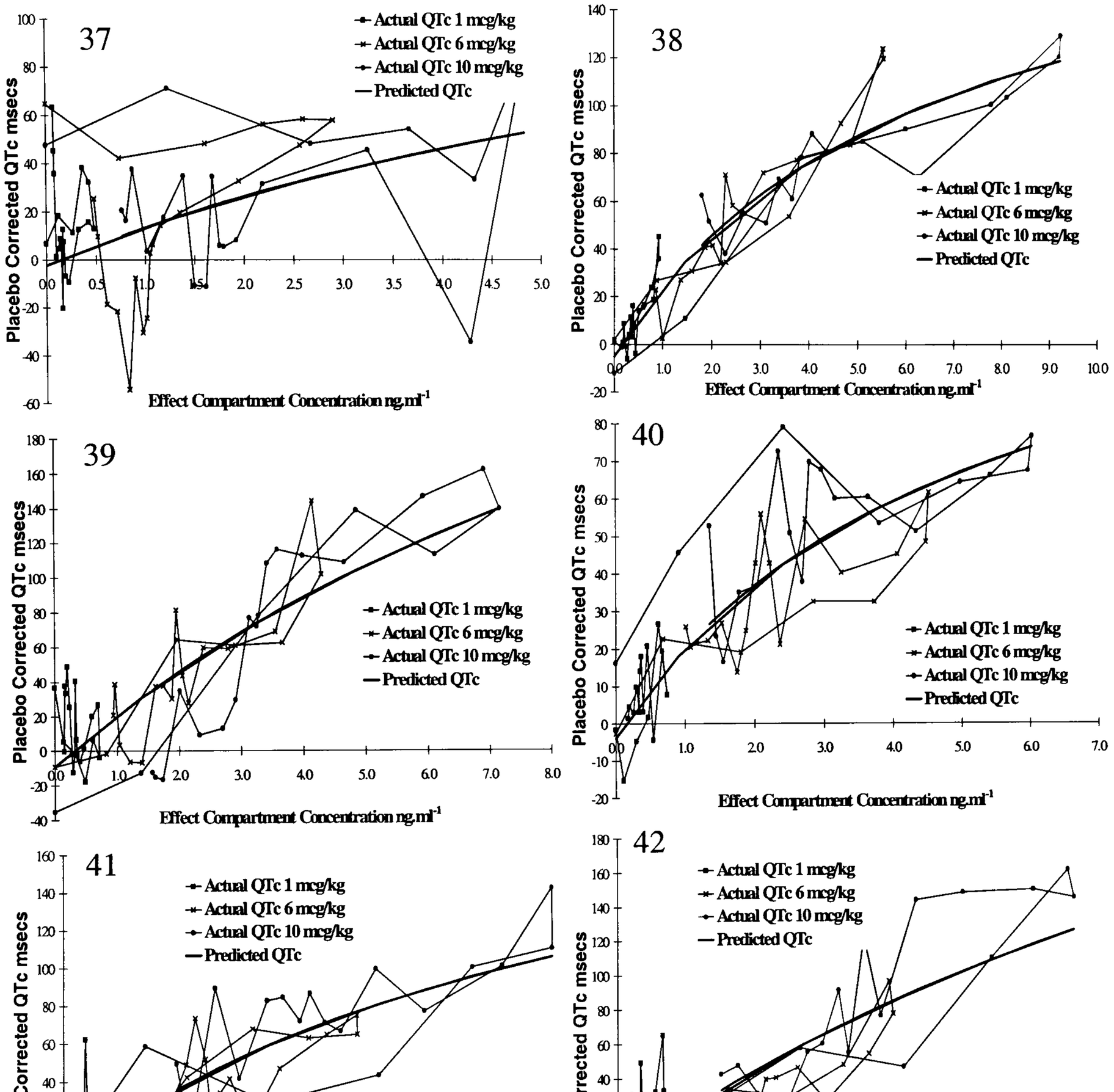


Figure 7.15 Individual placebo corrected QTc prolongation and predicted Emax response versus effect compartment concentration for the health volunteers recieving doses 1, 6, 10 mcg.kg^{-1}



7.7.8 Healthy volunteer vs IHD

Differences in the baseline, Keo and slope parameters, between healthy volunteers and IHD patients,were formally tested using the linear model (run 21, Table7.7). None of the parameter differences between the two groups were found to be significantly different (runs 23, 24 and 25, Table 7.9). Graphical analysis did not demonstrate any correlation between the investigated covariates and the individual parameter estimates of the pharmacodynamic model.

Table 7.9 Comparison between the population pharmacodynamic parameters for healthy volunteers and IHD patients using the FOCE with interaction estimation method

Run[1]	Parameter Tested	Run 21 Parameter Estimate	Parameter Estimate for the IHD Group	Parameter Estimate for the Volunteer Group	Obj Fun	Δ Obj Fun[2]	LRT[3]
23	Keo (hr^{-1})	3.79	3.68	3.86	9590	-0.07	p<0.8
	(SE%)	(14)	(19)	(14)			
24	Baseline (msecs)	1.61	3.02	0.99	9589	-1.26	P<0.3
	(SE%)	(88)	(71)	(111)			
25	Linear ($msecs.ml^{-1}$)	16.9	17.50	16.60	9590	-0.2	p<0.7
	(SE%)	(7)	(8)	(8)			

1) Differences in the parameter estimates between the groups are tested individually in runs 23 - 25 by comparing them to run 21.

2) Comparison with objective function from. run 21 Table7.7

3) LRT = Likelihood Ratio Test

7.8 Predictions and simulations

7.8.1 Comparison of a dose per kilogram regimen and a fixed dose regimen for termination of arrhythmia with an IV infusion

The influence of body weight on Cmax following different 0.5 hr infusion regimens using the average PK model

Based on the final average PK model prior to inclusion of the covariates (run 3), the predicted average concentration time profiles after an arbitrary dose of 12 $mcg.kg^{-1}.hr^{-1}$ are shown for a body weight range of 50 to 100 kg in Figures 7.16 a. Since the dose for the 100kg subject would be twice that for the 50 kg subject, the average peak concentration would be predicted to be 100% larger in subjects weighing 100kg (Figures 7.16 a). After correcting for the relationships between body weight and CL and Vss identified in the final covariate model (Table 7.6 run 18), the peak concentration was still predicted to be 70% higher in subjects weighing 100kg in comparison to subjects weighing 50 kg (Figure 7.16 b). The range in the predicted peak concentration across body weight could be further reduced by using a fixed dose regimen. In this case the peak concentration would be predicted to be 17% lower in subjects weighing 100kg in comparison to subjects weighing 50kg (Figure 7.16 c). Therefore, based on the covariate model it was predicted that the narrowest range in peak concentrations would be achieved by using a fixed dose regimen. However, this conclusion depends on the accuracy of the covariate relationships. A sensitivity analysis was performed to test whether the slope of the relationship between Vss and body weight altered the above conclusion. Even when the slope for the relationship between Vss and body weight was as high as $2.4L.kg^{-1}$ (i.e. intercept was zero) the peak concentrations following the per kilogram dosing strategy were still predicted to be 57% higher in 100kg subjects compared to 50kg subjects (Figure 7.17 a).

Figure 7.16 The populaton mean predicted concentration time profiles over the body weight range of the study for a 30 minutes infusion of a) 12mcg.kg^{-1}.hr^{-1} assuming no relationship between CL,Vss, V_1 and WT, b) 12mcg.kg^{-1}.hr^{-1} assuming the modelled relationships between WT and CL and Vss i.e. slopes of 0.114L.hr^{-1}.kg^{-1} and1.52L.kg^{-1}, c) a fixed dose (12mcg.kg^{-1}.hr^{-1}x 77.9kg) assuming the modelled relationships between WT and CL and Vss i.e. slopes of 0.114L.hr^{-1}.kg^{-1} and 1.52L.kg^{-1}

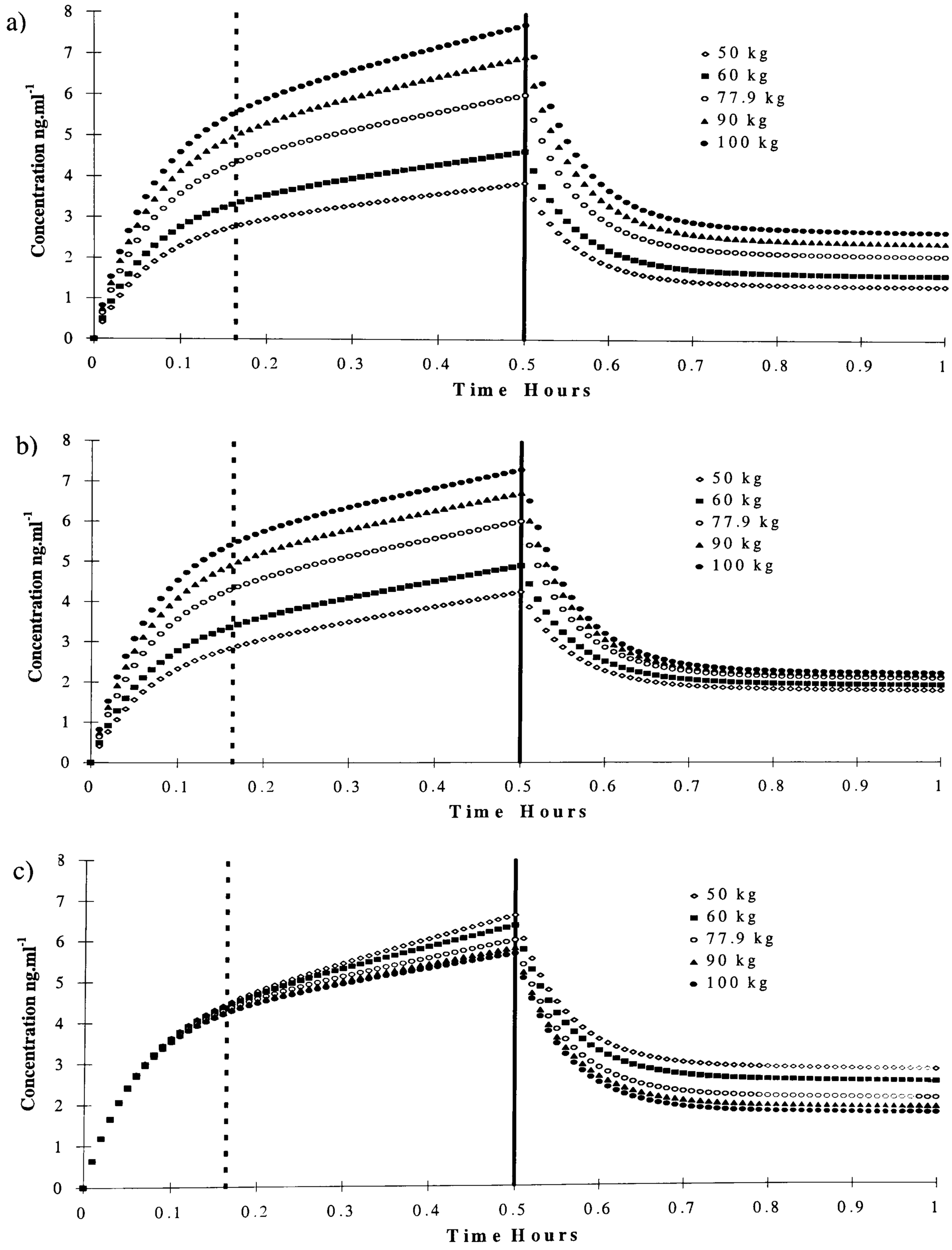
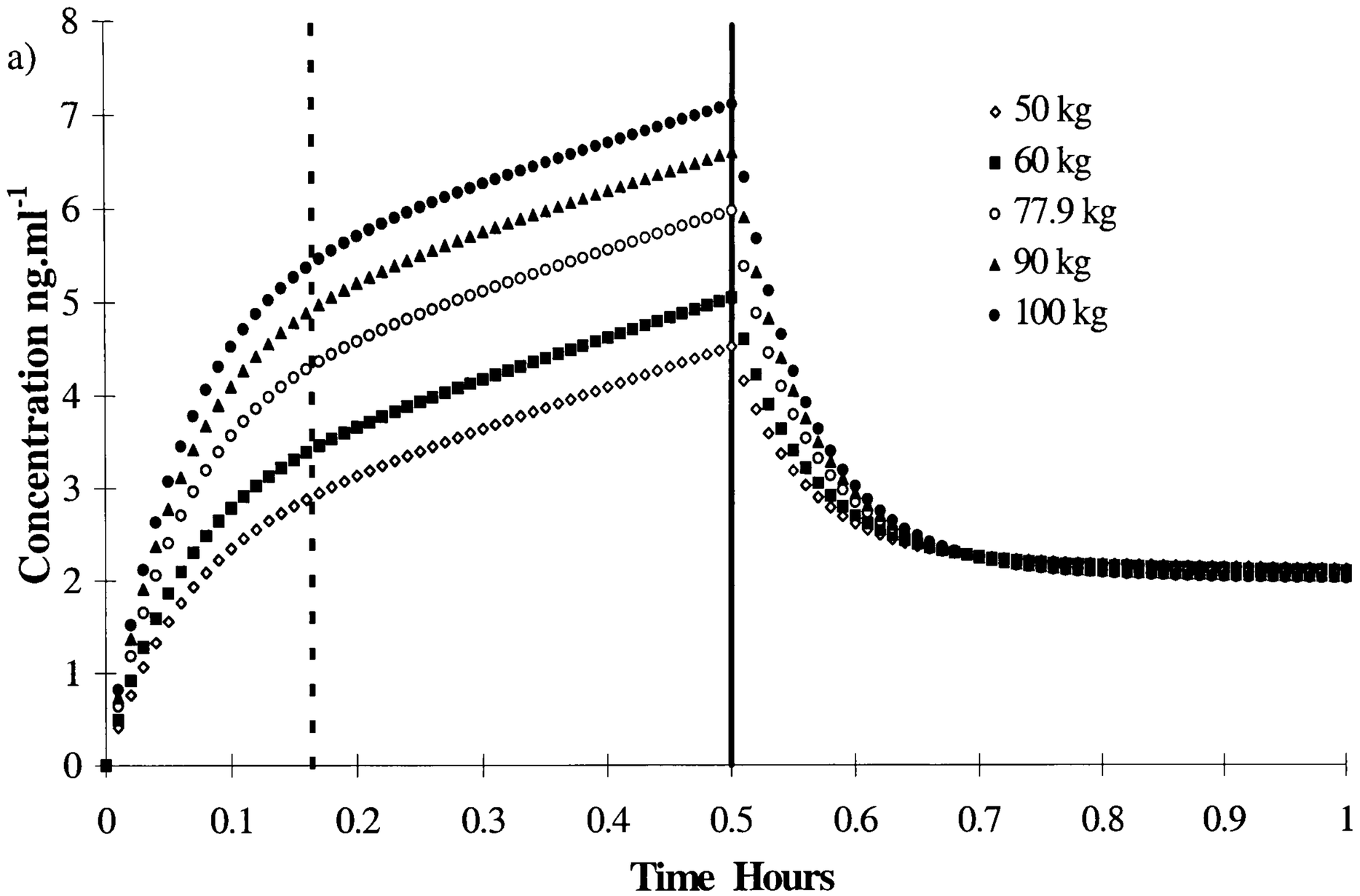


Figure 7.17 The populaton mean predicted infusion profiles for (a) a 30 minutes infusion of 12mcg.kg^{-1}.hr^{-1} over the body weight (WT) range for the study: assuming the slope for Vss was 2.4L.kg^{-1} and b) a fixed dose (12mcg.kg^{-1}.hr^{-1}x 77.9kg) for all weights assuming the slope for Vss was 2.4L.kg^{-1}. The estimated slope for CL vs body weight i.e. 0.114L.hr^{-1}.kg^{-1} was used in each case



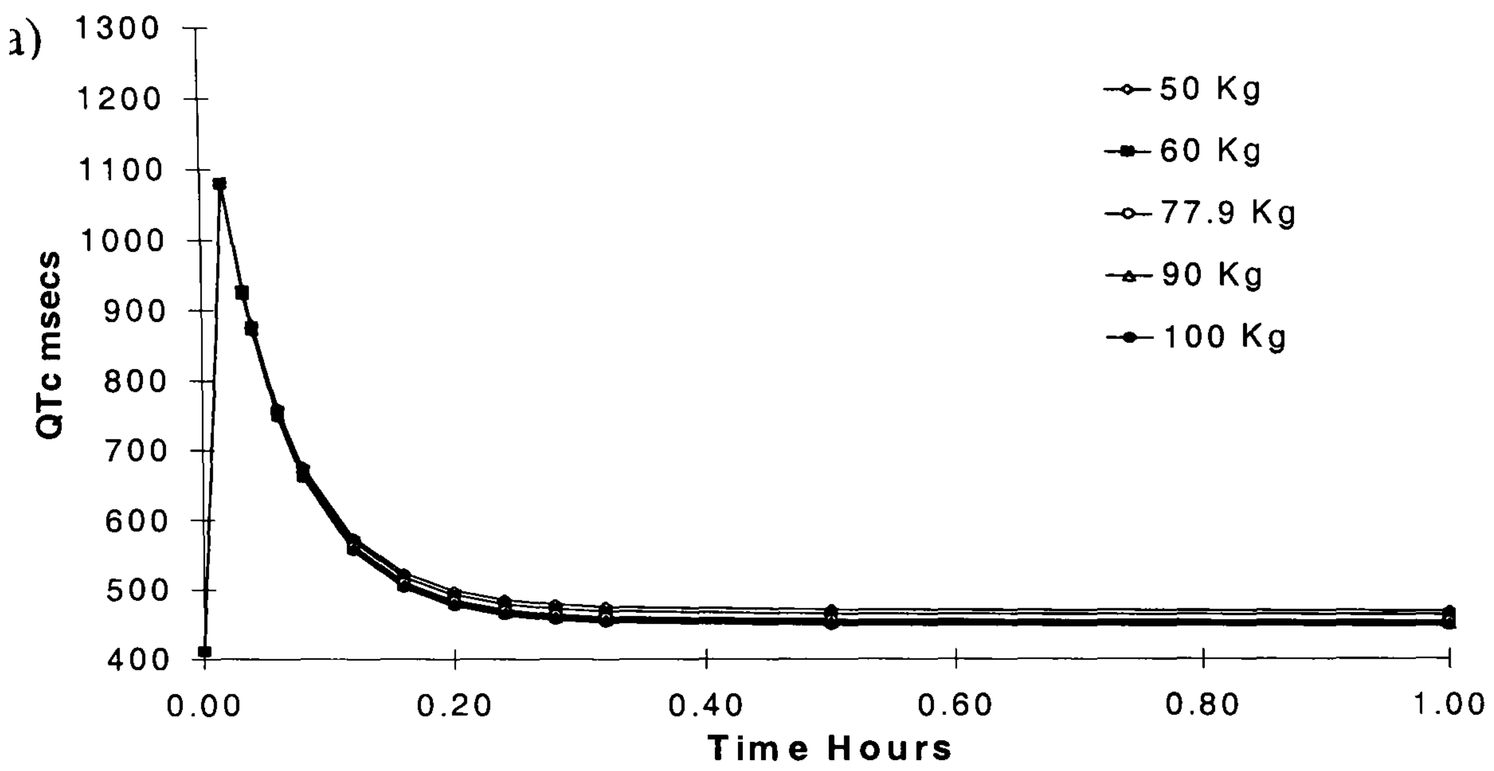
if

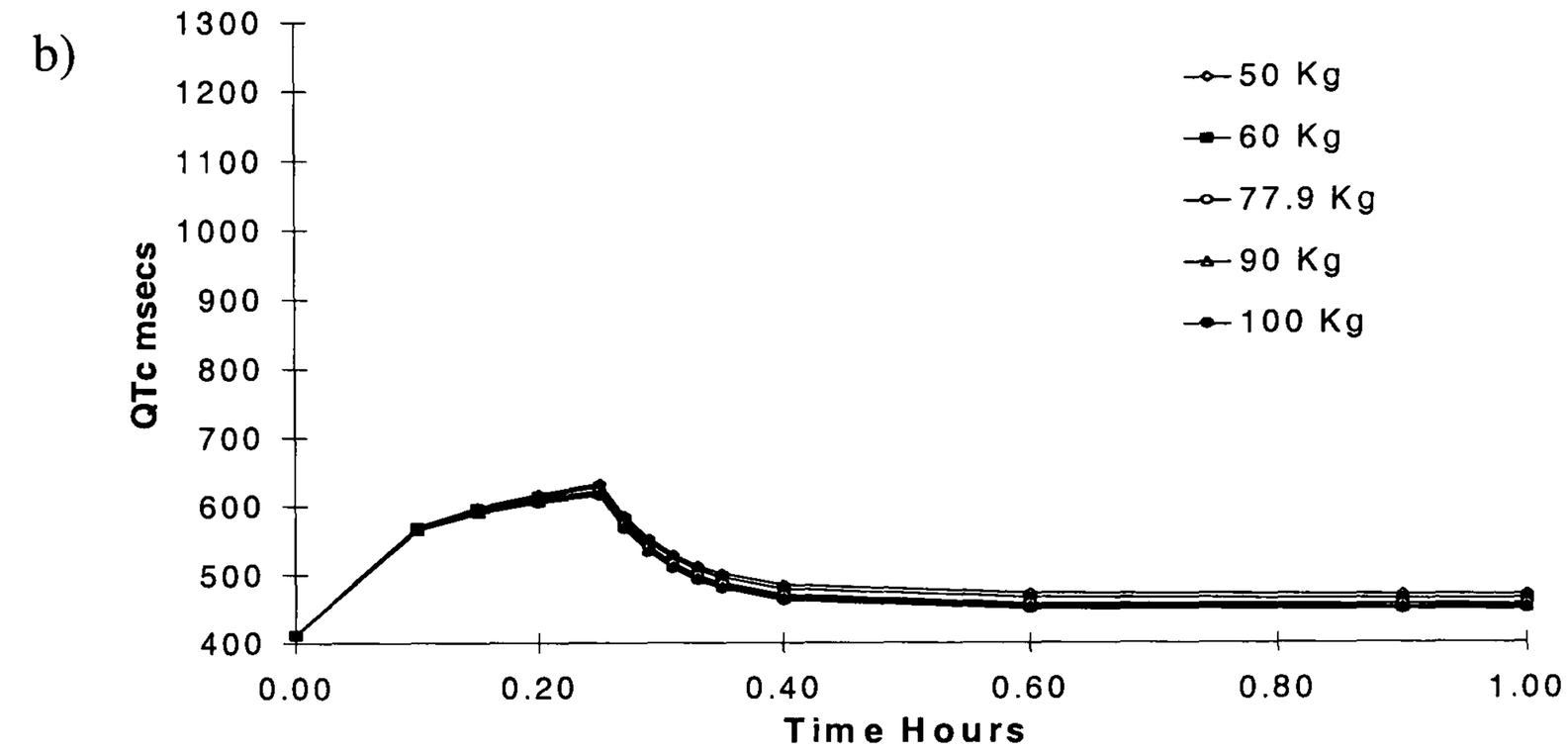
In comparison, the peak concentrations following the fixed dose regimen were predicted to be 27% lower in 100kg subjects compared to 50kg subjects (Figure 7.17 b). While the spread in concentration following the dose per kilogram regimen occurs over the initial 0.16 hr (up to the dashed line Figures 7.16a, 7.16b and 7.17.a), the concentrations following the fixed dose regimen are narrowest over this period (up to dashed vertical line Figures 7.16c and 7.17b). The variability at the end of the 0.5 hr infusion is more dependent on the initial volume of distribution (V_1) than Vss and therefore, the relationship between V_1 and body weight. In the present analysis, V_1 was not influenced by body weight, so the predicted concentration range was narrowest when dose was fixed across body weight.

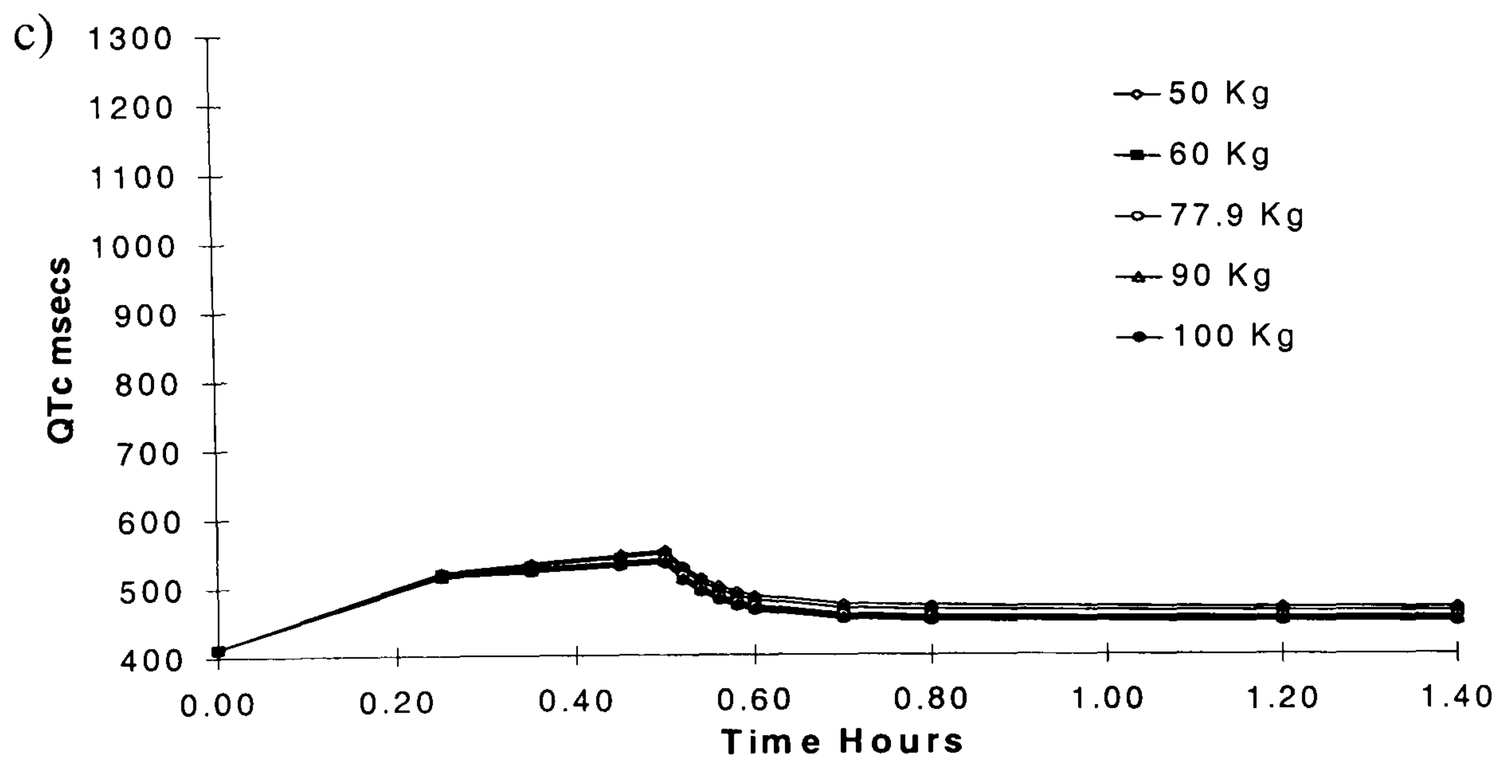
The influence of body weight and infusion time on peak QTc using the average PK\PD model

The predicted QTc following a fixed 600 mcg dose (Figure 7.18) and a 7.7mcg.kg^{-1} dose (Figure 7.19) over infusion times of 1, 15, 30 and 45 minutes were compared using the final average PK/PD model (combination of population parameters from run 18 and run 21). For mean study weight (77.9 kg), the total amount administered is the same for both regimens. Since a linear pharmacodynamic model was used, it mimicked the differences in plasma concentrations, so it is not surprising that the range of peak QTc intervals was predicted to be narrower with the fixed dose regimen. As expected, the peak QTc interval was predicted to be inversely related to the rate of administration and, therefore, the length of the infusion. However, the duration of infusion affected the expected range in peak predictions for each regimen differently. While there was a slight widening of the predicted range of peak QTc's with increasing infusion duration with the fixed dose regimen (figure 7.18), a considerable narrowing was predicted for the per kilogram regimen (figure 7.19). Therefore, the difference in the predicted ranges of peak QTc was less with the longer infusions.

Figure 7.18 Population mean predicted QTc following a fixed dose of 600mcg infused over a) 1 b) 15 c) 30 and d) 45 minutes







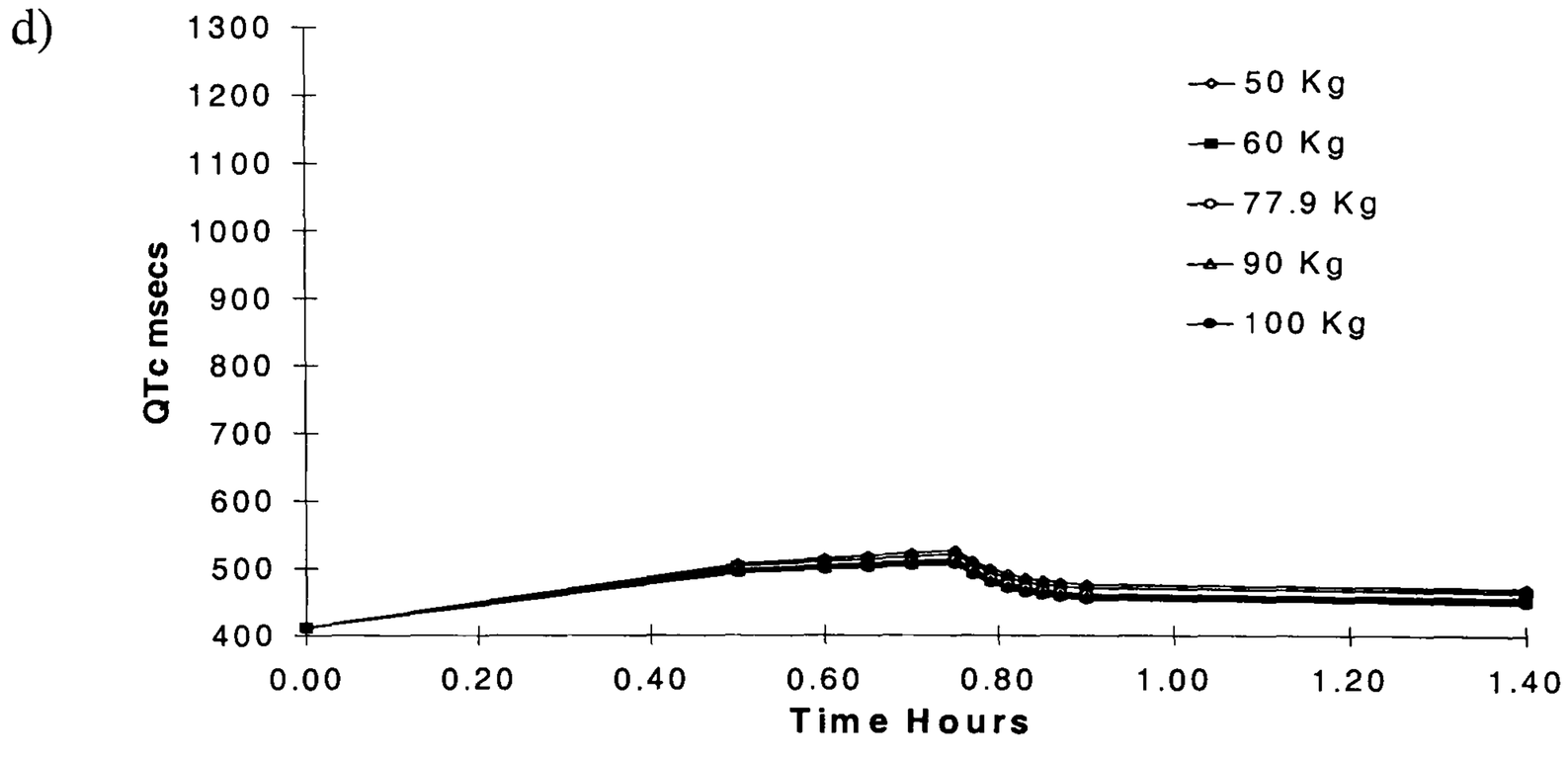
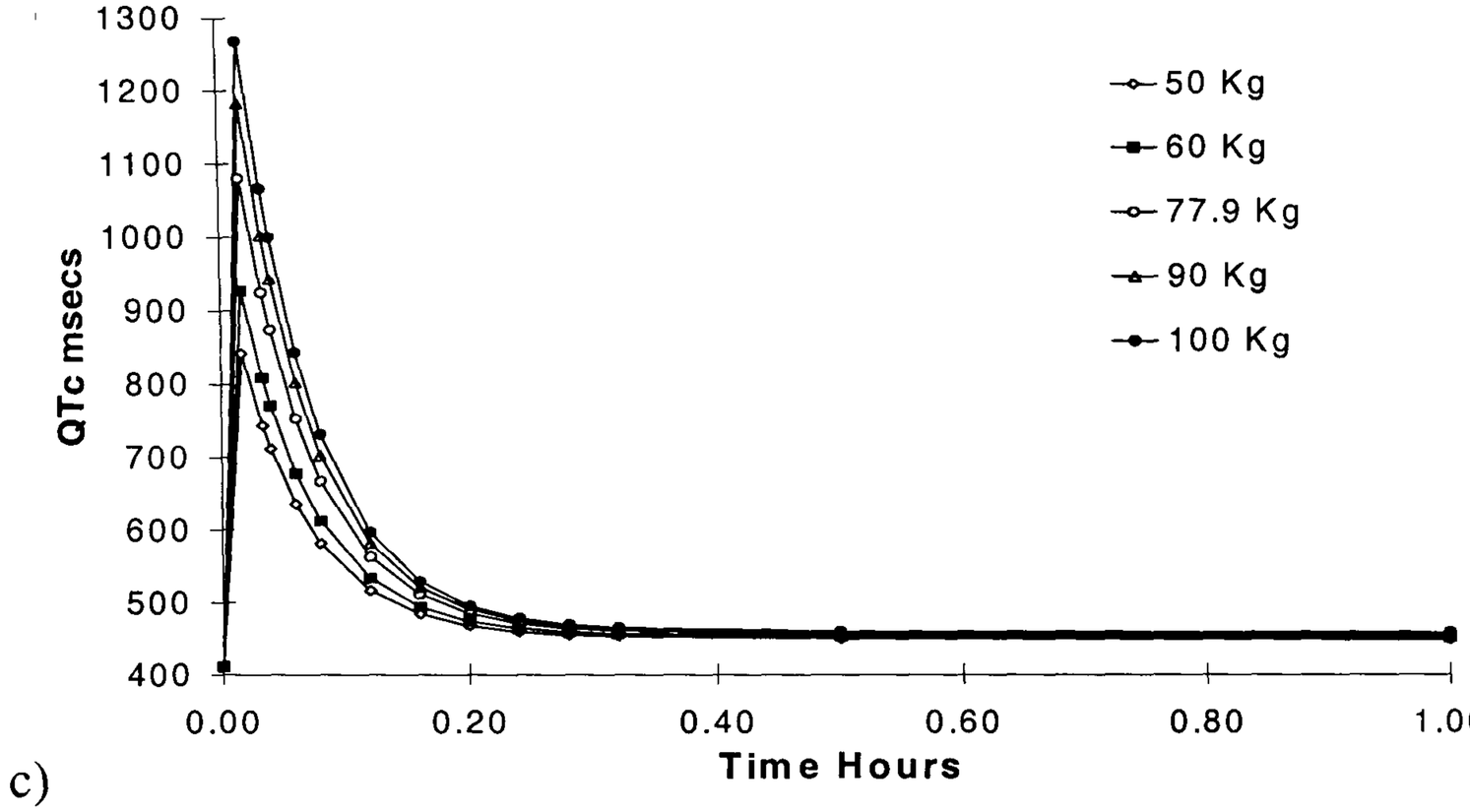
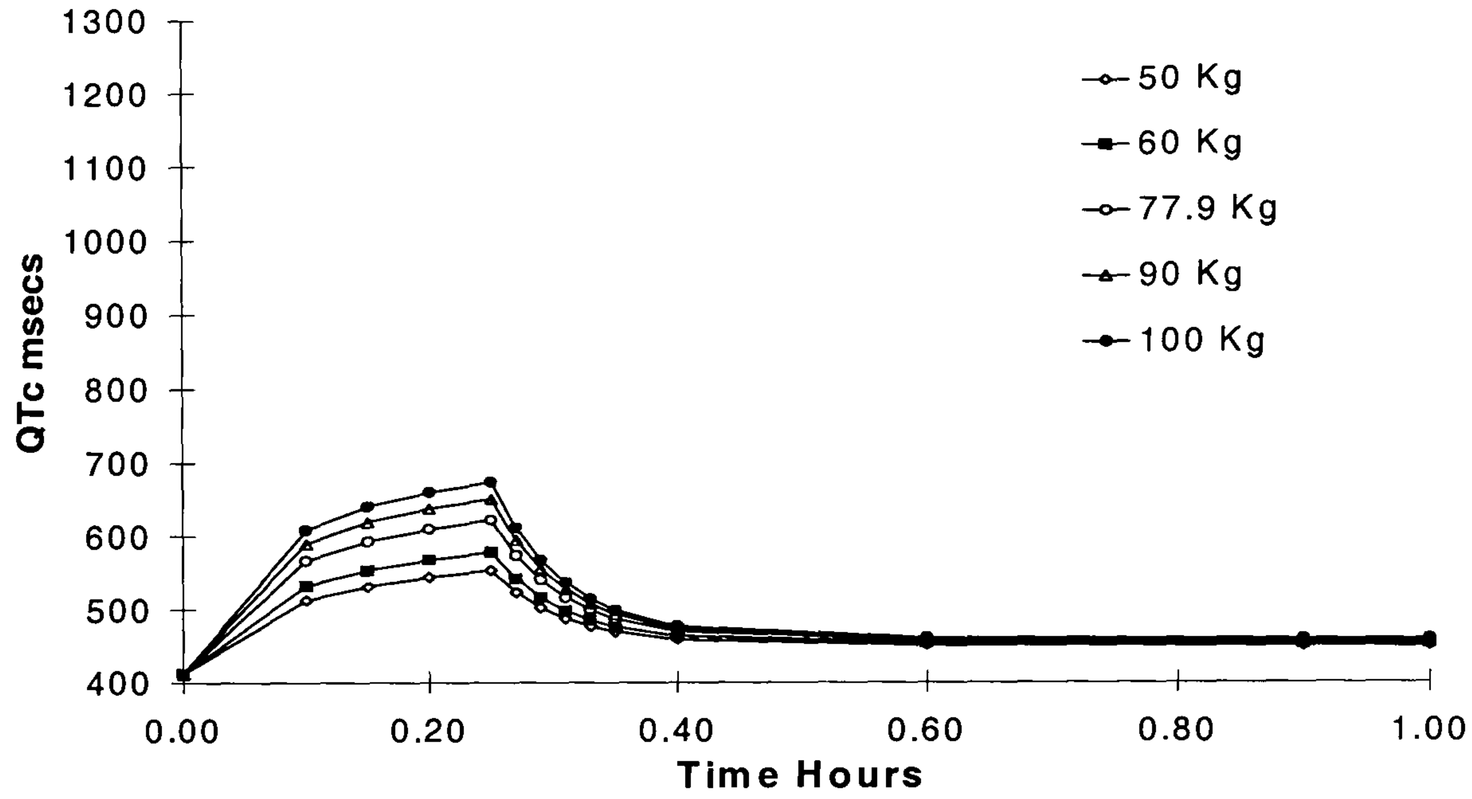


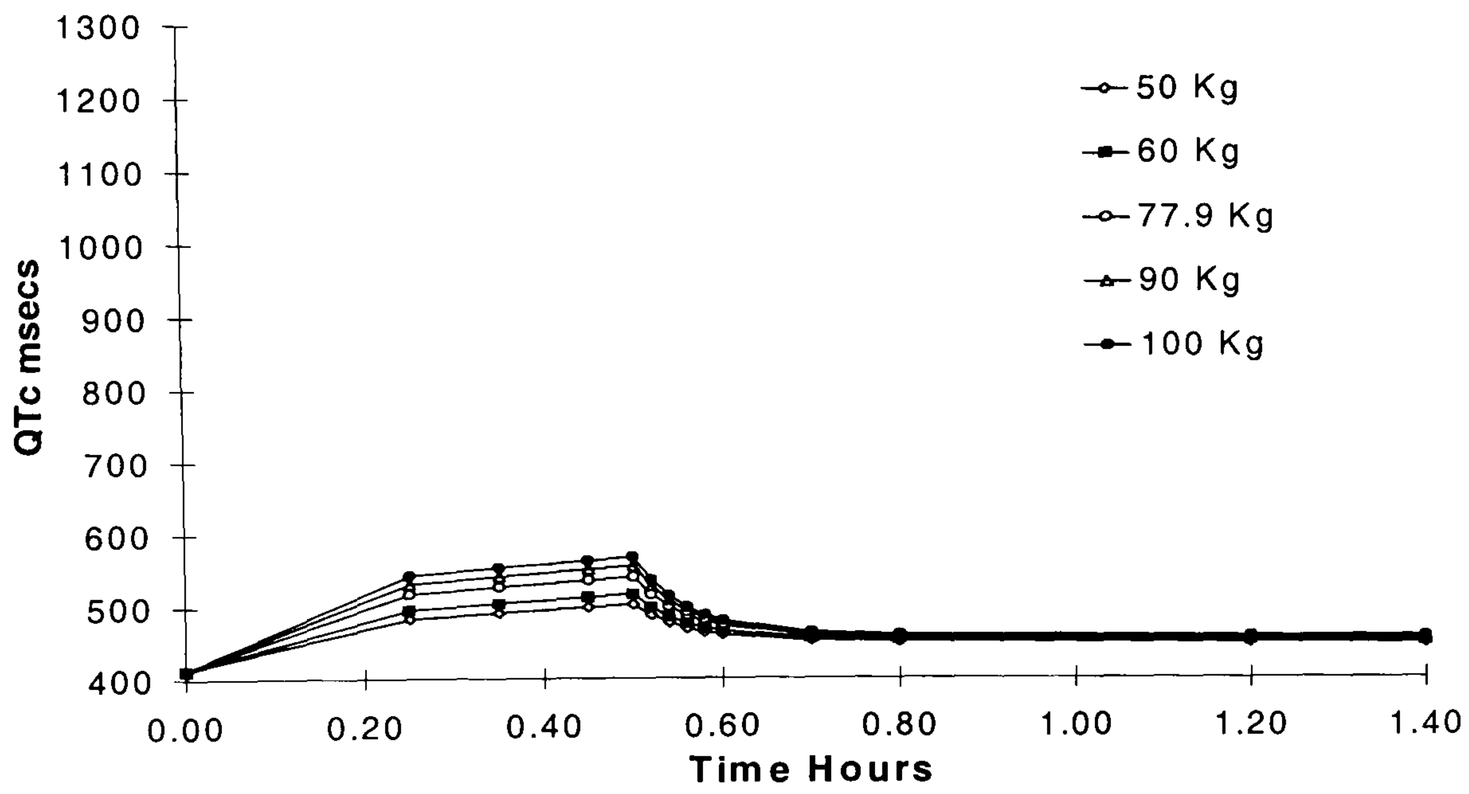
Figure 7.19 Population mean predicted QTc following 7.74 mcg.kg^{-1} infused over a) 1 b) 15 c) 30 and d) 45 minutes



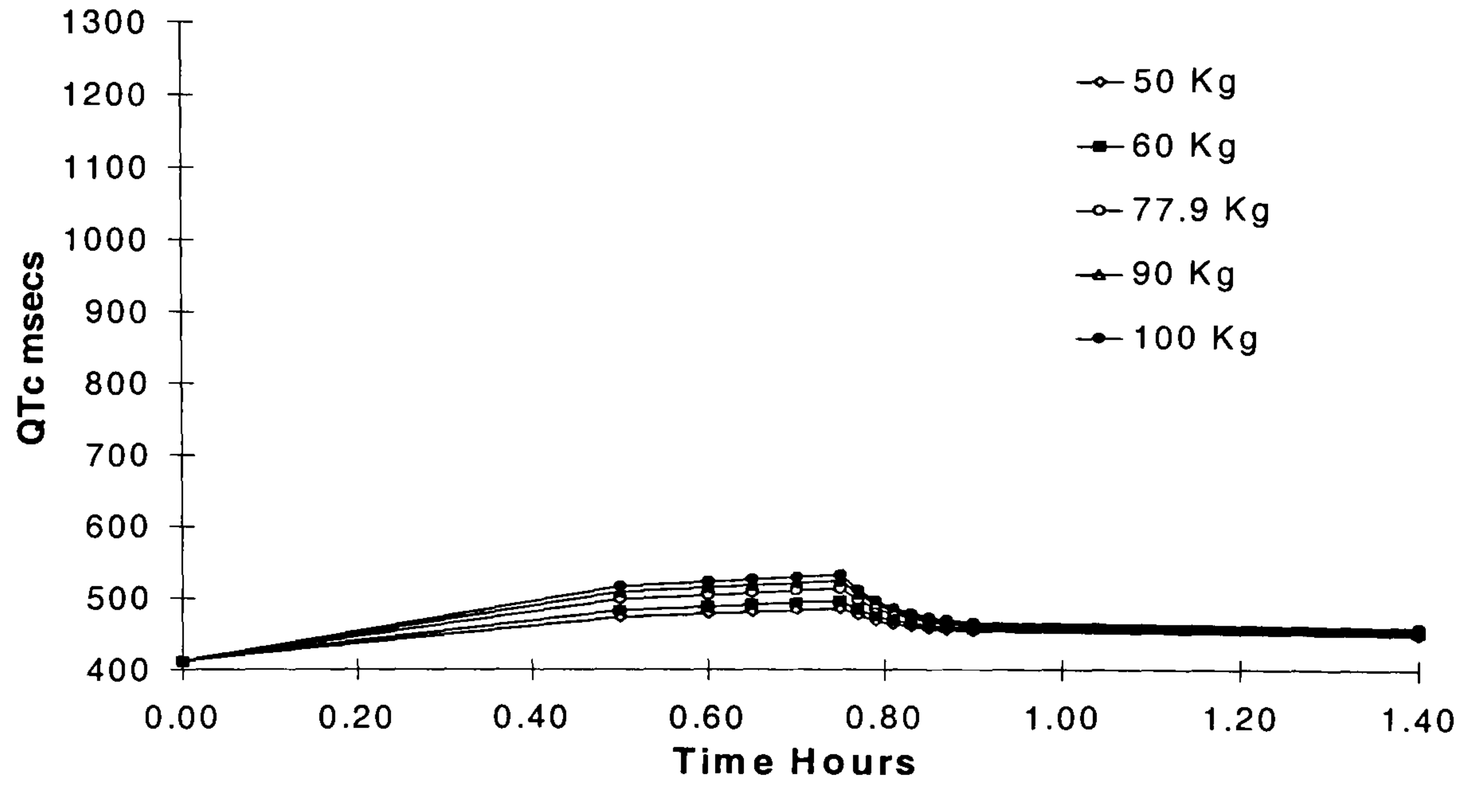
b)



c)



d)



The influence of body weight and infusion time on Cmax and QTc via simulations utilising the population parameter estimates

As before, a fixed 600 mcg dose and a 7.7mcg.kg^{-1} dose infused over 1, 15, 30 and 45 minutes were compared. However, in this instance the full PK/PD model (mean and variance estimates from runs 18 and 21) was used to simulate the Cmax and peak QTc for 1000 subjects. Body weight was randomly sampled from a normal distribution with mean and SD equal to 77.9 and 13.9kg, respectively. The results are shown in Table 7.10. In contrast to the results with the average PK/PD model, the differences between the two dosing strategies were much less obvious. While the SD and 5-95th percentile range for the Cmax and peak QTc (%change QTc) were still larger for the dose per kilogram regimen, the differences were less than would be expected from the comparisons shown in Figures 7.16 - 7.19. As before the difference between the SD's and 5-95th percentile ranges became less as the infusion time increased.

As previously discussed (see Section 7.1.6), there is an increased risk of TdP when QTc intervals are prolonged to greater than 25% from baseline or to absolute values of greater than 560msecs. The risk following a fixed 600mcg dose and a 7.74mcg.kg^{-1} dose infused over 1, 15, 30 and 45 minutes were compared. The percentage of subjects exceeding the criteria for increased risk of TdP are also shown in Table 7.10.

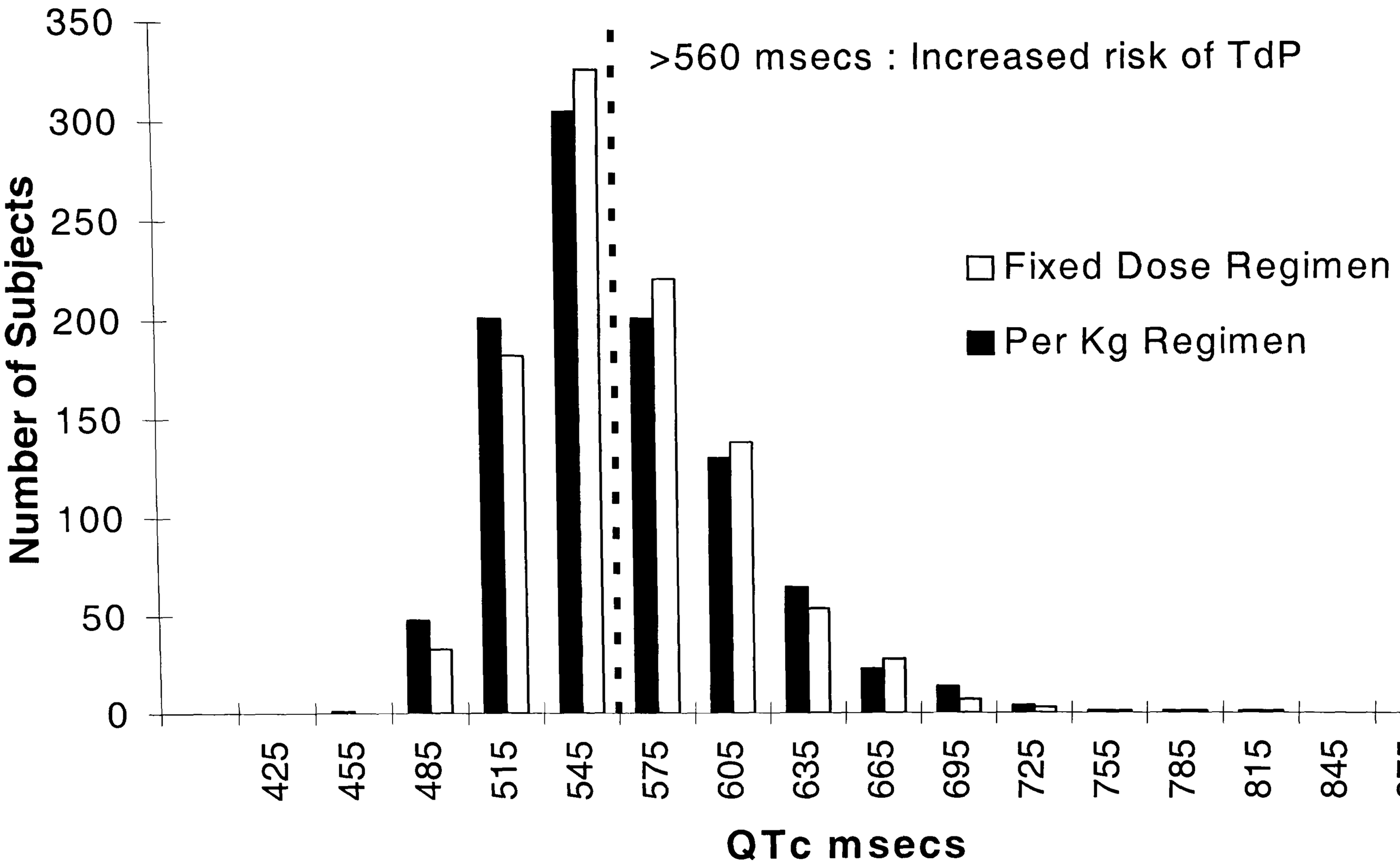
Following the one minute infusion, almost all subjects, regardless of dosage regimen, were predicted to be at risk of TdP. As expected, the percentage at risk was predicted to decrease with increasing infusion time. However, even the percentage at risk after a 45 minute infusion (24-25%\67%-69%) was still unacceptably large, and lower doses would be required. For this population, the percentage change in QTc criterion (>25%) was found to be more sensitive than the absolute value criterion (>560msecs). The percentage at risk in the whole population was very similar for the two regimens across all infusion times.

Table 7.10 Simulated Cmax and peak QTc (and % prolongation) for 1000 patients administered either 600mcg or 7.7 mcg kg^{-1} over 1, 15, 30 and 45 minutes

		1minute			15 minutes			30 minutes			45 minutes		
		Cmax $ng.ml^{-1}$	Peak msecs	Peak Δ%	Cmax $ng.ml^{-1}$	Peak msecs	Peak Δ%	Cmax $ng.ml^{-1}$	Peak msecs	Peak Δ%	Cmax $ng.ml^{-1}$	Peak msecs	Peak Δ%
Fixed Dose Regimen													
Mean		44.2	1165	181%	12.5	637	53%	7.9	564	36%	6.1	542	30%
SD		24.3	424	103%	3.5	72	18%	2.0	47	12%	1.5	39	10%
Max		170.5	3196	663%	25.4	1290	124%	15.1	1161	83%	12.8	1087	71%
Min		7.3	533	30%	5.0	496	13%	3.6	473	5%	2.2	468	4%
Median		38.7	1073	157%	12.1	623	50%	7.6	556	35%	5.9	535	29%
95th Percentile		93.3	2027	398%	18.8	767	87%	11.5	645	58%	9.0	603	48%
5th Percentile		15.2	676	63%	7.4	546	27%	4.9	508	18%	4.0	495	16%
>560/>25%	All		100%	100%		91%	97%		46%	82%		24%	69%
>560/>25%	WT <64		99%	100%		92%	96%		54%	82%		30%	73%
>560/>25%	WT >91.8		99%	100%		91%	94%		42%	78%		14%	58%
Per Kilogram Regimen													
Mean		44.3	1163	180%	12.4	635	53%	7.8	563	35%	6.0	540	30%
SD		26.8	454	111%	4.1	78	20%	2.3	50	13%	1.6	40	11%
Max		193.1	3191	647%	34.7	1315	146%	21.6	1176	88%	14.3	1098	71%
Min		4.6	492	20%	3.2	481	4%	2.8	469	-2%	2.3	461	-2%
Median		38.0	1050	154%	11.9	623	50%	7.5	555	34%	5.8	534	29%
95th Percentile		98.9	2161	418%	19.7	781	91%	12.0	647	59%	9.1	605	48%
5th Percentile		13.8	661	58%	6.7	534	26%	4.5	500	17%	3.7	492	15%
>560/>25%	All		99%	100%		86%	96%		44%	81%		25%	67%
>560/>25%	WT <64		96%	98%		66%	83%		18%	61%		7%	49%
>560/>25%	WT >91.8		100%	100%		97%	99%		70%	89%		43%	80%

The lack of difference was due to the overall shape of the two resultant distributions. For example, the distribution of peak QTc following the 30 minute infusion (Figure 7.20) demonstrated that, although there was a larger number of patients with peak QTc > 670msecs dosed according to the per kilogram regimen, a larger number of patients with peak QTc between 560 and 670msecs were dosed according to the fixed dose regimen. Together, approximately the same number of patients were predicted to have peak QTc's > 560msecs. Nevertheless, the relationship between percentage at risk (defined using either criterion) and body weight was different for the two regimens and the differences became more apparent as the infusion time increased (Table 7.10). Not surprisingly, patients with high body weight >91.8 Kg (mean +1SD) were predicted to be at much greater risk when dosed according to the per kilogram regimen. Although patients with low body weight <64Kg (mean -1SD) were predicted to be at greater risk when dosed according to the fixed dose regimen, the increase over that for the whole population was small i.e. For the 30 minute infusion 54% vs 46% for the absolute value criterion and 82% vs 82% for percentage change criterion, respectively.

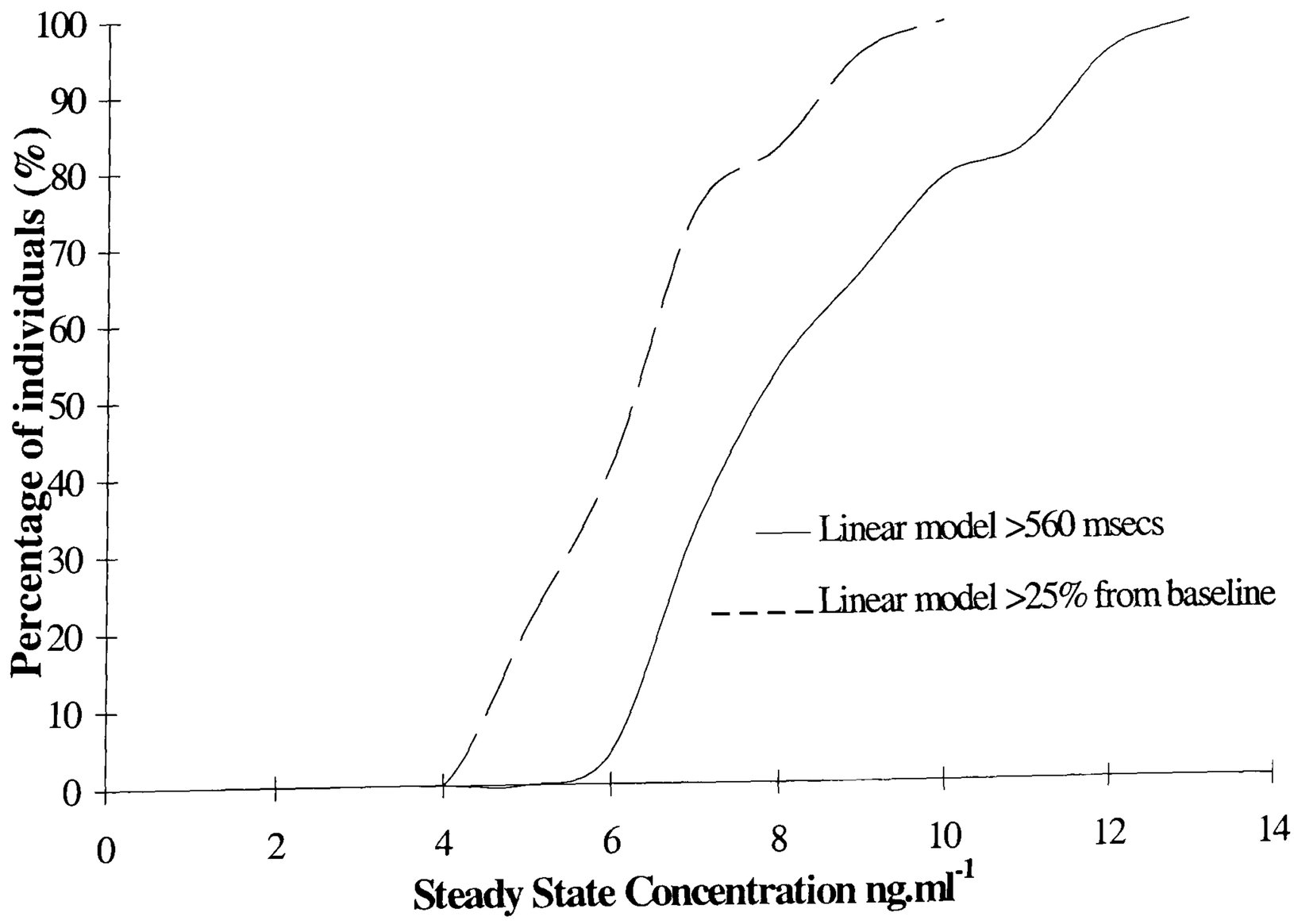
Figure 7.20 Simulated peak QTc for 1000 patients administered either 600mcg or 7.7 mcg kg^{-1} over 30 minutes



7.8.2 Steady state infusions and risk of TdP

Maintenance therapy with dofetilide for the prevention of arrhythmias will require the development of an oral formulation . While the absorption characteristics of dofetilide are needed to predict an oral dosage regimen, it is possible to use the current model to predict the number of subjects who would be at risk of TdP at various Css concentrations, and thereby predict the maximum tolerated Css. QTc intervals were predicted for various target steady state concentrations (1 to 20 $ng.ml^{-1}$) using the individual parameter estimates for the linear pharmacodynamic model (Table 7.8). The cumulative percentage of individuals with prolongations greater than 560msecs or +%25 from baseline were predicted. These were used to predict the percentage of subjects with an increased risk of TdP (Figure 7.21). The steep slopes define tight upper limits for the Css. An increased risk would appear to occur with concentrations above 4 $ng.ml^{-1}$ for the >+25% criteria and above 6 $ng.ml^{-1}$ for the >560 msecs criteria (Figure 7.21). The percentage change from baseline criteria was again shown to be the most sensitive criteria.

Figure 7.21 Cummulative frequency of individuals exceeding safety limits for QTc prolongation



7.9 Discussion

This analysis has demonstrated that the dose concentration effect relationship for dofetilide is the same in healthy volunteers and IHD patients. In general, much of early drug development is based on there being a concordance between healthy volunteers and patients with IHD. For example, the maximum tolerated dose and dose ranges for future patient studies are determined through investigation in healthy volunteers (see chapter 4). Furthermore, clinical pharmacology studies undertaken throughout drug development (i.e. drug interaction, food effect and bioequivalence studies) routinely utilise healthy volunteers as substitutes for the target patient population (see chapter 5). It is therefore important that potential differences in the PK/PD relationship for patients and healthy volunteers are investigated at an early stage in the drug development process.

While pharmacokinetics and pharmacodynamics for patients and healthy volunteers are similar for most marketed drugs, there have been some notable exceptions (Smith et al.. 1983; Boyd et al. 1989). In such cases it may be possible to use an integrated PK/PD model to adjust for differences, so that inferences for the target population could still be made from investigations in healthy volunteers.

7.9.1 Pharmacokinetic analysis

The bias in the fit of concentration data using the FO method can occur when models are highly non-linear (Chapter 3), as demonstrated with the multiple dose three compartment model (chapter 4.5.2). However, the single dose two compartment model used in this Chapter is relatively linear in comparison. The large number of observations per individual (average 66 per patient) may have caused the FO method to be particularly unstable and sensitive to inaccurate starting estimates. Rerunning the FO method using the parameters calculated by the FOCE method did not reduce the bias. The value of the FOCE method in population analysis is, therefore, further highlighted.

A combined exponential and additive model provided the best fit to the data. The SD of the additive component was estimated to be very similar to the limit of quantification of the assay (0.05 $ng.ml^{-1}$, information supplied by Pfizer Ltd). As discussed in chapter 4, this result demonstrates that the combined error model is useful when concentrations are measured at or around the limit of quantification.

A larger population is required to substantiate the covariate relationships which were determined. However, model development is a step-wise process where decisions are often made on limited data, so the extrapolations made here are consistent with routine practice. The relationship between clearance and body weight may reflect changes in renal and/or hepatic function. Renal impairment has been shown to greatly increase the exposure and compromise the safety profile of sotalol, a drug which is structurally similar to dofetilide (Dancey et al. 1997). Although there were no comparative estimates of CL in the literature, the terminal half-life for this study (9.0hrs) was within the range of 7.1 to 9.7hr^{-1} previously estimated) (Gemmill et al. 1991; Sedgwick et al. 1991; Tham et al. 1993; Le Coz et al. 1995).

The Vss estimate (185L) was less than that previously reported (228 to 267L) (Gemmill et al. 1991; Sedgwick et al. 1991; Tham et al. 1993; Le Coz et al. 1995). Since the average body weight in this study (77kg) was consistent with the other studies (68 to 74 kg), the final covariate model cannot explain the lower Vss.

The hydrophilic nature of dofetilide means that it does not readily distribute and accumulate into adipose tissue. It would therefore be expected that lean body weight would correlate better with Vss, but the covariate analysis demonstrated that actual body weight was the most influential covariate. It is possible that the calculation of lean body weight using height alone was too empirical, and that that an additional adjustment for body frame size (which was not available for this study) may have improved the estimates (Robinson et al. 1983).

The relationship between body weight and Vss was shown to be entirely due to an increase in the peripheral compartment volume. Since plasma volume has been shown to be related to body weight (Snyder et al. 1975) it may be expected that V_1 would also correlate with body weight. However, there may not be a direct relationship between V_1 and plasma volume, since V_1 may include distribution into the extravascular space.

7.9.2 PK/PD and baseline response

The demonstrated delay between the end of the infusion peak dofetilide concentration and the maximum QTc was consistent with previous studies investigating the pharmacokinetics and pharmacodynamics of intravenous dofetilide (Gemmill et al. 1991; Sedgwick et al. 1991; Rasmussen et al. 1992; Le Coz et al. 1995). The Tmax for the peak QTc prolongation was shown to be independent of dose, so the temporal delay was unlikely to be the result of an indirect effect (Dayneka et al. 1993; Jusko & Hui, 1994). Le Coz et al. have previously estimated the temporal displacement of dofetilide using an effect compartment (Le Coz et al. 1995). Their estimate of equilibrium half-life estimate was 6-7 minutes and therefore similar to that estimated here (9 minutes).

As previously discussed, many antiarrhythmic drugs show hysteresis after IV dosing (Chapter 1.2.4), and the estimation half-lives have been shown to be remarkably similar e.g. 2 minutes for disopyramide (Whiting et al. 1980), 6 minutes for procainamide (Galeazzi et al. 1976) and 8 minutes for quinidine (Holford et al. 1981). The delay mechanism underlying the distribution into the effect compartment may therefore be similar for many antiarrhythmic drugs and related to their shared activity on ion channels. In contrast to beta-blockers, which combine extracellularly with adrenergic receptors, ion channel blocking drugs exert their effects within the lipid membrane (Herbette et al. 1988) or on the intracellular face of the cardiac ion channel (Hondeghem & Katzung, 1977). Localised distribution has been postulated as an explanation of the differences in the time

course of the various antiarrhythmic effects of amiodarone (Roden, 1993). While the adrenergic effects are manifested soon after initiation of therapy (Mitchell et al. 1989; Kadish et al. 1990), slower diffusion to the potassium ion channel means that prolongation of the refractory period takes longer to develop (Ikeda et al. 1984; Mitchell et al. 1989). It is likely that a similar process governed the delay in the QTc prolongation following the IV administration of dofetilide.

The importance of taking placebo response into account in assessing the dose response relationship (Dobrilla & Scarpignato, 1994) and its particular importance to the assessment of cardiovascular drugs (Bienenfield et al. 1996) has been well documented. Diurnal variation in QT as a consequence of both temporal changes in sympathovagal tone and circulating catecholamines is also well recognised (Bexton et al. 1986; Murakawa et al. 1992; Hohnloser & Klingenheben, 1994). As previously discussed, the corrected QT interval (QTc) was used to account for heart rate changes during and between study days (see section 7.15). However, evidence of diurnal variation in QTc has also been demonstrated (Vervaet & Amery, 1993). The point for point subtraction of the smoothed placebo QTc from QTc intervals recorded during treatment with active drug was used to remove both placebo effects and any diurnal effects remaining after correction for heart rate.

The baseline QTc measurement was larger than that estimated by Le Coz et.al. (1995) (418 vs 365msecs, respectively). Since resting QTc intervals have been shown to increase with age (Goldberg et al. 1991; Reardon & Malik, 1996), the age difference between the two study groups (57.2 vs 23.4 years, respectively) may account for the difference in baseline QTc. Age related changes in the sympathovagal tone and circulating catecholamine levels may underlie this effect.

Although calculating QTc prolongation as change from baseline has been used to compare the effects of different doses of dofetilide (Sedgwick et al. 1991; Rasmussen et al. 1992

Tham et al. 1993), an adequate fit to the baseline corrected data could not be attained in this analysis. Holford and Sheiner, have previously suggested that when the baseline measurement is known with the same degree of certainty as other measurements it should be estimated as a parameter to prevent model mis-specification (Holford & Sheiner, 1982a, b). Correspondingly, the intraindividual variability was reduced by greater than 85% when the placebo corrected baseline was modelled as a parameter, rather than being used to correct the post dose observations.

In contrast to this analysis, Le Coz et.al (1995) determined that an Emax model was more appropriate than a linear model for the majority of subjects (for 8/10 subjects). The Emax and Ce_{50} estimates (121msecs, 57% CV and 2.2 $ng.ml^{-1}$, 26% CV, respectively) were less than those estimated when the Emax model was used in this analysis (193 , 38 %CV and 6.35 , 65%CV, respectively). Furthermore, the gradient at the Ce_{50} (Emax/ Ce_{50}) was 55msecs/ $ng.ml^{-1}$ in comparison to 35msecs/ $ng.ml^{-1}$ estimated in this analysis indicates that the healthy volunteers in Le Coz et al. study were more sensitive to dofetilide. Since healthy volunteers were used in both studies, the age difference between the two groups (23.4 vs 57.2 years, respectively) may account for the difference. While the effect of the ageing process on pharmacokinetics of antiarrhythmics has been well studied, less is known about age related changes in the pharmacodynamics (Storein, 1984; Nestico & Morganroth, 1986; Podrid et al. 1989; Hayakawa & Ino, 1994; Kim et al. 1994). Since changes in the pharmacokinetics confound the interpretation of changes in the pharmacodynamics (De Caprio et al. 1995) both need to be studied together .

It has been shown that the sensitivity of the potassium channel to class III agents decreases in patients with heart disease (Wit & Coromilas, 1993). Physiological differences between the aged and healthy heart have been shown to parallel the progression of heart disease (Yamaguchi & Ito, 1988; Assey, 1993). So it is possible that the difference in sensitivity to

dofetilide is due to the correlation between age and functional changes in the potassium ion channels, which occur in the absence of obvious organic disease.

Whether or not this is true, the older population did achieve QTc prolongations greater than the maximum predicted by Le Coz et.al. A recent analysis has shown that data up to 95 % of Emax is needed to obtain adequate precision (Dutta et al. 1996). Therefore, due to the safety implications of underestimating the QTc prolongation, and therefore the associated risk of TdP, it would appear to be more prudent to use a linear pharmacodynamic model in the prediction of QTc intervals following alternative dosage regimens. Furthermore, while receptor theory implies that a finite maximum could be observed, the therapeutic range for dofetilide is likely to be in the linear portion of the concentration response relationship.

7.9.3 Predictions and simulations

Comparison of a dose per kilogram regimen and a fixed dose regimen for termination of arrhythmia via IV infusion

Given that on average only 7 % of the total dose would be eliminated during the initial period of distribution, V_1 would have little effect on the time to steady state and the concentration measured during the later stages of a steady state infusion (Rowland & Tozer, 1989b). However, short intravenous infusions are generally used for the abolition of arrhythmias, so explaining why the variability in V_1 is important in the selection of a future dosing strategy. Since V_1 was found not to be related to body weight, the only fixed factors affecting the rise in concentration during the distribution phase were the rate of infusion and the administered dose. The relationship between Vss and body weight only begins to affect the rising concentration towards the end of the distribution phase (0.16 hrs i.e. 4 x $T1/2\alpha$). Therefore, the shorter the infusion, the more the peak concentration would be influenced by V_1, and the longer the infusion the more the concentration would be influenced by Vss. Therefore, the relationship between body weight and Vss has more influence on the longer infusions.

The interplay between covariate relationships, duration of infusion and dosing regimen was examined using predictions based on the average PK/PD model and simulations using the full PK/PD model. Even with a much larger slope for the relationship between weight and Vss, the range of peak concentrations (predicted using the average PK\PD model) for the per kilogram regimen (following a 30 minute infusion), was still twice that for the fixed dose regimen. A fixed dosage regimen was shown to provide less variable Cmax and Peak QTc measurements for infusions of up to at least 45 minutes of duration.

Slower infusion rates resulted in smaller maximal changes in QTc, and a lower risk of TdP (Table 7.10). While the therapeutic response of other antiarrhythmics has not previously been shown to relate to the level of QTc prolongation, it may be possible to determine this for dofetilide since it has only one mechanism of action. Successful termination of an arrhythmia may be related to the peak QTc or the percentage change from baseline. However, the rate of change of QTc may also be important for quick and effective termination. An optimal infusion time and dose for abolishing arrhythmias without inducing TdP may therefore exist.

The remaining interindividual and intraindividual variabilities were large in comparison to that explained by the covariate model. So inclusion of these estimates along with an expectation of the body weight distribution provides a more realistic comparison of the dosing regimens. Although the difference between the two regimens was less than would be predicted using the average PK/PD model, the full simulation approach confirmed that adopting a fixed dose strategy for short infusions would reduce the variability in Cmax and QTc prolongation. The reason for this not translating into an overall reduction in the risk of TdP was found to be due to the differences in the resultant distributions of QTc (Figure 7.20). The initial distributions for the PK/PD parameters (assumed to be log-normal) and the weight distribution (assumed to be normal) could therefore affect the comparison.

The sensitivity of the >25% change from baseline criterion compared to the > 560msecs criterion was entirely dependent on the baseline QTc. If the baseline was greater than 425msecs then the absolute QTc criterion would be evoked before the percentage change for baseline was exceeded by 25%. The absolute prolongation has been most often correlated with an increased risk of TdP (Neuvonen et al. 1979; Belton et al. 1982; Kuck et al. 1984), however, utilisation of both criteria provides added protection to the patient in clinical practice (Stratmann & Kennedy, 1987).

An important difference between the regimens is in who they place at risk The percentage at risk of TdP would be particularly high for heavy patients dosed according to the per kilogram regimen. Conversely, the fixed dosage regimen would associate a slightly higher risk of TdP with lighter patients. In deciding on the most appropriate regimen, consideration should be given to potential correlation's between body weight and other risk factors for TdP i.e. hypokalaemia and females. Furthermore the lighter patients may tend to be frailer and consequently less likely to recover from an episode of TdP.

Steady state simulation

The steady state simulation indicated that steady state concentrations (Css) above 4 $ng.ml^{-1}$ ($mcg.l^{-1}$) would be (based on the >25% change from baseline criterion) associated with a sharp increase in the percentage of patients at high risk of developing TdP. Oral dosage regimens for the prevention of arrhythmias could be designed with this level as a maximum for the target concentration range. However, it has been assumed that tolerance does not develop, in contrast Schwartz et al. (1989) have previously shown that the sensitivity of the myocardium to verapamil, measured by QTc prolongation, decreases upon multiple dosing. Thus further studies are required to assess the effect of duration of therapy on sensitivity of the myocardium to dofetilide.

7.10 Conclusions

There was no significant difference in pharmacokinetics and pharmacodynamics of dofetilide between patients with IHD and age matched healthy volunteers.

The population approach using NONMEM successfully identified both individual and population PK and PD responses, while providing a basic population model which could be used in the prospective assessment of the factors which affect dofetilide's QTc /safety profile. The FOCE method with "interaction" gave the least biased estimates for the parameters of the pharmacokinetic model. A two compartment model best described the pharmacokinetics and an effect compartment was used to account for the displacement between concentration and effect. A linear model best described the subsequent relationship between effect compartment concentration and QTc prolongation. The inclusion of baseline placebo QTc as a calculable parameter in the pharmacodynamic model substantially reduced intraindividual variability.

Simulations were used to identify steady state concentrations which increased the percentage of subjects with QTc prolongations above those associated with a high risk of TdP. The effect of dosage regimen and duration of infusion on Cmax, peak QTc and risk of TdP was also investigated via prediction and simulation. While dosing on the basis of per kilogram body weight is routinely used in therapeutics, this analysis would suggest that for a short infusion, the range and variability in Cmax and QTc measurements would be narrower following a fixed dose regimen. The importance of V_1 and body weight to this conclusion was highlighted.

The fact that lighter patients (who may also be frail and elderly) are most likely to be placed at risk with the fixed dose regimen may favour the dose per kilogram regimen. Nevertheless, the analysis indicates that caution is required when dosing heavy patients on a per kilogram basis, such that a maximum total dose should be imposed in subsequent

studies. Further investigation of the variability in the PK/PD relationship is required to identify other factors which can aid in the predictions of concentrations and QTc measurements and therefore allow optimisation of the dofetilide dosage used in the treatment and prevention of cardiac arrhythmias.

CHAPTER 8

GENERAL CONCLUSIONS

The analyses discussed in thesis have raised some general points to consider when applying nonlinear mixed effect modelling in the early phases of drug development. These are summarised in this chapter.

NONMEM Estimation Methods

In general the NONMEM software performed well. The FOCE methods substantially out performed the FO method in the determination of the most appropriate PK/PD model (Chapters 4 & 7) and of point and interval estimates for a bioequivalence test (Chapter 5). The increase in computation time was most troublesome when the problem required a complex nonlinear model and a large amount of data was available for each subject (Chapter 4). Unfortunately this is exactly the situation where the FOCE methods are most likely to be advantageous. However, it may be possible to substantially reduce the extraordinary run times by utilising user defined code (Chapter 4).

Comparison to Standard Approaches of Estimating Pharmacokinetic Parameters

Noncompartmental analysis was successfully used to determine the primary pharmacokinetic and pharmacodynamic parameters of interest and allowed drug accumulation (Chapter 4), bioequivalence (Chapter 5), dose response (Chapter 6) and PK/PD relationships (Chapter 7) to be investigated by standard methods. However, model based approaches were required in the prediction of steady state concentrations (Chapter 4), and in testing bioequivalence where only a few random plasma samples per individual were available (Chapter 5). Furthermore, a fuller understanding of the relationships underlying the dose response could be ascertained by adopting the model based approaches. In particular, a greater insight into potential covariate effects and a break down of the variability into either the PK/PD component of dose response relationship was possible (Chapter 6 &7). Together these provided a framework with which to predict and simulate responses to different dosage regimens in larger populations (Chapter 4, 6 &7).

Identification of Structural and Covariate Models

Although identification of the "best" structural model is known to be problematic when the data is sparse (Chapter 5 & 6), the average model can also be difficult to select when the data is rich (Chapter 4 & 7). While characterisation of individual plasma time profiles allows identification of a range of different structural models (Chapter 4), the fit of an individuals data to competing models can be explored using the individual estimates obtained from the population approach (Chapter 7). Furthermore, it is likely that an apparent qualitative change in the structural model between subjects is more likely to be an artefact of the encountered variability and, therefore, more readily dealt with by adopting a population approach.

Due to the small numbers of subjects, it was known from the outset that it would be difficult to accurately determine the underlying covariate relationships. Nevertheless, the identified covariate relationships allowed several hypotheses to be generated (Chapters 6 & 7). While these have to be confirmed in larger Phase II or Phase III studies, the approach is consistent with the concept of Phase I/II studies as the learning and theory testing stage of drug development. One way of increasing the confidence in the identified covariate effect is to test assumptions underlying the relationships. Sensitivity analysis was found to be useful in assessing the potential impact if the covariate effect be over or underestimated (Chapter 7). The subsequent clinical importance of these relationships was best ascertained through simulation (Chapter 6).

Study Design

Although the approach of using mixed effects modelling to interpret data from studies where the design has been compromised should be treated with caution, it was found to have utility in the example presented in this analysis (Chapter 7). On the other hand, a model based approach can also be jeopardised by studies designed without its application in mind (Chapter 6).

Predictions/Simulations

Prediction and simulation techniques are core to developing the understanding gained by applying a model based approach to a data problem. While a prediction using individual or typical parameter estimates can be useful in exploring the relationships uncovered in a particular analysis (Chapter 7), undertaking a formal simulation is recommended since it formally accounts for both fixed and random effects (Chapter 4, 6 and 7). It should, however, be remember that the simulation will only allow investigation of factors included in the model.

Model Assumptions

While simulation allows you to test for the affect of modelling assumptions on potential clinical outcomes (Chapter 6 and 7), the assumptions should first be tested during the modelling process. For example, error structure was found to change upon changing from modelled baseline to percentage reduction model (Chapter 6). Some assumptions may limit the applicability of the developed modelling techniques. For example, the Wagner Nelson approximation used to directly estimate Cmax from a two compartment model, was only valid for drugs where the terminal half-life was much longer than the distribution half-life (Chapter 5).

Baseline Correction

While baseline correction can reduce the model complexity (Chapter 6) it is best to start from a model where baseline is estimated as a parameter (Chapter 7). In Chapter 7, when a baseline correction was utilised, the underlying variability in baseline measurement prevented the Emax relationship from being identified as a potential model. Modelling the baseline greatly reduced the variability and allowed the Emax relationship to provide a similar fit to the linear relationship. In Chapter 6, a baseline correction was implicit in the percentage reduction model adopted to simplify the original absolute reduction from baseline model. While this showed that baseline total cholesterol could be a covariate of both Emax and D_{50} it did not allow the determination of which relationship was most important.

Hypothesis Testing -

Determination of statistical significance is generally based on the ability to reject the Null Hypothesis in favour of a suitable alternative hypothesis. Normally the probabililty (α) of making a type I error is a preselected acceptable level and the probability of making a type II error (β) is minimised by choosing an appropriate sample size. While Phase I/II studies can be powered to minimise Type II error in the determination of mean differences, they are not generally powered for the purposes of PK/PD modelling. This is a particular problem with the covariate analyses undertaken (Chapter 6 and 7).

Population PK/PD Modelling the Alternative Paradigm for Drug Development ?

In the analyses presented, the benefits of PK/PD modelling in the early phases of drug development was highlighted. While the application of nonlinear mixed effects modelling to bioequivalence testing (Chapter 5) may only provide an advantage in a limited number of circumstances, the presented applications in the areas of drug safety (Chapter 4), dose response (Chapter 6) and integrated PK/PD problems (Chapter 7) cover much of the early development. It can therefore be speculated that wider implementation of these techniques would improve decision making and allow information to be more effectively propagated across the phases of drug development.

Appendix

Appendix 1.1

Chapter 4: The NMTRAN user supplied PRED subroutines for implementation of equations 4.2 (run 8) and 4.5 (run 11).

Chapter 4 Run8

```
$PRED
TALPH=THETA(1)
TA=THETA(2)
TBETA=THETA(3)
TB=THETA(4)
TGAM=THETA(5)
TC=THETA(6)
TKA= THETA(7)

ALPH=TALPH*EXP(ETA(1))
A=TA*EXP(ETA(2))
BETA=TBETA*EXP(ETA(3))
B=TB*EXP(ETA(4))
GAM=TGAM*EXP(ETA(5))
C=TC*EXP(ETA(6))
KA= TKA*EXP(ETA(7))
TAU=12

A1=EXP(-ALPH*T)
A2=EXP(-N*ALPH*TAU)
A3=EXP(-ALPH*TAU)

A10=A*A1*(1-A2)/(1-A3)

B1=EXP(-BETA*T)
B2=EXP(-N*BETA*TAU)
B3=EXP(-BETA*TAU)
B10=B*B1*(1-B2)/(1-B3)

C1=EXP(-GAM*T)
C2=EXP(-N*GAM*TAU)
C3=EXP(-GAM*TAU)
C10=C*C1*(1-C2)/(1-C3)

K1=EXP(-KA*T)
K2=EXP(-N*KA*TAU)
K3=EXP(-KA*TAU)
K10=-(A+B+C)*K1*(1-K2)/(1-K3)

F=A10+B10+C10+K10
IPRED=F
Y=F*EXP(EPS(2))+EPS(1)
```

Chapter 4 Run11

```
$PRED
TALPH=THETA(1)
TA=THETA(2)
TBETA=THETA(3)
TB=THETA(4)
TGAM=THETA(5)
TC=THETA(6)

K=TALPH*EXP(ETA(1))
A=TA*EXP(ETA(2))
BETA=TBETA*EXP(ETA(3))
B=TB*EXP(ETA(4))
GAM=TGAM*EXP(ETA(5))
C=TC*EXP(ETA(6))

TAU=12

A1=EXP(-K*T)
A2=EXP(-N*K*TAU)
A3=EXP(-K*TAU)
A4=A*T*A1
A5=A*TAU*A1
A6=A4*(1-A2)/(1-A3)
A7=1/(1-A3)
A8=(A3-A2)*A7
A9=A5*A7*(A8-N*A2+A2)
A10=A6+A9

B1=EXP(-BETA*T)
B2=EXP(-N*BETA*TAU)
B3=EXP(-BETA*TAU)
B10=B*B1*(1-B2)/(1-B3)

C1=EXP(-GAM*T)
C2=EXP(-N*GAM*TAU)
C3=EXP(-GAM*TAU)
C10=C*C1*(1-C2)/(1-C3)

K10=-(A+B+C)*A1*(1-A2)/(1-A3)

F=A10+B10+C10+K10
IPRED=F
Y=F*EXP(EPS(2))+EPS(1)
```

Appendix 1.2

Chapter 5: The NMTRAN $PK subroutine for a two compartment model with the relative differences θ_{RD} in $\frac{F}{V_1}$ and Ka estimated using Equations 5.10 and 5.11, respectively (Run12); and the user supplied NMTRAN PRED subroutine parameterising the model in terms of CAmax using E.q. 5.23, 5.24, 5.26, 5.11 and 5.18 (run 13).

Chapter 5 Run 12

```
$SUBROUTINE ADVAN4 TRANS5
$PK
I=0
IF(FORM.EQ.2) I=1

FS2=(1-I)+I*(THETA(6)+1)
FKA=(1-I)+I*(THETA(7)+1)

TVBETA=THETA(1)
TVVS2=1/(THETA(2)*FS2)
TVKA=THETA(3)*FKA
TVAOB=THETA(4)
TALPHA=THETA(5)

BETA=TVBETA*EXP(ETA(1))
S2=TVVS2*EXP(ETA(2))
KA=TVKA*EXP(ETA(3))
ALPHA=TALPHA*EXP(ETA(4))

AOB=TVAOB
$ERROR
Y=F*EXP(ERR(1))
```

Chapter 5 Run 13

```
$PRED
IND=0
IF(FORM.EQ.2) IND=1
DR=1
IF(STD.EQ.1) DR=2
TIM=TIME*(1-IND)+(TIME-100)*IND

TALPHA=THETA(1)
CMNAT=THETA(2)
KAN=THETA(3)
TAOB=THETA(4)
TBETA=THETA(5)
KCMAX=THETA(6)
KKA=THETA(7)

ALPHA=TALPHA*EXP(ETA(1))
TCMAX=CMNAT*(1-
IND)+CMNAT*(1+KCMAX)*IND
CMAX=(TCMAX/DR)*EXP(ETA(2))
TKA=(1-IND)*KAN+IND*KAN*(1+KKA)
KA=TKA*EXP(ETA(3))
AOB=TAOB
BETA=TBETA*EXP(ETA(4))
K21=(AOB*BETA+ALPHA)/(AOB+1)
R1=(ALPHA-K21)/(KA-K21)
IF (R1.LE.0) EXIT 1 1
TPK=-1/(KA-ALPHA)*DLOG(R1)

A1=(KA-ALPHA)*(BETA-ALPHA)
A=(K21-ALPHA)/A1
B1=(KA-BETA)*(ALPHA-BETA)
B=(K21-BETA)/B1

AA1=EXP(-ALPHA*TPK)
AA2=EXP(-ALPHA*TIM)
BB1=EXP(-BETA*TPK)
BB2=EXP(-BETA*TIM)
KA1=EXP(-KA*TPK)
KA2=EXP(-KA*TIM)

IZ=A*AA1+B*BB1-(A+B)*KA1
JZ=CMAX/IZ
MZ=A*AA2+B*BB2-(A+B)*KA2
FUN=JZ*MZ
Y=FUN*EXP(ERR(1))
```

Chapter 5: The NMTRAN $PK subroutine for a two compartment model with the relative differences θ_{lnRD} (multiplicative model) in $\frac{F}{V_1}$ estimated using equations 5.11 and 5.28, respectively (run14); and the user supplied NMTRAN PRED subroutine parameterising the model in terms of CAmax using E.q. 5.23, 5.24, 5.26, 5.11 and 5.29 (run 15).

Chapter 5 Run 14

```
$SUBROUTINE ADVAN4 TRANS5
$PK
I=0
IF(FORM.EQ.2) I=1
FS2=I*(THETA(6))
FKA=(1-I)+I*(THETA(7)+1)
TVBETA=THETA(1)
TVVS2=(THETA(2)+FS2)
TVKA=THETA(3)*FKA
TVAOB=THETA(4)
TALPHA=THETA(5)

TVS2=EXP(TVVS2)
TS2=1/TVS2
BETA=TVBETA*EXP(ETA(1))
S2=TS2*EXP(ETA(2))
KA=TVKA*EXP(ETA(3))
ALPHA=TALPHA*EXP(ETA(4))
AOB=TVAOB
$ERROR
Y=F*EXP(ERR(1))
```

Chapter 5 Run 15

```
$PRED
IND=0
IF(FORM.EQ.2) IND=1
DR=1
IF(STD.EQ.1) DR=2
TIM=TIME*(1-IND)+(TIME-100)*IND

TALPHA=THETA(1)
CMNAT=THETA(2)
KAN=THETA(3)
TAOB=THETA(4)
TBETA=THETA(5)
KCMAX=THETA(6)
KKA=THETA(7)

ALPHA=TALPHA*EXP(ETA(1))

TCMAX=CMNAT+(KCMAX*IND)
TDMAX=EXP(TCMAX/DR)
CMAX=(TDMAX)*EXP(ETA(2))

TKA=(1-IND)*KAN+IND*KAN*(1+KKA)
KA=TKA*EXP(ETA(3))

AOB=TAOB
BETA=TBETA*EXP(ETA(4))
K21=(AOB*BETA+ALPHA)/(AOB+1)
R1=(ALPHA-K21)/(KA-K21)
IF (R1.LE.0) EXIT 1 1
TPK=-1/(KA-ALPHA)*DLOG(R1)
A1=(KA-ALPHA)*(BETA-ALPHA)
A=(K21-ALPHA)/A1
B1=(KA-BETA)*(ALPHA-BETA)
B=(K21-BETA)/B1
AA1=EXP(-ALPHA*TPK)
AA2=EXP(-ALPHA*TIM)
BB1=EXP(-BETA*TPK)
BB2=EXP(-BETA*TIM)
KA1=EXP(-KA*TPK)
KA2=EXP(-KA*TIM)
IZ=A*AA1+B*BB1-(A+B)*KA1
JZ=CMAX/IZ
MZ=A*AA2+B*BB2-(A+B)*KA2
FUN=JZ*MZ
Y=FUN*EXP(ERR(1))
```

Appendix 1.3

Chapter 6: The NMRAN user supplied PRED subroutines for implementation equations 6.1 to 6.5 i.e. the Placebo, Step, Steplinear, Emax and Emax percentage reduction models

Chapter 6 Step model

```
$PRED
IND=0
IF(DOSE.GT.0) IND=1
RED= THETA(2)*EXP(ETA(2))
PLAC=THETA(1)*EXP(ETA(1))
LR=PLAC-RED*(IND)
Y=LR*EXP(ERR(1))
```

Chapter 6 Steplinear model

```
$PRED
IND=0
IF(DOSE.GT.0) IND=1
RED1=THETA(2)+THETA(3)*DOSE
RED=RED1*EXP(ETA(2))
PLAC=THETA(1)*EXP(ETA(1))
LR=PLAC-RED*(IND)
Y=LR*EXP(ERR(1))
```

Chapter 6 Emax model

```
$PRED
B=THETA(3)+DOSE
A=THETA(2)*DOSE
RED=(A/B)*EXP(ETA(2))
PLAC=THETA(1)*EXP(ETA(1))
LR=PLAC-RED
Y=LR*EXP(ERR(1))
```

Chapter 6 Emax Percentage Reduction model

```
$PRED
B=THETA(2)+DOSE
A=THETA(1)*DOSE
RED=(A/B) *EXP(ETA(1))
LR=100-RED
Y=LR*EXP(ERR(1))
```

Appendix 1.4

Chapter 7: The NMTRAN $PK subroutine for a two compartment PK model with an effect compartment and either a linear or Emax PD model

Chapter 7 Linear PD model

```
$SUBROUTINE ADVAN5 TRANS1
$MODEL
COMP=(CENTRAL,DEFDOSE)
COMP=(PKCOMP)
COMP=(PDCOMP,DEFOBS)$PK

$PK
K10=VK1
K12=VK12
K21=VK21
S1=VS1
K13= 0.01*K10
K30= THETA(1)*EXP(ETA(1))
LINE=THETA(2)*EXP(ETA(2))
BASE=THETA(3)*EXP(ETA(3))
S3=S1*K13/K30


$ERROR
EFFC=F
IPRE=BASE+(LINE*F)
Y=IPRE+ERR(1)
```

Chapter 7 Emax PD model

```
$SUBROUTINE ADVAN5 TRANS1
$MODEL
COMP=(CENTRAL,DEFDOSE)
COMP=(PKCOMP)
COMP=(PDCOMP,DEFOBS)

$PK
K10=VK1
K12=VK12
K21=VK21
S1=VS1
K13= 0.01*K10
K30= THETA(1)*EXP(ETA(1))
EMAX=THETA(2)*EXP(ETA(2))
D50=THETA(3)*EXP(ETA(3))
BASE=THETA(4)*EXP(ETA(4))
S3=S1*K13/K30

$ERROR
EFFC=F
IPRE=BASE+(EMAX*F)/(D50+F)
Y=IPRE+ERR(1)
```

Glossary

Definitions: population and Statistical modelling theory terms and symbols

Symbol	Definition
ε_{ij}	Intraindividual random effect, accounts for the error between the model prediction for the jth observation for the ith individual and the actual observation
ε_i	the ith individual's vectors of ε values
η_{ki}	Interindividual random effects; accounts for the error between P_k and P_{ik}
η_i	the ith individual's vectors of η values
$v\sigma^2$	Variance of ε 's, related to predicted value by function
$\upsilon\omega_\kappa^2$	Variance of η_k 's, related to predicted value by function υ
Θ , θ_k	Vector of population mean parameter estimates; Kth parameter from the vector of population mean parameter estimates √
θ_{RD}	Relative difference between two means using an additive model
$\theta_{\ln RA}$	Ratio of two means using a multiplicative model
σ	v =1 (ζ=0) standard deviation (in observation units)
	$v = \hat{y}_{ij}^2$ (ζ=2) Coefficient of Variation (%)
ω_κ	For an additive model the standard deviation (in observation units) For exponential or proportional the coefficient of variation (%)
Ω	Variance - Covariance Matrix of interindividual random effects η
ζ	Fixed effect for the power function intraindividual variability model
χ^2	Chi squared test
Σ	Mathematical symbol to represent sum of
O_{els} OFV Obj. Fun.	Objective function value (-2 ln likelihood as estimated by NONMEM or -2LL)
$f()$	A general function of all arguments listed which includes a structural model that relates the independent variables
$g_k()$	A general function which relates P_k to Z_i through θ_k
$S(\quad)$	A general function incorporating interindividual and intraindividual submodels
P_i	ith Individuals vector of model parameters
P_{ik}	kth parameter from the ith Individuals vector of model parameters
P_k	the population average or typical parameter of the structural model
S	Variance - Covariance Matrix of intraindividual random effects (ε)
X_{ij}	All the independent variable information including the time of observations and dosage history
y_{ij}	The jth observation for the ith individual
$\hat{y}_{ij}$	The jth prediction for the ith individual
Z_i	Represents the vector of covariates for the ith individual

Symbol	Definition
ADD	Additive component of intraindividual error model
AIC	Akaike Information Criterion
%CV	Coefficient of variation
DV	Dependent variable
EPS	ε_{ij} in NONMEM syntax
ELS	Extended least squares
EXP	Exponential component of intraindividual error model
F	$\hat{y}_{ij}$ in NONMEM syntax
FO	First order estimation
FOCE	First order conditional estimation
GAM	General additive model
GOF	Goodness of fit plots
Interaction Method	FOCE method where Σ is based on the conditional estimates of $\hat{\eta}_i$
No Interaction Method	FOCE method where Σ is estimated based on the mean parameter model.
IIV	Interindividual variability
IOV	Interoccasion variability
IPRED	Individual predictions
IRES	Individual Weighted Residuals
IWRES	Individual Weighted Residuals
Likelihood ratio test (LRT)	The difference is approximately χ^2 distribution with degrees of freedom (df) equal to the difference in the number of free parameters. When one parameter is fixed in the reduced model a decrease in objective function value 3.84 is significant at $p<0.05$.
Posthoc	Individual posterior Bayes parameter estimates obtained after the ELS problem has been minimised v
PRED	Population predictions
PROP	Proportional component
RES	Residuals
SD	Standard Deviation
SE	Absolute Standard error
SE (%)	Absolute Standard error /population estimate *100
STS	Standard Two Stage Approach
WRES	Weighted Residuals
Y	y_{ij} in NONMEM syntax

Definitions : PK\PD Terms and Symbols

Symbol	Definition
$\alpha, \beta, \gamma, \delta,)$	Initial, intermediate and elimination rate constant (depending on number of compartments)
τ	The dosing interval
A_1	Amount of drug in the Central compartment
A_e	Amount of drug in the effect compartment
A_T	Total amount of drug in the central compartment at time T
A,B,C,D	coefficient constants
AUC	Area under the concentration time curve
$AUC_{0-\infty (inf)}$	Area under the concentration time curve between 0 and infinity
$AUC_{0-\tau}$	Area under the concentration time curve between 0 and τ
Ce	Concentration at the site of drug action (in the effect compartment)
Ce_{ss}	Concentration at the site of drug action at steady state (in the effect compartment)
Ce_{50}	Concentration at the site of drug action which gives 50% of the maximum response
C, Cp,(Ct)	Plasma concentration (Plasma concentration as function of time)
C_{ss}	Plasma concentration at steady state
CI	Confidence Interval
CL	Clearance
CL/F	Apparent clearance (oral plasma clearance)
Cmax	Maximum concentration
$Cmax^D$	the estimate of Cmax derived from the modelled parameters
CAmax	Maximum concentration at TApk
$Cmax^E$	Estimate of Cmax obtained directly from the model
$CAmax^E$	Estimate of CAmax obtained directly from the model
D_{50}	Dose which gives 50% of the maximum response
Emax	The maximum response
F	Bioavailability
Hysteresis	The temporal displacement between concentration and effect in a counterclockwise direction
k	Combined absorption and distribution rate constant
Ka	First Order rate constant for absorption
Ko	Zero order constant for absorption
Ke/K_{10}	Elimination rate constant
K_{12},K_{21}	inter-compartmental rate constants
K_{1e}	Rate constant governing the transfer to the effect compartment
Keo	Rate constant representing the loss from the effect compartment and accounting for the hysteresis
LR	Lipid response
MRT	Mean residence time
N	Number of doses given
PD	Pharmacodynamics
PK	Pharmacokinetics
Proteresis	The temporal displacement between concentration and effect in a clockwise direction
Q	Intercompartmental clearance
V	Volume of distribution

Symbol	Definition
t	time
T1/2 (half-life)	Terminal half-life
Tlag	Lag time for absorption
Tmax/Tpk	Time of maximal plasma concentration
TApk	Time at which the accumulative amount absorbed / V1 equals a maximum. IT approximates to Tpk when Ka>α >β
V_1	Volume of distribution of the central compartment
Ve	Volume of distribution of the effect compartment
Vss	Volume of distribution at steady state

Other Abbreviations

Symbol	Definition
AGE	Age of patient (years)
CLcr	Creatinine Clearance ($ml.min^{-1}$) estimated from the Cockcroft Gault relationship*
FDA	Food and Drug Administration
HDL	High density Lipoproteins ($mmol.L^{-1}$)
HMG COA	3-hydroxy-3-methylglutaryl Coenzyme A inhibitor
HR	Heart Rate
LDL	Low density Lipoproteins ($mmol.L^{-1}$)
LOQ	Limit of Quantification
QT	The ventricular refractory period i.e. the time between the depolarisation and repolarisation of the ventricular myocardium, It is measured from a standard electrocardiogram (ECG) in msecs
QTc	QT corrected for heart rate
RR	RR interval from the electrocardiogram
RBS	Ranitidine bismuth subcitrate
SCRT	Serum creatinine concentration ($mg.dl^{-1}$)
SD	Standard deviation
SEX	Gender (males 0, females 1)
TdP	Torsade de Pointes
WT	Body weight of patient (kg)
VLDL	Very Low density Lipoproteins ($mmol.L^{-1}$)
VT	Ventricular Tachycardia
VF	Ventricular Fibrillation

*Cockcroft Gault relationship:

$$CLcr = \frac{(140 - Age) \times Weight}{\text{serum creatinine}} \times [1.23(male); or 1.04(female)]$$

Publications and Presentations

Presentations

A population approach to dose vs response relationship for simvastatin in hypertensive hypercholesteroleamic patients
S.F. Marshall, H.L.Elliott, P.A. Meredith
British Pharmacological Society
London 5-7th January 1994

A population approach to dose versus response relationship for simvastatin in hypertensive hypercholesterolaemic patients
S.F. Marshall, H.L.Elliott, P.A. Meredith
Population Approach Group Europe
London 13-14th June 1994

Prediction of simvastatin pharmacological response: a population analysis
S.F. Marshall, H.L.Elliott, P.A. Meredith
Population Approach Group Europe
Frankfurt 9-10th June 1995

Application of mixed effects modelling to bioequivalence testing
S.F. Marshall, P.A. Meredith
NONMEM intermediate workshop
Uppsala 12-13th October 1995

Population pharmacokinetics and Pharmacodynamics of a novel anti-arrhythmic drug in healthy volunteers and ischaemic heart disease patients
S.F. Marshall, H.L.Elliott, P.A. Meredith
PK UK
Nottingham, 1-3rd November 1995

Publications

Marshall SF, Elliott HL, Meredith PA. A population approach to dose vs response relationship for simvastatin in hypertensive hypercholesteroleamic patients. 1994 Br J Clin. Pharmacol Vol 37 p494.

Marshall SF, Meredith PA, Elliott HL. Efficacy of low-Density-lipoprotein lowering with Statins. 1994 Lancet Vol 344 p683-684.

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