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Evaluation of the Effect of Vitamin D Pharmacokinetics on Bone Health Using a Multi-Scale Systems Pharmacology Model (MSPM)

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Alanna S. Ocampo-Pelland, PhD
University of Connecticut, 2016

Objectives: Relationships between Vitamin D dose, 25OHD, and indicators of bone health (serum calcitriol, parathyroid hormone (PTH), and bone mineral density at the lumbar spine (BMDLS)) were explored by considering the combined effect of Vitamin D3 and calcium administration (D3CA) on bone-health endpoints.
Methods: Population PK models were developed to predict D and 25OHD, and the final model was integrated with a previously published MSPM [1], describing calcium, PTH, and bone-remodeling. Public-source D and 25OHD PK data in healthy or osteoporotic populations, including 74 studies, representing 5684 individuals, were selected using PUBMED. Nonlinear-mixed-effects models were developed simultaneously for D and 25OHD PK (NONMEM v7.2). Model development explored 1- and 2-compartment models with linear (CL) or non-linear (CLNL) clearance. Using R's *mrgsolve* package [2], a scaling factor for calcitriol production and exponent-gamma term, related to osteoclast bone resorption, were estimated, translating predicted changes in bone-markers to changes in BMDLS (*Nelder-Mead* method in *stats* R package). Population-level simulations evaluated changes in BMDLS/PTH, following various 1-year regimens of D3CA administration and quantified the relationship between BMDLS/PTH and 25OHD concentration.

Results: D2/D3 parent and metabolite were described by 2-compartment models with numerous shared estimates. D3 CLNL resulted in inverse 25OHD3 relationships with dose and baseline; D2 did not exhibit nonlinearity. A power-model structure described the 25OHD-calcitriol conversion ($\theta 1 = 638.1$, $\gamma = 0.038$), reflecting the apparent self-inhibition of calcitriol on its own production. Consistent with known biology, results from population-level simulations indicated that, for fixed D3 doses, BMDLS/PTH increased/decreased with increasing calcium administration (BMDLS PCFB range: 0.1 to 1.7%; PTH PCFB range: -4 to -30% for D3 doses 400-5000 IU/d, calcium doses 0-300 mg/d for 1 year). Also, BMDLS/PTH changed by +2%/-52.7% for 25OHD concentrations 43-102 nmol/L.

Conclusions: This is the first model that simultaneously integrates D3/D2 data, describing the system kinetics as a single unit and the first to quantitatively evaluate D3CA administration effect on bone-health endpoints. Future utility of results includes comparing effects of D3 or D3CA administration during clinical trials of other anti-osteoporosis therapeutics and quantifying D3 effects in other disease states like renal osteodystrophy.

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Doctor of Philosophy Dissertation

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Acronyms

24OHD 24-hydroxylated Vitamin D.

24OHD2 24-hydroxylated Vitamin D2.

25OHD total 25-hydroxylated Vitamin D.

25OHD2 25-hydroxylated Vitamin D2.

25OHD3 25-hydroxylated Vitamin D3.

BL baseline.

BMD bone-mineral density.

BMDLS bone-mineral density at the lumbar spine.

BMI body mass index.

BSAP bone-specific alkaline phosphatase.

CFB change from baseline.

CHEMI chemiluminescence-based assays.

CI confidence interval.

CKD-MBD chronic kidney disease mineral bone disorder.

CLNL non-linear clearance.

CPBA competitive protein binding assays.

CTX C-terminal telopeptide.

CV coefficient of variation.

CWRESI individual conditional weighted residuals.

D total Vitamin D.

D2 Vitamin D2.

D3 Vitamin D3.

D3CA Vitamin D3 plus calcium supplementation.

DBP Vitamin D binding protein.

DRI Dietary Reference Intake.

EA Estimated Average.

EAR Estimated Average Requirement.

FOCE-I first-order conditional estimation with interaction.

GOF goodness of fit.

HPLC-MS high pressure liquid chromatography tandem mass spectometry.

HPTBD hyperparathyroid bone disease.

IOM Institute of Medicine.

IV intravenous.

KDIGO Kidney Disease: Improving Global Outcomes.

MBMA model-based meta-analysis.

MD multiple dose.

MSPM multi-scale systems pharmacology model.

NA-CLPBA Nichols Advantage-automated protein binding assay.

OF osteitis fibrosa.

P1NP procollagen I intact N-terminal propeptide.

PCFB percent change from baseline.

pcVPC prediction-corrected visual predictive checks.

PK pharmacokinetics.

PO oral.

PPK population pharmacokinetics.

PTH parathyroid hormone.

RDA Recommended Dietary Allowance.

RIA radioimmunoassays.

RSE relative standard error.

SA sensitivity analysis.

SD single dose.

SE standard errors.

SIGDIG significant digits.

VDR Vitamin D receptor.

Dedication

With love to my family and friends and to the One for whom "nothing is impossible" (Luke 1:37). Thank you for everything.

"Non nisi te, Domine." - St. Thomas Aquinas, patron of students

Consummatum est (John 19:30).

Chapter 1

Introduction

1.1 Objectives

1.1.1 *Overall Project Objectives*

It is well known that total Vitamin D (D) plays an important role in maintaining bone health, but what still remains elusive is identifying an appropriate dose for maintaining endpoints relevant to bone health (e.g., 25OHD, bone-mineral density (BMD)). In the past, multi-scale systems pharmacology models [3] have proven very useful in helping to predict the therapeutic response of the body to a drug. A population pharmacokinetics (PPK) model for Vitamin D and 25OHD was developed to describe the metabolism of this hormone as a tool to explore dosing ranges for Vitamin D deficient and non-deficient subjects. Specifically, this Vitamin D model was incorporated into an existing MSPM that described calcium homeostasis and bone remodeling [1], to explore the effect of Vitamin D therapy on relevant bone-health endpoints.

1.1.2 *Vitamin D and 25OHD PK Model Objectives*

Vitamin D and its metabolite, 25OHD, play an important role in maintaining bone health. This hormone has also become an active research focus in other areas [4], [5] including diabetes [6], [7], cancer [8], [9], heart disease [10], and various inflammatory diseases, such as multiple sclerosis [11]. Clinical studies investigating these relationships with D and 25OHD often vary in dosing regimen, assays, demographics, and control of endogenous D3 production. This has lead to sometimes uncertain and conflicting dose and exposure-related associations with D and 25OHD

[12], [13]. Dose-equivalency of D3 and D2 with regard to their abilities to raise 25OHD levels is also under debate. Therefore, a PPK model was developed to predict mean D3, D2, 25OHD3 and 25OHD2 exposure from varied doses and administration routes. These findings were used to investigate non-linearity in D and 25OHD kinetics, to explore the relationship between D dosage and attainment of threshold values of 25OHD over a range of clinical D doses, and to compare D3 and D2's ability to maintain 25OHD levels. Finally these findings were used to understand sources of 25OHD exposure variability related to parent-metabolite baseline, weight, and assay types, as applicable. The details of this portion of the model development and corresponding results are discussed in Chapters 3-4.

1.1.3 *MSPM Integration Objectives*

The last objective was to integrate the Vitamin D PK model with a previously-published MSPM [1] that described calcium, PTH, and bone-remodeling. In the past, the systems model has been used to evaluate the effect of therapies (e.g., teriparatide, denosumab [1], sclerostin antibodies [14]) on BMD, fracture, and bone markers, in different therapeutic areas that effect the quality of bone, including menopausal transition [15], osteoporosis [14], [16], and chronic kidney disease [17]. The model has also been used to design estrogen suppression therapies to provide pain relief and minimize bone loss in patients with endometriosis [18]. This analysis extends this MSPM to include an oral input for Vitamin D therapy so as to evaluate the effect of supplementation with or without calcium on bone-health endpoints, including calcitriol, PTH, and BMDLS.

1.2 Background

1.2.1 *Vitamin D Metabolism*

Vitamin D3 is synthesized from 7-dehydrocholesterol in the skin via its interaction with sunlight [19], [20]. A minimal amount of D3 is attributed to diet [12]. Vitamin D2 is not made endogenously but is man-made by exposing the sterol, ergosterol, to ultraviolet light. Certain lichen and fungi also naturally produce Vitamin D2 [21]. Because of the increased risk in skin cancer from exposure to UVB radiation (290-320 nm), daily D supplements, between 400 and 1000 IU, are commonly used to maintain levels of D and its metabolite. For the same reason, the majority of studies done

on D are restricted to evaluating the response due only to dietary intake, with the amount of daily sun-exposure controlled. As a reflection of the available data, this model will also focus on the response to supplemented dietary D, with the assumption of limited sunlight.

Following oral administration, Vitamin D is hydroxylated in the liver into 25(OH)D, the main marker for D deficiency, and then again in the kidney by the 1-alpha hydroxylase enzyme into calcitriol ($1,25(OH)_2D$), the active form of D, which stimulates calcium absorption from the gut and kidney [12], [22] (Figure 1.1). Circulating $1,25(OH)_2D$ has a high affinity for Vitamin D binding protein (DBP), which transports 25(OH)D into intestinal cells, where it binds to a Vitamin D receptor (VDR). This receptor-ligand complex binds to a Vitamin D responsive element on a responsive gene, such as those involved in the transcription and translation of osteocalcin, calcium-binding protein, or 24-hydroxylase [22]. Calcium-binding protein mediates the absorption of calcium from the gut via an ATP-dependent mechanism against the natural calcium gradient. When dietary calcium is insufficient, Vitamin D, along with PTH, stimulates osteoclast-mediated bone resorption and renal reabsorption of calcium from the distal tubule in the kidney [20].

Figure 1.1 also describes the feedback between physiological compartments, which together regulate calcium-bone homeostasis. Increased levels of PTH and decreasing levels of phosphate increase the manufacture of $1,25(OH)_2D$ by increasing the amount of 1-alpha hydroxylase. $1,25(OH)_2D$ has a negative feedback on PTH [23], acting as an important regulator of PTH levels. Finally, increasing levels of FGF-23 will cause a decrease in levels of $1,25(OH)_2D$ and phosphate. Imbalance between these tightly regulated compartments is what causes different bone disease states including osteoporosis, osteomalacia, and renal osteodystrophy.

1.2.2 *Vitamin D Deficiency and Related Diseases*

Vitamin D deficiency is related to several bone diseases (Table 1.1) and treatment often includes administration of some form of Vitamin D. Osteomalacia and rickets (in children) are among the more commonly occurring diseases due to Vitamin D deficiency. Nutritional rickets is most common in undeveloped countries, whereas the hereditary Vitamin D-dependent rickets is most prevalent in more developed countries. Each may be cured by the proper administration of either oral or intravenous metabolites of Vitamin D or the infusion of calcium. In the case of chronic kidney disease, Vitamin D deficiency and subsequent chronic kidney disease mineral bone disorder

(CKD-MBD), are secondary to the initial renal failure. CKD-MBD, formerly referred to as renal osteodystrophy, is a new term that was coined in 2006 by Kidney Disease: Improving Global Outcomes (KDIGO) in order to better describe the complex interconnections between abnormal mineral metabolism, increased bone fragility, impaired linear bone growth, and vascular calcification in patients with CKD [24]. Specifically, CKD-MBD is defined as "a systematic disorder of mineral and bone metabolism due to CKD manifested by either one or a combination of the following: abnormalities of calcium, phosphorus, PTH, or Vitamin D metabolism, abnormalities in bone turnover, mineralization, volume, linear growth, or strength, and vascular or other soft tissue calcification" [25]. Renal osteodystrophy is now defined as only a precise component of CKD-MBD, specifically anything related to "an alteration of bone morphology in patients with CKD...which is quantifiable by histomorphometry of bone biopsy" [25]. There are different types of bone disease, secondary to renal dysfunction, which may be classified according to levels of bone turnover, mineralization, and bone volume (Table 1.1). These, along with rickets, are also described in more detail in Table 1.1. Focus will be placed on renal bone disease, related to increased levels of parathyroid hormone, specifically mild hyperparathyroid bone disease (HPTBD) and the more severe form, osteitis fibrosa (OF), and their relationship to Vitamin D.

In patients with HPTBD or OF, low renal mass causes a decrease in the amount of phosphate excreted, leading to hyperphosphatemia. Excess phosphate in the blood leads to large amounts of calcium ion binding, causing hypocalcemia, which causes an increase in PTH synthesis, through decreased activation of the parathyroid calcium sensor. Low renal mass also contributes to a decrease in the synthesis of calcitriol, which leads to decreased expression of the VDR on the parathyroid gland, desensitizing the gland to the inhibitory effect of the active form of Vitamin D. High PTH levels lead to large amounts of bone turnover and erosion. At the same time, low levels of calcitriol cause a decrease in the absorption of calcium from the gut, further contributing to hypocalcemia [26]. Traditional therapy for HPTBD or OF usually includes phosphate binders to treat the hyperphosphatemia, followed by administration of a calcitriol analog to correct the hypocalcemia and suppress PTH secretion.

The current project's focus is to develop a model to describe the PK of Vitamin D and 25OHD and then to provide a dietary input of Vitamin D to the larger MSPM to explore background dosing regimens for osteoporosis drug trials. However, future applications of the model may include

investigation of Vitamin D treatments for other disease states, such as those listed in Table 1.1.

1.2.3 *Previous Mathematical Model Describing 25OHD3 Kinetics*

There has been little pharmacokinetic modeling work done to characterize the kinetic disposition of D, 25OHD, and other related metabolites. This is, in part, due to the lack of data for all forms of the Vitamin except for 25OHD. One report used a one compartment model for 25OHD3 to estimate the steady-state cholecalciferol input required to maintain a given serum 25OHD3 concentration [27]. That model was simplified using a steady-state assumption so that the input rate to the system was equal to the output rate and it characterized a linear relationship between 25OHD3 increment and D3 dosage. Limitations included a relatively narrow D3 dose range (0, 1000, 5000, 10000 IU/d), the exclusion from the analysis of collected D3 PK data, which could have better characterized the hepatic conversion of D3 to 25OHD3, and consistently high metabolite baselines in all subjects (average 25OHD3 baseline = 70 nmol/L). The current analysis included data measuring 25OHD3 following metabolite dosing and a broader range of dosing regimens (wide dose range, single and multiple doses, and both IV and oral administration) that allowed for a investigation of more complex model structure including nonlinear relationships.

1.2.4 *D3 and 25OHD3 Dose-Response Relationship*

The 25(OH)D3 cutoff for Vitamin D deficiency, assuming minimal sun exposure, was set by the Institute of Medicine (IOM) new Dietary Reference Intake (DRI) in 2011 at 50 nmol/L (20 ng/mL) for healthy adults <70 years of age [13]. This reference was based on a meta-analysis of studies, which indicated that BMD decreased and fracture-risk increased below this level. Based on a regression analysis done on studies that dosed D3 and measured serum 25OHD3 for patients above 49.5 degrees latitude (minimal sun exposure), D3 doses 400-600 IU/d were found sufficient by the IOM to meet this 50 nmol/L cutoff in 97.5% of the population. Earlier reports had specified lower cutoffs for Vitamin D3 deficiency for adults at 30 nmol/L (12 ng/mL) [28], and, most recently, very high levels of cutoffs have been recommended at <75 nmol/L [19], [22], [29], [30]. The IOM, however, in reviewing Vitamin D3- 25OHD3 dose-exposure response studies from the last several decades, have found that there is not consistent evidence for benefit >75 nmol/L. Discrepancies in meeting IOM 25OHD3 cutoff recommendations, given D3 dosing recommendations, can be

attributed to confounding variables across studies including differences in subject weight, assay type, sun exposure, skin pigmentation, latitude, genetics, sunscreens, and cultural differences in dress.

Since Vitamin D is lipid soluble, it has a large volume of distribution, causing it to be sequestered in the fat, lowering the concentration of 25OHD3 in the serum [31], [32] . Consequently, obese individuals have been shown to have statistically lower serum 25OHD3 concentrations than normal individuals, for a given dose [33], [34]. In another study in obese individuals, it was shown that the 25(OH)D response to Vitamin D3 is directly related to dose and body size with $\sim$ 2.5 IU/kg required for every unit increment in 25(OH)D [35].

In addition to variability attributable to body size differences, there is considerable evidence which points to the large discrepancies in measurements of 25OHD3 across different assay types [36], [37], [38], [39], [40], [41]. Investigations across decades of Vitamin D3 research have measured 25OHD3 with several assay types, including CPBA [42], [43], RIA [44], [45], CHEMI [36], and, most recently and effectively, HPLC-MS [46]. Currently there is no standardization for 25OHD3 assay measurements; however, the Vitamin D Standardization Program, a collaborative effort begun in 2010 by the Office of Dietary Supplements of the National Institutes of Health, is making an effort at developing a standardization program [47].

1.2.5 *D2 vs. D3*

When Vitamin D2 was first synthesized in the lab, it was assumed to be equivalent to Vitamin D3 in its effectiveness in raising 25OHD levels [21]. Recent studies comparing the efficacy of Vitamin D2 and D3 in raising metabolite levels, have yielded inconsistent results, with the majority demonstrating that D3 is more effective than D2 in raising 25OHD levels. Because of these findings several researchers have urged that all supplementation be in the form of D3, but D2 (Drisdol) still remains the primary form of treatment for severely deficient patients. The current analysis gathers data from across these comparative studies to provide a model-based meta-analysis approach to compare D2 and D3's effectiveness in raising 25OHD levels.

D2 and D3 are structurally similar; D2 includes an additional methyl group on carbon 24 (C-24) and a double bond between carbons 22 and 23 (insert reference). Several hypotheses explaining why D2 and D3 differ in their abilities to raise 25OHD levels have been proposed. Some

studies have demonstrated differences in D2 and D3's affinity for DBP. Hollis et al showed D3 and its metabolites to have a greater affinity for DBP, except in the case of the active form [48]. The extra methyl group at C-24 in the D2 hydrocarbon creates steric hindrance, which alters its ability to bind to DBP. Nilsson et al revealed that D3 had a higher association constant for DBP than D2 (2.8*108 vs. 1.3*108) [49]. With less protein binding, both D2 and 25OHD2 may have shorter circulating half-lives and higher clearances; therefore, D2 would not be expected to maintain 25OHD concentrations as high as D3. Another suggestion is that there is a difference in conversion of parent to metabolite for D2 and D3, resulting from differences in enzyme affinity. One study showed that mitochondrial Vitamin D 25-hydroxylase (CYP27A1) converts D3 to 25OHD3 five times as fast as it does D2 to 25OHD2 [50]. The same study showed that microsomal enzymes hydroxylated D3 but not D2. However, later findings indicated that both D2 and D3 are hydroxylation substrates for cytochrome P450, CYP2R1 in humans [51], [52]. Finally, the difference in side chain chemistry for D2 and D3 leads to different metabolic fates for each, including the 24-hydroxylation of D2 before it is 25-hydroxylated, which is not possible for D3 [21], [53], [54], [55]. Around 20-50% of available D2 in the liver may be converted to this form [21], leaving less parent to be converted to the 25OHD form.

Studies have also shown that these differences in metabolic fates may lead to differences in D2 and D3's bioefficacy. When $1,24(OH)_2D2$ is formed from 24-hydroxylated Vitamin D (24OHD) in the kidney it has a lesser affinity for the VDR than do $1,25(OH)_2D3$ and $1,24(OH)_2D3$ [54], [21]. Also the formation of $1,24,25(OH)_3D2$ causes deactivation, whereas $1,24,25(OH)_3D3$ needs to undergo additional side-chain oxidation to become deactivated [54], [21]. Additionally, $1,24,25(OH)_3D3$ has an affinity for the VDR ($\sim 40\%$ more than $1,25(OH)_2D3$), causing significant biological activity.

1.2.6 *BMD and serum PTH Responses to Vitamin D and Calcium Supplementation*

As mentioned above, the final objective of this analysis was to incorporate the PK models for D2, D3, 25OHD2, and 25OHD3 into a multi-scale systems calcium-bone homeostasis model to evaluate the effect of Vitamin D PK on BMDLS and other bone markers, especially serum PTH with or without calcium therapy. The PK models and the systems model connected at the level of calcitriol, which is the main metabolite that follows 25OHD and is the "active" form of the hormone, having the most direct biological effects on the system. Therefore, the immediate goal for linking the two

models was to model the conversion of 25OHD to calcitriol.

In general, current clinical evidence indicates that the effect of Vitamin D3 with or without calcium supplementation on BMD is small, with D3CA being more effective than Vitamin D3 alone. In their meta-analysis of the effect of Vitamin D with or without calcium on BMD at different sites, the IOM "concluded that there is good evidence that Vitamin D3 plus calcium supplementation resulted in small increases in BMD at the lumbar spine, total body, femoral neck, and total hip" [13]. The majority of the studies analyzed by the IOM (total 17) administered both Vitamin D3 (300-800 IU/d) and calcium (377-1450 mg/d) to late post-menopausal women for 2-3 years. In these studies, positive increases in BMD ranged 0.25-2.98% and 0.5-2.90% at the lumbar spine and femoral neck, respectively. Only two studies that were analyzed administered Vitamin D3 alone, with one showing a significant increase in BMD at the femoral neck (1.47%, SD = 6.13%) after two years of administering 400 IU/d D3 [13].

There has also been debate about the relationship between 25OHD and serum PTH levels. The IOM concluded that, while certain studies have suggested that PTH plateaus around serum 25OHD levels of 75 nmol/L, a review of the literature shows that PTH plateaus are reached at different levels of serum 25OHD ranging from 37.5 to 125 nmol/L [13].

Therefore, by integrating the Vitamin D PK model with the MSPM, the current analysis quantified the relationship between Vitamin D3 with or without calcium supplementation and change in BMDLS and serum PTH, allowing for dosing recommendations to be made for reaching particular endpoint thresholds.

1.3 Innovation and Utility

This is the first model that simultaneously integrates D3, D2, 25OHD3, 25OHD2 individual and arm-level data to describe the kinetics of this parent-metabolite system as a single unit in a MBMA. It is also the first model to quantitatively evaluate the effect of Vitamin D3 and calcium administration on bone-health endpoints as mediated through calcium and PTH homeostatic mechanisms. The model-based results may be useful for evaluating differences in D2 and D3 PK, as well as for examining the proposed IOM and other dosing recommendations along with additional considerations, for example, based on baseline values or assay type (for 25OHD3). The results from the integrated PK and systems models may be useful when comparing the concomitant

effects of Vitamin D3 and calcium administration during clinical trials of other anti-osteoporosis therapeutics and can also be investigated to quantify Vitamin D3 effects in other disease states such as CKD-MBD.

Figure 1.1: Metabolism of Vitamin D and its role in calcium-bone homeostasis

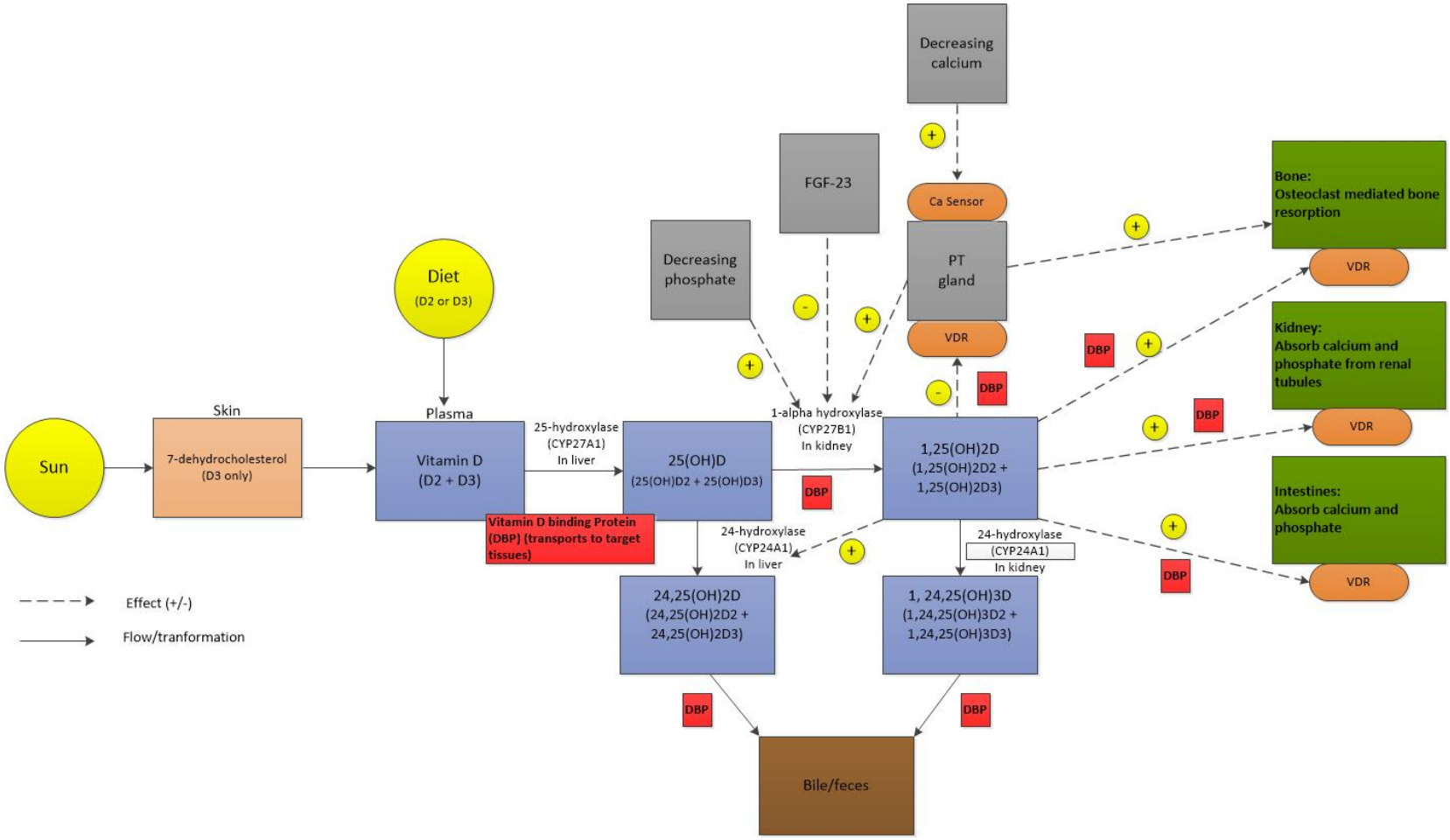


Table 1.1: Vitamin D and bone disease

Disease	**Cause**	**Clinical Signs**	**Biochemical Signs**	**Treatment**
Nutritional rickets	poor diet or lack of sun exposure	bone deformities	low 25(OH)D levels, elevated PTH levels	Vitamin D supplementation
Vitamin D-Dependent Rickets Type I	mutation of the 1-alpha-hydroxylase gene	bone deformities, muscle weakness, growth failure, hypotonia	hypocalcemia, increased PTH levels, normal 25(OH)D levels, decreased $1,25(OH)_2D$ levels, low phosphate levels	0.5-2 mcg/day of intravenous $1,25(OH)_2D$
Vitamin D-Dependent Rickets Type II	mutations of the VDR gene	total body alopecia, bone deformities	hypocalcemia, increased PTH levels, normal 25(OH)D levels, high $1,25(OH)_2D$ levels, high alkaline phosphatase levels	daily intravenous infusion of 0.5-1.0 mg/m^2 of elementary calcium
Renal Bone Disease: adynamic bone disease	decreased numbers of osteoblasts and osteoclasts and hypo-responsiveness of osteoblasts to PTH $\rightarrow$ low bone turnover	bone deformation, bone pain, fracture	high PTH (less than in HPTBD or OF), low calcitriol levels, high phosphate levels, hypercalcemia	IV/oral calcitriol, calcimimetics, phosphate binders
Renal Bone Disease: mild HPTBD/OF	low renal mass low levels of calcitriol and hyperphosphatemia $\rightarrow$ hypocalcemia $\rightarrow$ secondary hyperparathyroidism $\rightarrow$ high bone turnover	bone deformation, bone pain, fracture	high PTH levels, low calcitriol levels, hypocalcemia, hyperphosphatemia, high alkaline phosphatase	IV/oral calcitriol, calcimimetics, phosphate binders
Renal Bone Disease: osteomalacia [56]	defective bone mineralization: increased lag time between osteoid deposition and its mineralization	bone deformation, bone pain, fracture	low calcitriol and 25OHD levels	calcitriol

Chapter 2

Specific Aims

The following were presented as specific aims of the current analysis during the project prospectus evaluation in July 2013:

1. To model the pharmacokinetics-pharmacodynamics (PK-PD) of Vitamin D with the purpose of studying the "classical role" of Vitamin D in bone health.
   (a) PK: Add to existing MSPM a new model for calcitriol's two precursor forms: D3/D2 (parent) and 25OHD3/25OHD2.
   (b) PD: Model exposure-response relationship between Vitamin D and relevant endpoints, such as bone-markers (PTH, calcitriol, calcium, phosphate) and BMD. To date there has not been much written about the relationship between calcitriol's precursor forms and bone health endpoints [13].
   (c) To quantify population PK and PD parameters for this system, including typical values and random inter-arm and residual variabilities, where possible. The latter would be done in a meta-analysis context.
   (d) If possible, to identify relevant covariates (e.g., weight/body mass index (BMI), race, dietary Vitamin D intake, baseline 25OHD levels, concomitant calcium dosing, Vitamin D or 25OHD assay differences) which are predictive of the unexplained random variability in the data.
2. To determine if there is a difference between Vitamin D2 and Vitamin D3 (and corresponding metabolites) kinetics and to quantify that difference using the model's parameterization.
   (a) To determine how this difference affects each type's ability to increase and/or maintain 25OHD levels.
   (b) To determine how this difference affects each type's ability to reduce bone loss (BMD)

3. To recommend Vitamin D (D2 and D3) dosing and a 25OHD cutoff for healthy subjects based on determined bone-health indicators.
   (a) To recommend Vitamin D dosage for adequate maintenance of 25OHD (currently defined as >= 50 nmol/L (20 ng/ml)) [13] and reduction in bone loss (BMD) and to compare these model recommendations to the IOM's 2011 DRI values of 600-800 IU/day (Recommended Dietary Allowance (RDA) to cover 97.5 % of the general population's needs) [13].
   (b) To recommend a 25OHD deficiency cutoff, based on the model's exposure-response prediction for Vitamin D and bone health indicators and to compare the model's recommendation to the IOM's cutoff for Vitamin D deficiency at 50 nmol/L (20 ng/mL) of 25OHD, as opposed to the previously accepted 75 nmol/L (30 ng/mL) [13].
4. To determine how knowledge about the relationship between Vitamin D and BMD may impact future clinical trial design.
   (a) To perform sensitivity analysis on trial design elements (dietary Vitamin D intake, weight, age, 25OHD baseline) to determine which factors most influence BMD (hip and spine) percent change from baseline (PCFB). The results from this analysis can help to determine inclusion criteria for enrollment in clinical trials that dose Vitamin D
   (b) To conduct a clinical trial simulation to calculate the sample size (n) needed to detect a BMD delta (PCFB treated - PCFB placebo) for a given Vitamin D dose, power, and test size (alpha), with denosumab used as an example therapeutic.

Chapter 3

Model-based Meta-analysis for Development of a Population-Pharmacokinetic (PPK) Model for Vitamin D3 and its 25OHD3 Metabolite Using Both Individual and Arm-Level Data

The following chapter, as well as the discussion related to it in Chapter 6, are taken from *Journal of Pharmacokinetics and Pharmacodynamics* 2016 Apr;43(2):191-206.

3.1 Methods

3.1.1 *Study Selection*

Studies were selected using specific search criteria in PUBMED to identify public-source PK data for D3 and 25OHD3. The database was searched from June 2010 until August 2014. To the extent possible, PRISMA guidelines were followed for reporting this meta-analysis [57]. To qualify for inclusion, studies had to include interventions that differed only in Vitamin D3 content in healthy or osteoporotic adult subjects. Studies of individuals with disorders that could affect bone and calcium metabolism (e.g., chronic kidney disease) were excluded. Three types of studies of varying designs (e.g., randomized, non-randomized, blinded, open label) were used in the analysis: 1) D3-D3 = D3 administered, D3 concentrations reported; 2) 25D3-25D3 = 25OHD3 administered, 25OHD3 concentrations reported; or 3) D3-25D3 = D3 administered, 25OHD3 concentrations reported. All

concentration units were converted to nmol/L. Principal summary measures collected included mean absolute concentrations or mean CFB concentrations, if absolute baselines were reported. Assay type was also collected as well as mean weight or BMI, if available. The assay types collected did not necessarily distinguish between Vitamin D2 and D3 and their metabolites; however, subjects generally would have very low levels of D2 and 25OHD2 unless they were actively taking regular supplements. Study inclusion criteria usually required that subjects refrain from any kind of Vitamin D supplementation prior to the study. Search terms included the following: "vitamin D3", "cholecalciferol", "25-hydroxyvitamin D3", or "25OHD3", "randomized." The reference lists of studies found by these searches were also used to find additional qualifying studies. Data were extracted from published reports using digitizing software (Arizona GraphClick, version 2.9.2). Each unit of data was digitized three times and the average of the three trials was taken as the final values to be used in the analysis.

3.1.2 *Graphical Data Evaluation*

A graphical exploration of the observed concentration data was conducted to consider the dispositional characteristics (e.g., one- or two-compartmental) of D3 and 25OHD3. The data were also visually inspected for dose-proportionality (linear vs. non-linear kinetics).

3.1.3 *Nonlinear Mixed Effects Pharmacokinetic Analysis of Parent and Metabolite*

The PK analysis was conducted using nonlinear mixed effects modeling (NONMEM® software, version 7.2, ICON Development Solutions, Hanover, MD), using first-order conditional estimation with interaction (FOCE-I). Model development and qualification proceeded by first considering a sequential and then a simultaneous modeling process. Modeling parent (D3-D3), metabolite (25D3-25D3) and parent-metabolite (D3-25D3) data separately was done to explore different model structures and to investigate the possibility of non-linear kinetics. The simultaneous modeling process was used for the final model.

The model was implemented as a system of ordinary differential equations to describe mass transfer between compartments. Model development for both D3 and 25OHD3 included consideration of 1- and 2-compartment models with first-order absorption and linear or non-linear clearances. The parameters considered for estimation for the parent and metabolite (m) models

included central volume (Vc or Vcm, L), peripheral volume (Vp or Vpm, L), inter-compartmental clearance (Q or Qm, L/h), and baseline D3 or 25OHD3 concentration in the central compartment ($DBASE$ or $DBASEm$, nmol/L). Parent-metabolite conversion parameters included maximum rate of metabolism ($Vmax$, nmol/h) and Km (the parent concentration at which the rate of metabolism is half of $Vmax$, nmol/L). Initial D3 or 25OHD3 amounts in the central ($A0_{D3}$, $A0_{25D3}$) and peripheral ($A0_{P,D3}$, $A0_{P,25D3}$) compartments, as well as the constant endogenous D3 input rate ($ENDOG$, nmol/h) to the central parent compartment, were calculated as functions of other PK parameters. $ENDOG$ was assumed to represent natural production of D3 from sunlight as well as from any dietary sources not related to the supplemented dosing. Despite the potential for seasonal variation in $ENDOG$, this rate was considered constant, given the lack of data pertaining to seasonal sunlight exposures in the individual study publications.

This MBMA included both individual subject and arm-level data with random effects for each. These unit-level random effects entered at the same level in the model hierarchy. Observation-level (residual) random effects were also included. The hierarchical random-effects model was adjusted to accommodate the mixed data by weighting the unit-level random effects (η_{kui}) and the residual random effects (ϵ_{uij}) by the inverse square-root of the unit size [58].Therefore, data from the larger units were weighted more heavily during the estimation process. Separate unit-level and residual random effect variances were estimated for arms and individuals, respectively, in the final parent-metabolite model.

For both parent and metabolite, an exponential variance model was used to describe the unexplained random variability of PK parameters across units (individuals or arms) in the form:

$$P_{\mathrm{i}} = \theta_{\mathrm{k}} \cdot e^{\frac{\eta_{\mathrm{kui}}}{\sqrt{nARM_{\mathrm{ij}}}}} \tag{3.1}$$

where P_i is the estimated parameter value for the ith unit, k is the typical population value of parameter k, η_{kui} are the unit-level random effects for unit i and parameter k, u is an indicator for whether the data unit is an individual or an arm, and nARM_{ij} is the size of the unit for unit i at time-point j ($\mathrm{nARM}_{ij} = 1$ for individuals). Models were explored using various random effects covariance structures.

A proportional residual variance structure for parent and metabolite was used to describe

residual variability:

$$C_{\text{obs,ij}} = C_{\text{pred,ij}} \cdot e^{\frac{\epsilon_{\text{uij}}}{\sqrt{nARM_{\text{ij}}}}} \tag{3.2}$$

where $C_{obs,ij}$ is the observed concentration in unit i at time-point j, $C_{pred,ij}$ is the unit predicted concentration, and ϵ_{uij} is the proportional residual random error (proportional, due to the first-order approximation).

GOF for each model was assessed by the examination of the following criteria: visual inspection of diagnostic scatter plots (observed vs. predicted concentration, observed and predicted concentration vs. time, individual conditional weighted residuals (CWRESI) vs. predicted concentration or time (not shown in results)), the precision of the parameter estimates, as measured by asymptotic standard errors derived from the covariance matrix of the estimates, successful minimization with at least 2 significant digits in parameter estimates, changes in the minimum value of the objective function, and changes in the estimates of unit-level and residual variability for the specified models.

Data programming and simulations were implemented in R (v 3.1.1) [59] via the *mrgsolve* package (version 0.3040) [60]. This package uses R as the interface to a DLSODA differential equation solver (ODEPACK, November 12, 2003 version), within a C++ wrapper.

3.1.4 *Covariate Data Exploration and Modeling*

A measure of body size (weight or BMI) and assay type were pre-specified as covariates of interest prior to model development, given general clinical interest in these effects. Both weight and BMI were collected in an effort to find a measure for body size, since studies reported one or the other or both. Graphical evaluations were used to explore relationships of model residuals or unit-predicted (post-hoc) random effects relative to potential covariates. These plots guided structural development of covariate relationships when sufficient covariate data were available to include such effects.

3.1.5 *Model Qualification*

PcVPC were performed to assess the model performance when describing the central tendency and the variability in the observed 25OHD3 data. Inter-unit and residual variability were included by sampling 1000 replicates from the inter-unit and residual variability distributions for parent and metabolite from the final model. Both simulated and observed data were summarized to find

the median and a particular prediction interval (typically 5th to 95th percentiles) to describe the observed distribution of the data in a time bin. Particular confidence interval (CI)s around the simulated median, 5th and 95th percentiles were chosen so as to adequately describe the range of the observed data and to ensure visual clarity when plotting. Observed data at time points that went beyond the largest time bin median values were plotted at the last time bin median point.

A posterior predictive check was also conducted to qualify the final model's ability to describe the central tendency of the data for each unit (individual or treatment arm) and to illustrate the precision of the model predictions for the central tendency, given the final parameter estimates (fixed effects and residual error) and their estimation precision. The unit-level random effects distributions were not randomly sampled for these simulations. Rather the unit-level random effects were fixed to the empirical Bayes estimates from the final model for each unit, making the predictions conditional on these values. The check was done for each unit, separately. Several doses only had one or two units associated with them. Uncertainty was included around the final parameter estimates by sampling 500 replicates from a multivariate normal distribution centered at the final parameter estimates and having covariance equal to the covariance matrix of the estimates for the final model. For each unit, the median 25OHD3 prediction, as well as the 25th and 75th percentiles were plotted and overlaid with the observed data.

3.1.6 *Simulations*

Simulations of typical response (e.g. no random inter-unit or residual variability) were used to explore the effect of metabolite baseline on metabolite response. These typical response predictions were made for 25OHD3 concentrations resulting from Vitamin D3 doses of 400, 600, 800, 1000, 2000, 5000, or 10,000 IU/d administered for 1 year. Metabolite baselines of 10, 30, 50 and 80 nmol/L were investigated.

Simulations investigating the impact of assay type on 25OHD3 concentration (relative to the standard assay measurements by HPLC-MS) included uncertainty in the assay bias parameters. Uncertainty was included around these parameters by drawing 500 replicates from a multivariate normal distribution centered at the final parameter estimates and having covariance equal to the covariance matrix of the estimates for the final model. All other fixed effects parameters were fixed to their final estimates and no random or residual variability was included. Differences in median

25OHD3 concentration after 1 year of daily D3 dosing were examined graphically for a typical D3 dose range of 400, 800 or 2000 IU/d.

3.2 Results

3.2.1 *Study Selection*

Overall 57 studies with 5395 individuals were represented in this analysis; this was comprised of 25 individual-level profiles and 111 treatment arm mean profiles (Table 3.1). The total number of observations analyzed was 801 samples (total number of D3-D3 observations = 54; 25D3-25D3 = 131; D3-25D3 = 616). The total average sample size per unit was 6 observations (average samples per D3-D3 unit = 5; 25D3-25D3 = 5; D3-25D3 = 8). Both intravenous (IV), oral (PO), single dose (SD) and multiple dose (MD) data were used, with D3 and 25OHD3 dosing (400-300,000 IU/d and 15-1000 ug/d, respectively). A more detailed description of individual studies, as well as a graphical display of all the observed data is available in Appendix B (Table B, Figures B.1 - B.3)

3.2.2 *Graphical Data Evaluation*

There were two linear concentration segments apparent in the observed log-D3 and log-25OHD3 concentrations over time (Figures B.1 and B.2 in Appendix B); this suggested two-compartment dispositional PK for both parent and metabolite. To confirm these visualizations, both one- and two-compartment structures were considered during the model building process. Furthermore, dose-normalized D3 and 25OHD3 concentration data were non-superimposable (Figure B.4 in Appendix B). These less than dose-proportional concentration-time profiles supported investigations of non-linear PK.

3.2.3 *Nonlinear Mixed Effects Pharmacokinetic Analysis of Parent and Metabolite*

The simultaneous modeling process was used for the final model, where the metabolite PK parameters were fixed to values estimated using just the metabolite dosing data (25D3-25D3) and the parent and conversion parameters were estimated simultaneously using both the D3-D3 and D3-25D3 datasets. All models were successfully minimized to 2 significant digits. Convergence difficulties were experienced for significant digits (SIGDIG) >2, which was considered to be a reflection of both

the complexity of the model and the sparsity of the data used to fit the model. The NONMEM control streams and outputs for the final 25D3-25D3 and D3-25D3 models are found in Appendix B, respectively.

The metabolite dosing data from 11 individuals and 7 treatment arms across 5 different studies (Table 3.2, Figure B.2 in Appendix B) supported a two-compartment model with first-order elimination to describe 25OHD3 PK. Non-linear clearance for the metabolite was considered but not supported by the data. The metabolite-only data included both IV and PO dosing; PO absolute bioavailability ($F_{metabolite}$) was estimated to be very near the reference IV data with typical value $F_{metabolite}$= 0.998 (95% CI (0.867, 1.13)). In the final metabolite model, random effects were included on $DBASEm$ (ω^2_{DBASEM}) and Km (ω^2_{CLM}). Off-diagonal covariance elements were assumed to be zero. Separate residual errors were included for single and multiple dose data.

The final parent-metabolite model included 2-compartment models for parent and metabolite with a Michaelis Menten-type non-linear clearance from parent to metabolite (Figure 3.1). The model was fit using the D3-D3 (12 treat arms across 7 studies) and D3-25D3 (14 individuals and 103 treatment arms across 53 studies) datasets. Parent PK parameters, as well as parent-to-metabolite conversion parameters ($Vmax$, Km), were estimated simultaneously (Tables 3.3- 3.5), while the metabolite PK parameters (25D3-25D3) were fixed to the final estimates (Table 3.2) from the metabolite model discussed above. GOF plots for the final parent-metabolite model and for D3 (Figure 3.2) indicated an adequate description of the data.

$$\frac{d(A_{\text{gut}})}{dt} = -ka \cdot A_{\text{gut}} \tag{3.3}$$

$$\frac{d(A_{\text{D3}})}{dt} = ENDOG + ka \cdot A_{\text{gut}} - (CLNL + Q) \cdot C_{\text{D3}} + Q \cdot C_{\text{P,D3}} \tag{3.4}$$

$$\frac{d(A_{\text{P,D3}})}{dt} = Q \cdot C_{\text{D3}} - Q \cdot C_{\text{P,D3}} \tag{3.5}$$

$$\frac{d(A_{\text{25D3}})}{dt} = CLNL \cdot C_{\text{D3}} - (CLm + Qm) \cdot C_{\text{25D3}} + Qm \cdot C_{\text{P,25D3}} \tag{3.6}$$

$$\frac{d(A_{\mathrm{P,25D3}})}{dt} = Qm \cdot C_{\mathrm{25D3}} - Qm \cdot C_{\mathrm{P,25D3}} \tag{3.7}$$

$$ENDOG = Vmax \cdot (\frac{DBASE}{Km + DBASE}) \tag{3.8}$$

$$CLNL = \frac{Vmax}{Km + C_{\mathrm{D3}}} \tag{3.9}$$

$$A0_{\mathrm{gut}} = 0 \tag{3.10}$$

$$A0_{\mathrm{D3}} = DBASE \cdot Vc \tag{3.11}$$

$$A0_{\mathrm{P,D3}} = DBASE \cdot Vp \tag{3.12}$$

$$A0_{\mathrm{25D3}} = CLNL0 \cdot DBASE \cdot \frac{Vcm}{CLm} \tag{3.13}$$

$$A0_{\mathrm{P,25D3}} = CLNL0 \cdot DBASE \cdot \frac{Vpm}{CLm} \tag{3.14}$$

$$CLNL0 = \frac{Vmax}{Km + DBASE} \tag{3.15}$$

$$DBASEm = \frac{Vmax * DBASE}{(Km + DBASE) * CLm} \tag{3.16}$$

The final model structure is specified in Equations 3.3 - 3.16. A_{gut} is the amount (nmol) of D3 in the gut; A_{D3}/A_{25D3} is the amount (nmol) of D3/25OHD3 in the central compartment; $A_{P,D3}/A_{P,25D3}$ is the amount (nmol) of D3/25OHD3 in the peripheral compartment. C_{D3} is the concentration of D3 (nmol/L) in the central compartment; $C_{P,D3}$ is the concentration (nmol/L) of D3 in the peripheral compartment; C_{25D3} is the concentration of 25OHD3 (nmol/L) in the central compartment; $C_{P,25D3}$ is the concentration (nmol/L) of 25OHD3 in the peripheral compartment;

CLNL is the non-linear clearance of D3 (L/h); the input rate for 25OHD3 was assumed to be equal to this nonlinear elimination rate (*CLNL*C_{D3}, as in Equations 3.4 and 3.6), with *CLNL0*DBASE* being the formation rate at baseline.

In the final model, unit-level random effects were included on *Vmax* and *DBASE*. For the 25OHD3 endpoint, separate unit-level random effects and residual errors were included for individual ($\omega^2_{Vmax,i}$, $\sigma^2_{metab,i}$) vs. arm ($\omega^2_{Vmax,g}$, $\omega^2_{DBASE,g}$, $\omega^2_{DBASE-Vmax,g}$, $\sigma^2_{metab,g}$) data. Having only one random effect or residual error for both types of data caused an under-predictive bias in the individual-level prediction for individuals (not shown). Separate variances were included for individuals vs. arms to correct an under-prediction bias in the individual-level prediction (not shown).

3.2.4 *Covariate Analysis*

Due to the known discrepancies between analytical methods, assay was included as a covariate in the final parent-metabolite model. Exploratory graphics of this relationship included CWRESI vs. population prediction 25OHD3 from the final parent-metabolite base model (not shown). The ranges of the residuals were similar between assays; RIA residuals appeared negatively biased (more below zero) and CHEMI residuals were generally positively biased (more above zero). HPLC-MS residuals were the smallest in magnitude and their distribution was the most symmetrical about zero.

Both proportional and constant biases, as well as different residual error variances, were included on the prediction for 25OHD3 (Y_{25D3}), as seen below in Equations 3.17-3.20.

$$If(ASSAY = HPLC - MS)Y_{25\mathrm{D3}} = C_{25\mathrm{D3}} \cdot e^{\frac{\epsilon(1)}{\sqrt{nARM_{\mathrm{ij}}}}} \tag{3.17}$$

$$If(ASSAY = RIA)Y_{25\mathrm{D3}} = PAB_1 \cdot C_{25\mathrm{D3}} \cdot e^{\frac{PRA_1 \cdot \epsilon(1)}{\sqrt{nARM_{\mathrm{ij}}}}} + AAB_1 \tag{3.18}$$

$$If(ASSAY = CPBA)Y_{25\mathrm{D3}} = PAB_2 \cdot C_{25\mathrm{D3}} \cdot e^{\frac{PRA_2 \cdot \epsilon(1)}{\sqrt{nARM_{\mathrm{ij}}}}} + AAB_2 \tag{3.19}$$

$$If(ASSAY = CHEMI)Y_{25\mathrm{D3}} = PAB_3 \cdot C_{25\mathrm{D3}} \cdot e^{\frac{PRA_3 \cdot \epsilon(1)}{\sqrt{nARM_{\mathrm{ij}}}}} + AAB_3 \tag{3.20}$$

In the above equations, PAB_1, PAB_2, and PAB_3 are the estimated proportional assay biases for RIA, CPBA, and CHEMI, respectively. AAB_1, AAB_2, and AAB_3 are the estimated additive assay biases for those same respective assays. PRA_1, PRA_2, and PRA_3 are the estimated assay precisions. All predictions of observed data measured by RIA, CPBA, and CHEMI were made relative to HPLC-MS predictions. The addition of assay as a covariate to the model slightly improved the fit for the arm-level MD 25OHD3 data at the lower concentrations (not shown). The greatest improvement in fit was found in the individual-level metabolite data, which were all measured by HPLC-MS. CPBA had the largest assay coefficients for both the proportional (1.50) and the additive (-22.1 nmol/L) biases (Equation 3.19), while RIA showed the least difference relative to HPLC-MS (PAB_1 = 1.01, AAB_1= 2.50 in Equation 3.18).

In general, the model more precisely estimated parameters related to RIA than to CPBA and CHEMI. PAB_3 (Equation 3.20, % relative standard error (RSE) = 38.5%) was the least precisely estimated proportional bias compared to PAB_1 (Equation 3.18, %RSE = 7.94%) and PAB_2 (Equation 3.19, %RSE = 5.68%). Both AAB_1 and AAB_3 had high %RSE (Equation 3.18, %RSE = 126%; Equation 3.20, %RSE = 138%); however, the greater magnitude of AAB_3 (Equation 3.20, AAB_3 = -10.2 nmol/L) vs. AAB_1 (Equation 3.18, AAB_1 = 2.49 nmol/L) caused the large uncertainty around AAB_3 to have a greater impact on the prediction variability, as was seen in the posterior visual predictive check (Figure B.5 in Appendix B). CPBA had the largest precision estimate (Equation 3.19, PRA_2 = 1.51) compared to RIA (Equation 3.18, PRA_1 = 1.45) and CHEMI (Equation 3.20, PRA_3 = 1.16). The %RSEs around the precision estimates for CPBA (Equation 3.19, %RSE =10.9%) and CHEMI (Equation 3.20, %RSE = 19.7%) are higher than for RIA (Equation 3.18, %RSE = 8.76%). Assay was not included on the parent prediction because all treatment arm data, except for one (RIA), were measured by HPLC-MS.

3.2.5 *Model Qualification*

The pcVPC was implemented for individuals and arms separately (Figure 3.3) as well as for each of the different assay types (Figure B.8 in Appendix B). Notably, assay type considered only arm-level data since all individual-level observations were measured by HPLC-MS. Overall the model described the central tendency of the metabolite data for both arm- and individual-level data (Figure 3.3a-3.3b), as most of the observed medians fall within the simulated 80% confidence interval

for the median. However, the model over-estimated the inter-unit variability for the arm-level data (Appendix B, Figure B.10), particularly the lower 5th percentile; the model did, however, reasonably predict the variability in the individual data (Figure 3.3c). Across assays, the model again described the central tendency of the data about equally well for RIA, CPBA, and HPLC-MS, with CHEMI showing more of an under-predictive trend at later time points (B, Figure B.8).

Posterior predictive checks were used to investigate the model's ability to describe the central tendency of the parent and metabolite data for individuals, arms, doses, and assays (Figures B.5-B.7 in Appendix B), given the precision of the final parameter estimates. Due to the sparsity of the data, the middle 50% of the simulated median was plotted. The model predicted the central tendency of observations measured by RIA and HPLC-MS. The prediction intervals around observations measured by CPBA and CHEMI, however, were very wide because the bias and precision parameters (Equations 3.17-3.20, Table 3.4) related to those assay types were less precisely estimated, as discussed in the previous section. In general, this model more confidently predicted the central tendency of the given data when it was measured by RIA or HPLC-MS. There was a great deal of uncertainty in the predictions made for observations measured by assays CPBA or CHEMI.

3.2.6 *Simulation*

The population-level 25OHD3 concentrations after 1 year of D3 dosing at various amounts were simulated across different metabolite baselines (Figure 3.4). Assay type was fixed to HPLC-MS for these simulations. The non-linear kinetics of the system were apparent as inverse relationships of 25OHD3 response, with both D3 dose and metabolite baseline.

The impact of assay bias and precision was also explored through simulation: median 25OHD3 concentrations following 1 year of oral D3 dosing were compared from each assay (Figure 3.5). In these simulations, metabolite baseline was fixed to 40 nmol/L. With increasing dose, the difference in metabolite measurements for different assays, relative to HPLC-MS measurements, increases. In general, a persistent positive bias was observed for all three assays relative to HPLC-MS. The 25th and 75th percentiles of the predictions were included as prediction intervals around the median to consider if there was truly a difference between assay types, given the known confidence intervals of the assay bias and precision parameters. The model-derived graphics

distinguished differences in concentration measurements only for CPBA and HPLC-MS, as neither of those assays' prediction intervals overlapped for any of the doses. At higher doses (e.g. >= 2000 IU/d), differences between RIA and CPBA in addition to CPBA and HPLC-MS were also apparent. However, the prediction interval around CHEMI's median 25OHD3 concentration was so wide that it encompassed all the other assays' prediction intervals across all doses. Therefore, there was not enough information about CHEMI's precision and bias parameters to distinguish any real difference between concentrations measured by CHEMI versus the other three assay types.

Table 3.1: Summary of studies used in MBMA for D3 and 25OHD3 (RT = route; REG = regimen)

Treatment	Endpoint	Doses	RT/REG	Individuals	Arms	Total Subjects	Studies
25OHD3	25OHD3	15-100 μg/d	IV, PO/ SD, MD	11	7	65	5
D3	D3	400-100,000 IU/d	PO/SD, MD	0	12	168	7
D3	25OHD3	400-300,000 IU/d	PO/SD, MD	14	103	5173	53
Totals				25	111*	5395*	57*

*Totals listed may not equal the sum of the counts in the previous rows due to inclusion of individuals with more than one endpoint

Figure 3.1: Final compartmental models for D3 and 25OHD3

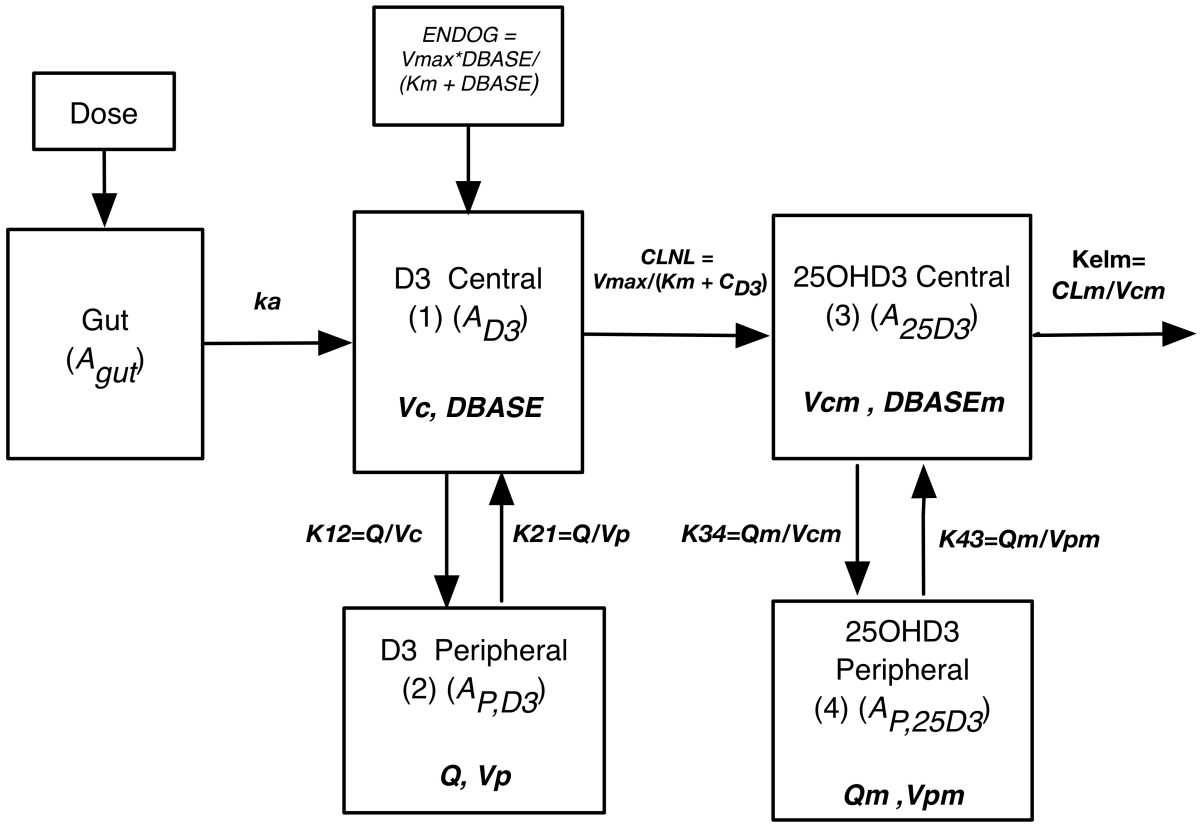


Figure 3.2: GOF diagnostics for parent-metabolite model (a-b, total D3-25D3 dataset), the parent model (c-d, D3-D3 dataset) and the metabolite model (e-f, 25D3-25D3 dataset), stratified by data type. Figures a-b do not include data from a treatment arm (n = 14, D3 dose = 50000 IU/d) in Barger-Lux et al (Appendix B reference [17]) at t = 0 and t = 1.8 months. Observed concentration data in [17] were unusually high (25OHD3 concentration = 710 nmol/L at 1.8 months) relative to other arms/individuals given the same dose (maximum concentration up through 2 months = 71 nmol/L)

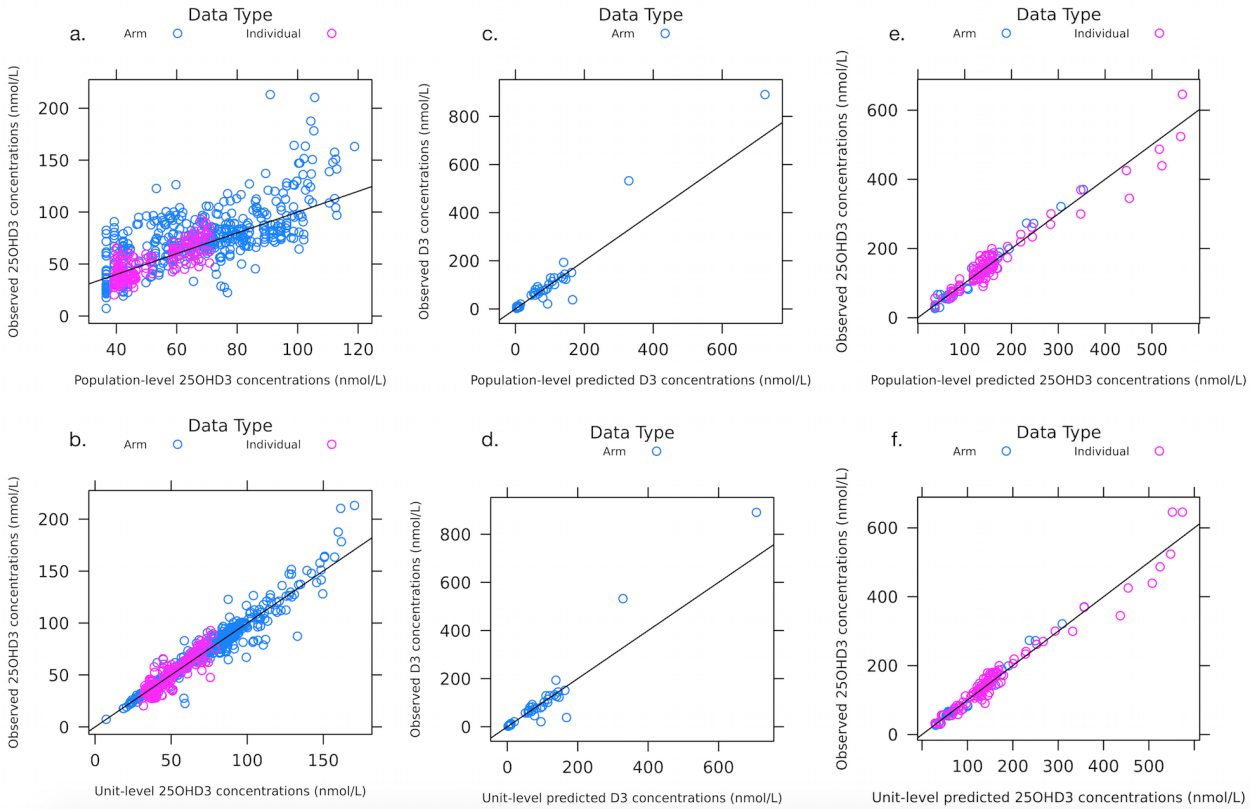


Table 3.2: 25OHD3 PK parameter estimates from final metabolite model (dataset: 25D3-25D3)[1]

Parameters	Description	Estimate (units)	SE	%RSE
Model: Metabolite				
Fixed effects				
kam	Absorption rate constant	0.323, FIX (h^{-1})	NA	NA
CLm	1st order clearance	0.0153 (L/h)	0.001	9.15
VCm	Central volume of distribution	4.35 (L)	0.145	3.33
Vpm	Peripheral volume of distribution	6.87 (L)	0.865	12.6
Qm	Inter-compartmental clearance	0.0507 (L/h)	0.00947	18.7
$DBASEm$	Metabolite baseline concentration	36.6 (nmol/L)	3.19	8.72
$F_{metabolite}$	Bioavailability	0.998	0.067	6.70
Random effects (% coefficient of variation (CV))				
ω^2_{DBASEm}	Variance of inter-unit random effects on $DBASEm$	0.207 (45.5)	0.085	40.8
ω^2_{CLm}	Variance of inter-unit random effects on CLm	0.0031 (5.57)	0.014	458
Residual effects (% CV)				
σ^2_{SD}	Variance of residual error for SD data	0.009 (9.49)	0.003	34.5
σ^2_{MD}	Variance of residual error for MD data	0.048 (21.9)	0.022	45.1

[1] All unit-level random and residual effects for arm data are weighted by n: $\omega^2_a = \omega^2_{a,raw}$ /n and $\sigma^2_b = \sigma^2_{b,raw}$ /n, where ω^2_a is the unit-level variance for parameter a and σ^2_b is the residual variance for b (SD or MD) for median unit size (n); $\omega^2_{a,raw}$ is the un-weighted unit-level variance for parameter a and $\sigma^2_{b,raw}$ is the un-weighted residual variance for b that NONMEM reports. Random effect standard errors (SE) for arm data ($COV MATRIX = S) are also weighted by n: SE(ω^2_a) = SE($\omega^2_{a,raw}$/n); SE(σ^2_b) = SE($\sigma^2_{b,raw}$ /n). The median n for the 25D3-25D3 dataset is 9. The covariance matrix of the estimates, as well as model code files, are included in Appendix A.

Table 3.3: Fixed effects structural D3 PK parameter estimates from final parent-metabolite model (datasets: D3-D3, D3-25D3)[2]

Parameters	Description	Estimate (units)	SE	%RSE
Model: Parent, Parent-Metabolite				
ka	D3 absorption rate constant	0.323, FIX (h^{-1})	NA	NA
Vc	D3 central volume of distribution	15.6 (L)	2.49	16.0
Vp	D3 peripheral volume of distribution	2333 (L)	279	12.0
Q	D3 inter-compartmental clearance	0.185 (L/h)	0.006	3.24
$DBASE$	D3 baseline concentration	3.75 (nmol/L)	0.288	7.68
$Vmax$	D3 maximum rate of elimination	1.62 (nmol/h)	0.087	5.38
Km	Michaelis-Menten constant	6.38 (nmol/L)	0.718	11.2

[2] All fixed effects estimates are relative to bioavailability

Table 3.4: Fixed effects 25OHD3 assay covariate parameter estimates from final parent-metabolite model (datasets: D3-D3, D3-25D3)[3]

Parameters	Description	Estimate (units)	SE	%RSE
Model: Parent, Parent-Metabolite				
PAB_1	Proportional 25OHD3 prediction bias for RIA	1.01	0.080	7.94
PAB_2	Proportional 25OHD3 prediction bias for CPBA	1.50	0.085	5.68
PAB_3	Proportional 25OHD3 prediction bias for CHEMI	1.37	0.526	38.4
AAB_1	Additive 25OHD3 prediction bias for RIA	2.49 (nmol/L)	3.16	127
AAB_2	Additive 25OHD3 prediction bias for CPBA	-22.1 (nmol/L)	4.79	21.7
AAB_3	Additive 25OHD3 prediction bias for CHEMI	-10.1 (nmol/L)	14.1	140
PRA_1	Precision for RIA	1.45	0.127	8.76
PRA_2	Precision for CPBA	1.51	0.164	10.9
PRA_3	Precision for CHEMI	1.16	0.228	19.7

[3] All fixed effects estimates are relative to bioavailability

Table 3.5: Random effects parameter estimates from final parent-metabolite model (datasets: D3-D3, D3-25D3)[4]

Parameters	Description	Estimate (%CV)	SE	%RSE
Model: Parent, Parent-Metabolite				
Inter-unit random effects				
$\omega^2_{Vmax,g}$	Variance of inter-unit random effects on *Vmax* for group data	0.113 (33.6)	0.023	20.3
$\omega^2_{DBASE,g}$	Variance of inter-unit random effects on *DBASE* for group data	0.46 (67.8)	0.100	21.7
$\omega^2_{DBASE-Vmax,g}$	Covariance of inter-unit random effects on *Vmax* and *DBASE* for group data	-0.028 (16.7)	0.046	164
$\omega^2_{Vmax,i}$	Variance of inter-unit random effects on *Vmax* for individual data	0.017 (13.0)	0.012	70.5
Residual random effects				
$\sigma^2_{metabolite,i}$	Variance of residual error for individual metabolite data	0.028 (16.7)	0.002	7.14
$\sigma^2_{metabolite,g}$	Variance of residual error for group metabolite data	0.01 (10)	0.001	10
$\sigma^2_{parent,SD}$	Variance of residual error for SD parent data	0.289 (53.8)	2.43	8.4
$\sigma^2_{parent,MD}$	Variance of residual error for MD parent data	0.016 (12.6)	0.005	31.2

[4] All unit-level random and residual effects for arm data are weighted by n: $\omega^2_a = \omega^2_{a,raw}$ /n and $\sigma^2_b = \sigma^2_{b,raw}$ /n, where ω^2_a is the unit-level variance for parameter a and σ^2_b is the residual variance for b (parent or metabolite,SD or MD) for median unit size (n); $\omega^2_{a,raw}$ is the un-weighted unit-level variance for parameter a and $\sigma^2_{b,raw}$ is the un-weighted residual variance for b that NONMEM reports. Random effect SE for arm data ($COV MATRIX = S) are also weighted by n: SE(ω^2_a) = SE($\omega^2_{a,raw}$/n); SE(σ^2_b) = SE($\sigma^2_{b,raw}$ /n). The median n for the collective D3-D3 and D3-25D3 dataset is 19. The covariance matrix of the estimates, as well as model code files, are included in Appendix A.

Figure 3.3: Prediction-corrected visual predictive checks for a) both arm- and individual-level multiple-dose 25OHD3 data (D3-25OHD3 dataset) b) arm-level multiple-dose data only c) individual-level multiple-dose data only. Solid red line is simulated median; solid blue lines are the simulated 5th and 95th percentiles; red band is the simulated 80% confidence interval around the simulated median; light blue bands are the simulated 80% confidence intervals around the simulated 5th and 95th percentiles of the median; black dots are the observed medians at a given time bin; black vertical bars are the observed 5th & 95th percentiles of the observed data; black horizontal bars indicate time bin ranges; green triangles are raw data; size of the triangles is proportional to sample size

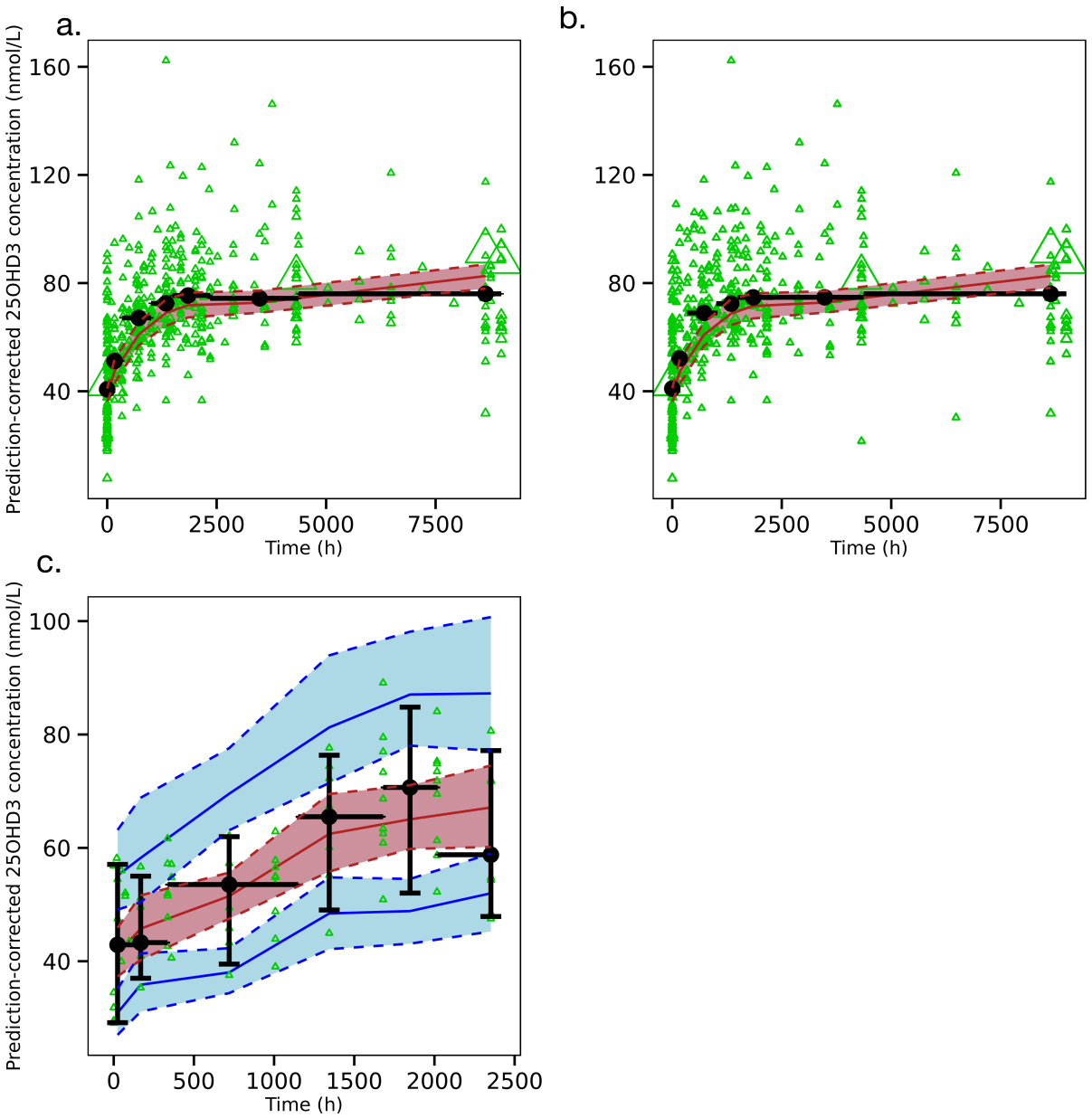


Figure 3.4: Population-level simulation results: a) the effect of metabolite baseline on simulated 25OHD3 response b) the effect of metabolite baseline on simulated 25OHD3 response on semi-log scale (x-axis) c) the effect of metabolite baseline on simulated 25OHD3 CFB d) the effect of metabolite baseline on simulated 25OHD3 CFB on semi-log scale (x-axis)

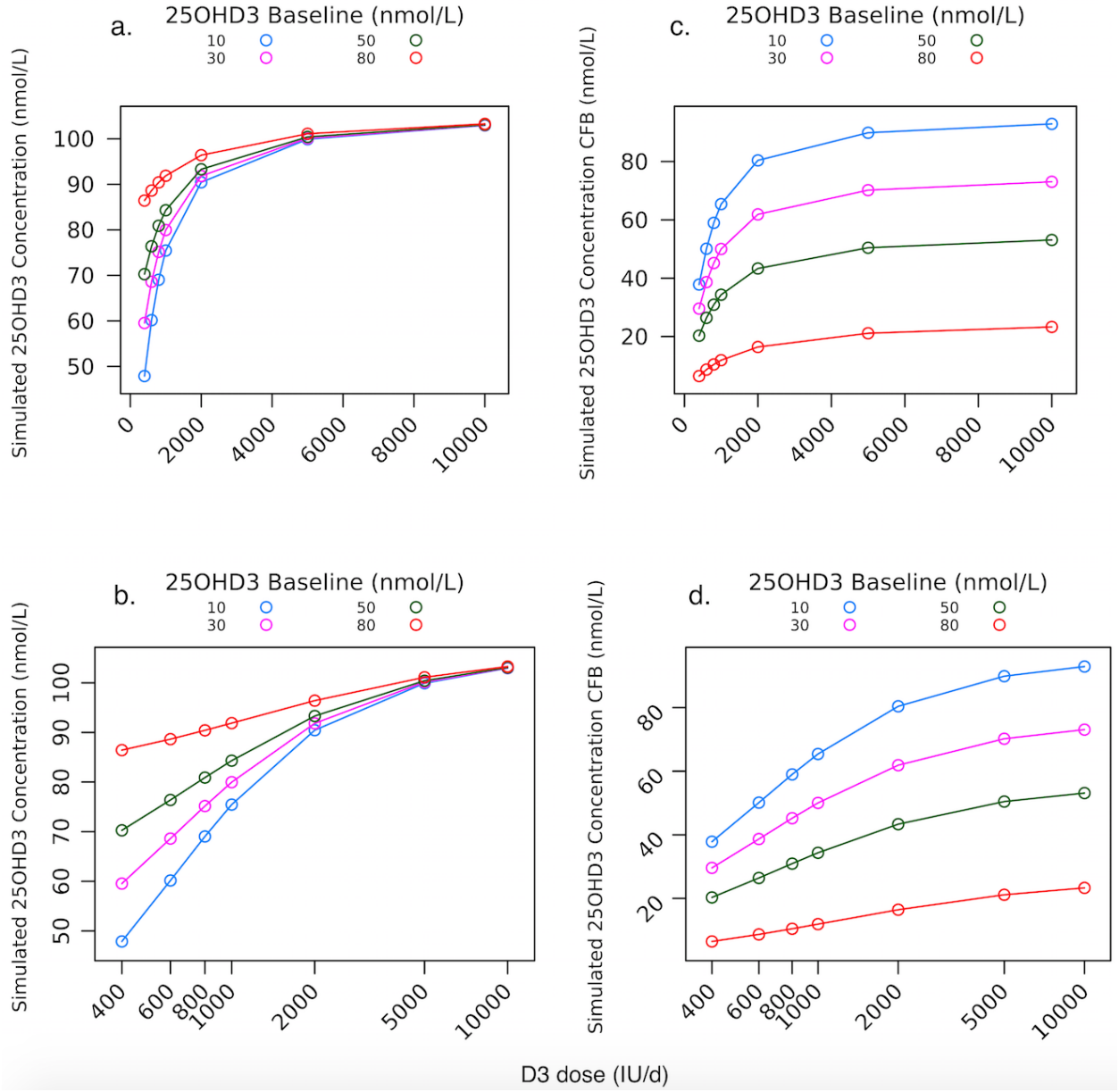
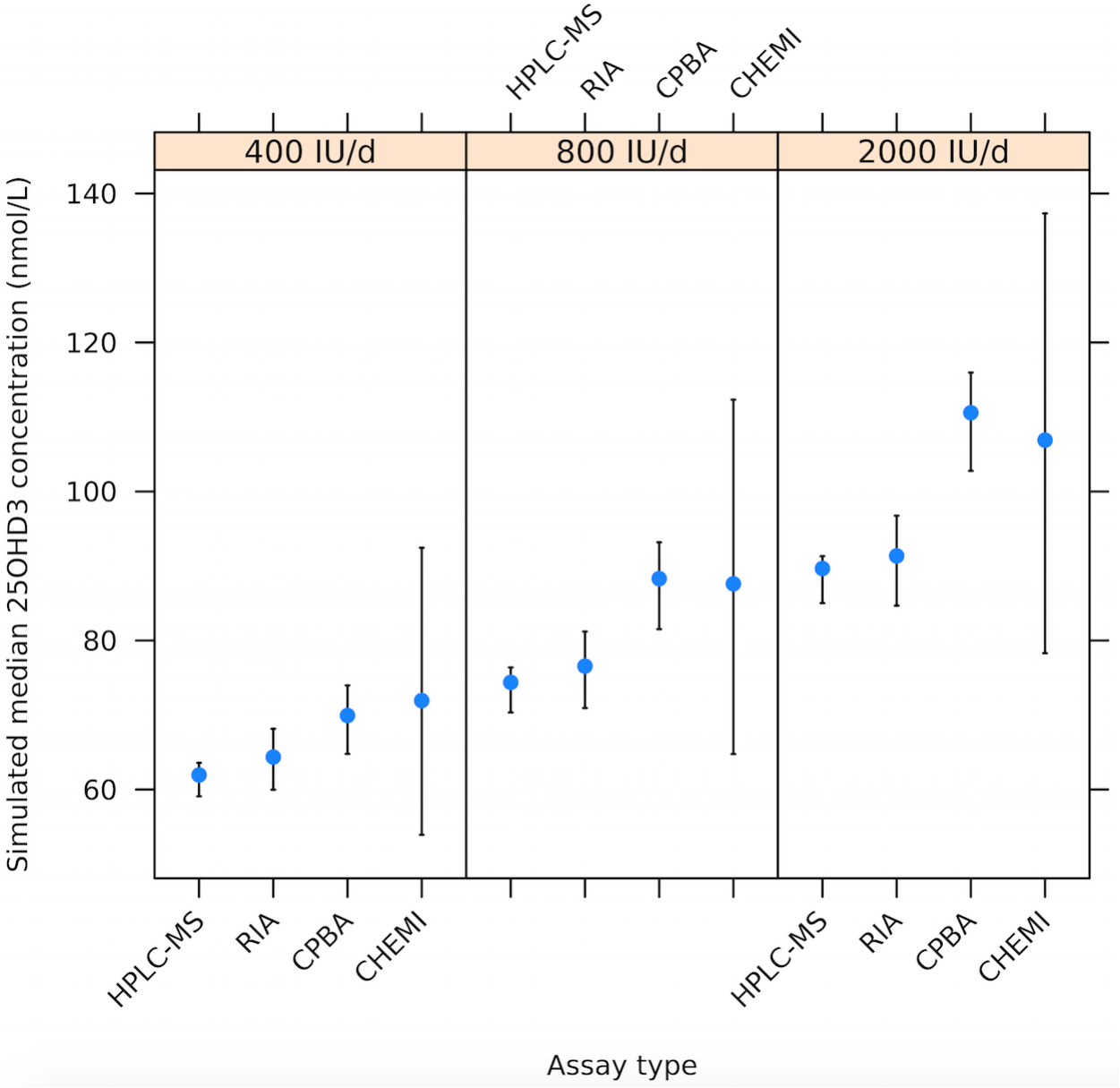


Figure 3.5: The effect of assay type on 25OHD3 measurement after daily D3 dosing for 1 year (m = 500 replicates). The vertical bars indicate the 25th and 75th percentiles of the simulated 25OHD3 median; simulations included uncertainty around the assay fixed effects parameters



Chapter 4

D2-25OHD2 Model

4.1 Methods

4.1.1 *Study Selection*

Studies were selected using specific search criteria in PUBMED to identify public-source PK data for D2 and 25OHD2. The database was searched from June 2010 until October 2015. Studies up through August 2014 were used to fit the model, while data from studies after this date were reserved for use in external validations of the model. To the extent possible, PRISMA guidelines were followed for reporting this meta-analysis [57]. To qualify for inclusion, studies had to include interventions that differed only in vitamin D2 content in healthy or osteoporotic adult subjects. Studies of individuals with disorders that could affect bone and calcium metabolism (e.g., chronic kidney disease) were excluded. Two types of studies of varying designs (e.g., randomized, non-randomized, blinded) were used in the analysis: 1) D2-D2 = D2 administered, D2 concentrations reported or 2) D2-25D2 = D2 administered, 25OHD2 concentrations reported. Principal summary measures collected included mean absolute concentrations or mean CFB concentrations, if absolute baselines were reported. Assay type was also collected as well as mean weight or BMI, if available. Search terms included the following: "vitamin D2", "ergocalciferol", "25-hydroxyvitamin D2", or "25OHD2", "randomized". The reference lists of studies found by these searches were also used to find additional qualifying studies. Data were extracted from published reports using digitizing software. Each unit of data was digitized three times and the average of the three trials was taken as the final values to be used in the analysis.

4.1.2 Graphical Data Evaluation

A graphical exploration of the observed concentration data was conducted to consider the dispositional characteristics (e.g., one- or two-compartmental) of D2 and 25OHD2. The data were also visually inspected for dose-proportionality (linear vs. non-linear kinetics).

4.1.3 Nonlinear Mixed Effects Pharmacokinetic Analysis of D2 and 25OHD2

The PK analysis for D2 and 25OHD2 was conducted using nonlinear mixed effects modeling (NONMEM® software, version 7.3, ICON Development Solutions, Hanover, MD), with first order FOCE-I. The parent (D2-D2) and metabolite (D2-25D2) data were modeled simultaneously.

The model was implemented as a system of ordinary differential equations describing mass transfer between compartments. Model development for both D2 and 25OHD2 included consideration of 1- and 2-compartment models with first-order absorption and linear or nonlinear clearances. The parameters considered for estimation for the parent and metabolite (m) models included central volume (*Vc2* or *Vcm2*, L), peripheral volume (*Vp2* or *Vpm2*, L), inter-compartmental clearance (*Q2* or *Qm2*, L/h), and D2 or 25OHD2 baseline concentration in the central compartment (*DBASE2* or *DBASEm2*, nmol/L). The duration of a zero-order bolus infusion into the D2 gut compartment was also considered for estimation (*D1*). Initial D2 or 25OHD2 amounts in the central ($A0_{D2}$, $A0_{25D2}$) and peripheral ($A0_{P,D2}$, $A0_{P,25D2}$) compartments were calculated as functions of other PK parameters.

This MBMA included both individual and arm-level data with random effects for each. These unit-level random effects entered at the same level in the model hierarchy. Observation-level or residual random effects were also included. The hierarchical random-effects model was adjusted to accommodate the mixed data by weighting the unit-level random effects (η_{kui}) and the residual random effects (ϵ_{uij}) by the inverse square-root of the unit size [58]. Therefore, data from the larger unit was weighted more heavily during the estimation process. Separate unit-level and residual variances were estimated for arms and individuals, respectively, in the final parent-metabolite model.

For both parent and metabolite, an exponential variance model was used to describe the

unexplained random variability of PK parameters across units (individuals or arms) in the form:

$$P_{\mathrm{i}} = \theta_{\mathrm{k}} \cdot e^{\frac{\eta_{\mathrm{kui}}}{\sqrt{nARM_{\mathrm{ij}}}}} \tag{4.1}$$

where P_i is the estimated parameter value for the ith unit, θ_k is the typical population value of parameter θ_k, η_{kui} are the unit-level random effects for unit i and parameter k, u is an indicator for whether the data unit is an individual or an arm, and $nARM_{ij}$ is the size of the unit for unit i at time-point j ($nARM_{ij} = 1$ for individuals). Models were explored using various random effects covariance structures.

A proportional residual variance structure for parent and metabolite was used to describe residual variability:

$$C_{\mathrm{obs,ij}} = C_{\mathrm{pred,ij}} \cdot e^{\frac{\epsilon_{\mathrm{uij}}}{\sqrt{nARM_{\mathrm{ij}}}}} \tag{4.2}$$

where $C_{obs,ij}$ is the observed concentration in unit i at time-point j, $C_{pred,ij}$ is the unit predicted concentration, and ϵ_{uij} is the proportional residual random error.

GOF for each model was assessed by the examination of the following criteria: visual inspection of diagnostic scatter plots (observed vs. predicted concentration, observed and predicted concentration vs. time, conditional weighted residuals vs. predicted concentration or time), the precision of the parameter estimates, as measured by asymptotic standard errors derived from the covariance matrix of the estimates, successful minimization with at least 2 significant digits in parameter estimates, changes in the minimum value of the objective function, and changes in the estimates of unit-level and residual variability for the specified models.

Data programming and simulations were implemented in R (v 3.1.1) [59] via the mrgsolve package (version 0.3040, Metrum Research Group, Tariffville, CT) [60]. This package uses R as the interface to a DLSODA differential equation solver (ODEPACK, November 12, 2003 version), within a C++ wrapper.

The PK analysis for D3 and 25OHD3 followed the methodology described above for D2 and 25OHD2 model development (Chapter 3). In order to simulate total 25OHD concentration values the two parent-metabolite models were combined and their metabolite outputs summed (total 25OHD = 25OHD2 + 25OHD3).

4.1.4 Model Qualification

pcVPC were performed to assess the model performance when describing the central tendency and the variability in the observed 25OHD2 data. Inter-unit and residual variability were included by sampling 1000 replicates from the inter-unit and residual variability distributions for parent and metabolite from the final model. Both simulated and observed data were summarized to find the median and a specific prediction interval (typically 5th to 95th percentiles) to describe the observed distribution of the data in a time bin. Simulated values were similarly summarized in order to describe the range of the observed data and to ensure visual clarity when plotting. Observed data at time points that went beyond the largest time bin median values were plotted at the last time bin median point.

A global sensitivity analysis (SA) was also performed on estimated parameters to evaluate the sensitivity of mean 25OHD2 and 25OHD3 concentrations to uncertainty in those parameter estimates. Uncertainty was included around the final parameter estimates by sampling ∼2000 replicates from a multivariate normal distribution centered at the final parameter estimates and having covariance equal to the covariance matrix of the estimates for the final model. The unit-level random effects distributions were not randomly sampled for these simulations. A local SA was performed on those parameters that were fixed in the final D2-25OHD2 model. The SEs that were estimated for these parameters in either the D3-25OHD3 model or the D2-D2 model (not shown) were used for the analysis; parameter values within +/- 2 SEs of the final parameter values were chosen for inclusion in the local SA.

External validations were attempted for 25OHD3 and 25OHD2, using clinical data that was not used to fit the model. External prediction-corrected visual predictive checks assessed the model performance in a similar fashion as was described above for the data used to fit the model.

4.1.5 Simulations

The relative effectiveness of D2 vs. D3 in raising metabolite levels was evaluated using population-level simulations of 25OHD2, 25OHD3 and total 25OHD concentrations, with uncertainty included around fixed effects parameter estimates. These simulations were performed for both the final D2-25OHD2 and D3-25OHD3 models. The 25OHD concentration-dose curves (simulated median 25OHD concentration, 5th and 95th percentiles) following D2 or D3 administration (<= 4000 IU/d)

for 3 months were compared, and each form's effectiveness in reaching certain 25OHD threshold values (>= 40 nmol/L, >= 50 nmol/L, >= 75 nmol/L) was evaluated.

4.2 Results

4.2.1 Study Selection

Overall 17 studies with 278 individuals were represented in this analysis; this was comprised of 15 individual-level profiles and 18 treatment arm mean profiles (Table 4.1). PO, SD and MD data were used, with D2 dosing (400-100000 IU/d). The external validation of 25OHD3 included 27 studies with 2363 individuals (46 arm units, no individual-level data) (Table 4.2). A more detailed description of individual studies, as well as a graphical display of all the observed data, is available in Appendix C (Table C, Figure C.1).

4.2.2 Graphical Data Evaluation

Biphasic kinetics was apparent in the observed log-D2 concentrations over time for two individuals, who had observations past 12 days (Appendix C, Figure C.1a). The two-compartment disposition was not visible in the remaining 7 units of data in the D2-D2 dataset, which had observations only up to 2.5 days. The 25OHD2 data did not include single dose or IV bolus-dose data to visually observe the compartmental disposition. Dose-normalized 25OHD2 observed concentration data showed some non-superimposability (Appendix C, Figure C.2b), but not as much as was observed for 25OHD3 (Appendix B, Figure B.4a). The dose-normalized curves for D2 indicated dose-proportionality (Appendix C, Figure C.2a). This inconsistency between the dose-normalized curves for D2 and 25OHD2 was investigated by considering both linear and non-linear clearances for D2.

4.2.3 Nonlinear Mixed Effects Pharmacokinetic Analysis of D2 and 25OHD2

The final model for D2 and 25OHD2 consisted of two compartment models with linear clearance for each moiety and with first order oral absorption for D2 (Figure 4.1, Equations 4.3-4.11). Parent and metabolite parameters were estimated simultaneously (Tables 4.3 - 4.4). The parent peripheral clearance ($Q2$=0.186 L/h) and volume ($Vp2$=2320 L) were fixed to estimates similar to those from

the D3 model in Chapter 3. The metabolite central volume ($Vcm2$=15 L) was fixed, for model identifiability, to the value estimated for the parent central volume when the parent data (D2-D2) was fit alone (not shown); the parent absorption rate constant ($ka2 = 0.323\ h^{-1}$) was fixed under this same condition. GOF plots for the final parent-metabolite model (Figure 4.2) indicated general goodness-of-fit, with some under-prediction of peak D2 observations.

$$\frac{d(A_{\text{gut2}})}{dt} = -ka2 \cdot A_{\text{gut2}} \tag{4.3}$$

$$\frac{d(A_{\text{D2}})}{dt} = ka2 \cdot A_{\text{gut2}} - (CL2 + Q2) \cdot C_{\text{D2}} + Q2 \cdot C_{\text{P,D2}} \tag{4.4}$$

$$+ + + \frac{d(A_{\text{P,D2}})}{dt} = Q2 \cdot C_{\text{D2}} - Q2 \cdot C_{\text{P,D2}} \tag{4.5}$$

$$\frac{d(A_{\text{25D2}})}{dt} = CL2 \cdot C_{\text{D2}} - (CLm2 + Qm2) \cdot C_{\text{25D2}} + Qm2 \cdot C_{\text{P,25D2}} \tag{4.6}$$

$$\frac{d(A_{\text{P,25D2}})}{dt} = Qm2 \cdot C_{\text{25D2}} - Qm2 \cdot C_{\text{P,25D2}} \tag{4.7}$$

$$A0_{\text{D2}} = DBASE2 \cdot Vc2 \tag{4.8}$$

$$A0_{\text{P,D2}} = DBASE2 \cdot Vp2 \tag{4.9}$$

$$A0_{\text{25D2}} = DBASEm2 \cdot Vcm2 \tag{4.10}$$

$$A0_{\text{P,25D2}} = DBASEm2 \cdot Vpm2 \tag{4.11}$$

In the above equations, A_{gut2} is the amount (nmol) of D2 in the gut; A_{D2} and $A_{P,D2}$ are the amounts of D2 (nmol) in the central and peripheral compartments, respectively; A_{25D2} and $A_{P,25D2}$ are the amounts of 25OHD2 (nmol) in the central and peripheral compartments,

respectively.

In the final model, inter-unit random effects were included on *VC2*, *DBASE2*, and *DBASEm2*. For the 25OHD2 endpoint, separate unit-level random effects and residual errors were included for individual ($\omega^2_{DBASEm2,i}$, $\sigma^2_{metab,i}$) vs. arm ($\omega^2_{DBASEm2,g}$, $\sigma^2_{metab,g}$) data. Including the separate random effects for individuals and arms improved the unit-level prediction for both arms and individuals.

Weight/BMI and assay were considered for inclusion as covariates in the 25OHD2 model because of general clinical interest, but the former was not included because of incomplete data reporting. Only 7 out of 30 data units, across 5 studies, reported BMI (mean = 27.7 kg/m^2, range =23.2-32.8); one study reported weight (59.5 kg). Assay was not included because of a lack of range in assay types, as most observations were measured by HPLC-MS.

4.2.4 Model Qualification

The 25OHD2 pcVPC was implemented for individuals and arms separately (Figure 4.3). As was the case for 25OHD3, overall the model described the central tendency of the metabolite data for both arm- and individual-level data, as most of the observed medians fall within the simulated 80% confidence interval for the median. However, the model over-estimated the inter-unit variability for both arm- and individual-level data at the 95th percentile and under-estimated the variability at the 5th percentile (not shown).

An external validation of the D3-25OHD3 model was performed using 25OHD3 PK data that was not included in the dataset used to fit the model (post August 2014). Once again, the model performed well, predicting the central tendency of the data, with some over-prediction at later time points (Appendix C, Figure C.3). External checking for the D2-25OHD2 model was not implemented because the literature search post August 2014 produced very little PK data of this type. Four studies were found which administered D2 and measured a form of the 25-hydroxylated metabolite (25OHD, 25OHD2, or 25OHD3) [61], [62], [63], [64]. From these studies, the 25OHD2 form of the metabolite was measured in only two out of the six units. The rest of the studies measured mostly 25OHD with a few measuring 25OHD3.

A global SA was performed on all the estimated parameters in the final models (D3-25OHD3 model: *DBASE*, *Vc*, *Vp*, *Q*, *Vmax*, *Km*, *CLm*, *Vcm*, *Vpm*, *Qm*; D2-25OHD2 model: *DBASE2*,

Vc2, *CL2*, *D1*, *Vpm2*, *Qm2*, *CLm2*, *DBASEm2*) to evaluate the effect of uncertainty in those parameters on conclusions about 25OHD2 and 25OHD3 exposure (25OHD3 assay = HPLC-MS) after 3 months of daily D2 or D3 dosing (400, 800, 1000, 2000, 4000, or 10000 IU/d), respectively (Figure 4.4). 25OHD3 concentrations at 3 months were most sensitive to uncertainty associated with *Vc* (trend line change in 25OHD3 concentration at 2000 IU/d D3 = 20 nmol/L, *Vc* range: 7.53-24.23 L (16.7 L change)), *Vmax* (change in 25OHD3 concentration = 17 nmo/L, *Vmax* range: 1.32-1.92 nmol/h (0.63 nmol/h change)), *CLm* (change in 25OHD3 concentration = -52 nmo/L, *CLm* range: 0.011-0.02 L/h (0.009 L/h change)), and *Qm* (change in 25OHD3 concentration = 17 nmo/L, *Qm* range: 0.02-0.083 L/h (0.063 L/h change)). Sensitivity increased with dose for parameters directly associated with the non-linear clearance of D3 (*Vmax* (at 400 and 10000 IU/d 25OHD3 change in concentration: 5 vs. 25 nmol/L), *Km* (-4 vs. 10 nmol/L)), *DBASE* at t = 0 (5 vs. 20 nmol/L)) and for *CLm* (-35 vs. -50 nmol/L). The 25OHD3 metabolite concentrations at 3 months proved relatively insensitive to changes in all other parameters. These results remained consistent across different 25OHD3 assay types (Appendix C: Figures C.4, C.5).

The results from both the global (Appendix C: Figure C.6) and local (Appendix C: Figure C.7) sensitivity analyses for the D2-25OHD2 model show that conclusions about 25OHD2 concentrations at 3 months are generally insensitive to uncertainty across all parameters. The one exception is uncertainty in *Vcm2*.

4.2.5 Simulations

Simulations of total 25OHD after 3 months of daily D2 or D3 dosing (Table 4.5, Figure 4.5, Appendix C: Figure C.8) show that, for an equivalent dose, D2 was less effective than D3 in raising 25OHD levels for low metabolite baselines (<= 20 nmol/L) and low parent doses (<2000 IU/d). The differences in metabolite concentration for given doses diminish with increasing dose and baseline, until D2 eventually became more effective than D3 in raising 25OHD levels. At an extremely deficient metabolite baseline of 5 nmol/L, 400 IU/d of D3 is sufficient (83.6% probability) for getting 50% of the population to a 25OHD concentration of >= 40 nmol/L (IOM Estimated Average (EA) [13]) by 3 months (median 25OHD = 43.5 nmol/L; 90% CI (38.0, 50.7)). At this same baseline, 800-1000 IU/d of D2 would be required to achieve this target (21.7%, 99.8% probability, respectively) in the same timeframe (25OHD median = 38.6, 45.3 nmol/L, respectively; 90% CIs

(36.0, 41.8), (42.4, 49.1)). At baseline values indicating more mild deficiency (20-30 nmol/L) 400 IU/d of D2 was sufficient (53.9%, 100% probability, respectively) for reaching 40 nmol/L by 3 months (median 25OHD = 40.3, 50.3 nmol/L; 90% CI (36.1, 46.4), (44.6, 58.6)). Across baselines, for doses >2000 IU/d, the rate of change in 25OHD after D3 supplementation decreased (at baseline (BL)= 5 nmol/L: rate = 0.043 nmol/L/IU/d for D3 doses 400-1000 IU/d, rate = 0.001 nmol/L/IU/d for D3 doses 2000-4000 IU/d; at BL= 30 nmol/L: rate = 0.03 nmol/L/IU/d for D3 doses 400-1000 IU/d, rate = 0.000875 nmol/L/IU/d for D3 doses 2000-4000 IU/d). In the case of D2, the rate stayed constant across doses and baselines (0.034 nmol/L/IU/d), whereby D2 to became comparable to and then more effective than D3 in continuing to raise 25OHD values for doses >= 1500-2000 IU/d.

Some clinicians may opt for keeping their patients above higher 25OHD thresholds (e.g. >= 50 nmol/L or >= 75 nmol/L) in order to assure adequate bone health. For these higher thresholds as well, at lower baselines, D3 was more efficient at achieving the targeted concentrations. At BL = 5 nmol/L, 600 IU/d and 2000 IU/d of D3 would be adequate (84.7%, 86.7% probability, respectively) for getting 50% of the population to >= 50 nmol/L or >= 75 nmol/L by 3 months (median 25OHD = 54.5, 86.3 nmol/L, respectively; 90% CI (47.7, 63.4), (76.0, 98.8)). These same targets would require D2 doses of >1000 IU/d and >2000 IU/d at the same baseline and timeframe. For higher baselines, the median 25OHD concentration after daily D2 doses of 2000 IU/d surpassed the 75 nmol/L threshold by a greater magnitude than was achieved subsequent to an equivalent D3 dose.

Table 4.1: Summary of studies used in MBMA for D2 and 25OHD2 (RT = route; REG = regimen)

Treatment	Endpoint	Doses (IU/d)	RT/REG	Individuals	Arms	Total Subjects	Studies
D2	D2	10000, 25000, 50000	PO/ SD	3	6	54	6
D2	25OHD2	400-100,000	PO/SD, MD	12	12	224	11
Totals				15	18	278	17

Table 4.2: Summary of studies used in 25OHD3 external validation (RT = route; REG = regimen)

Treatment	Endpoint	Doses (IU/d)	RT/REG	Individuals	Arms	Total Subjects	Studies
D3	25OHD3	200-200000	PO/ MD	0	46	2363	27

Figure 4.1: Final compartmental models for D2 and 25OHD2

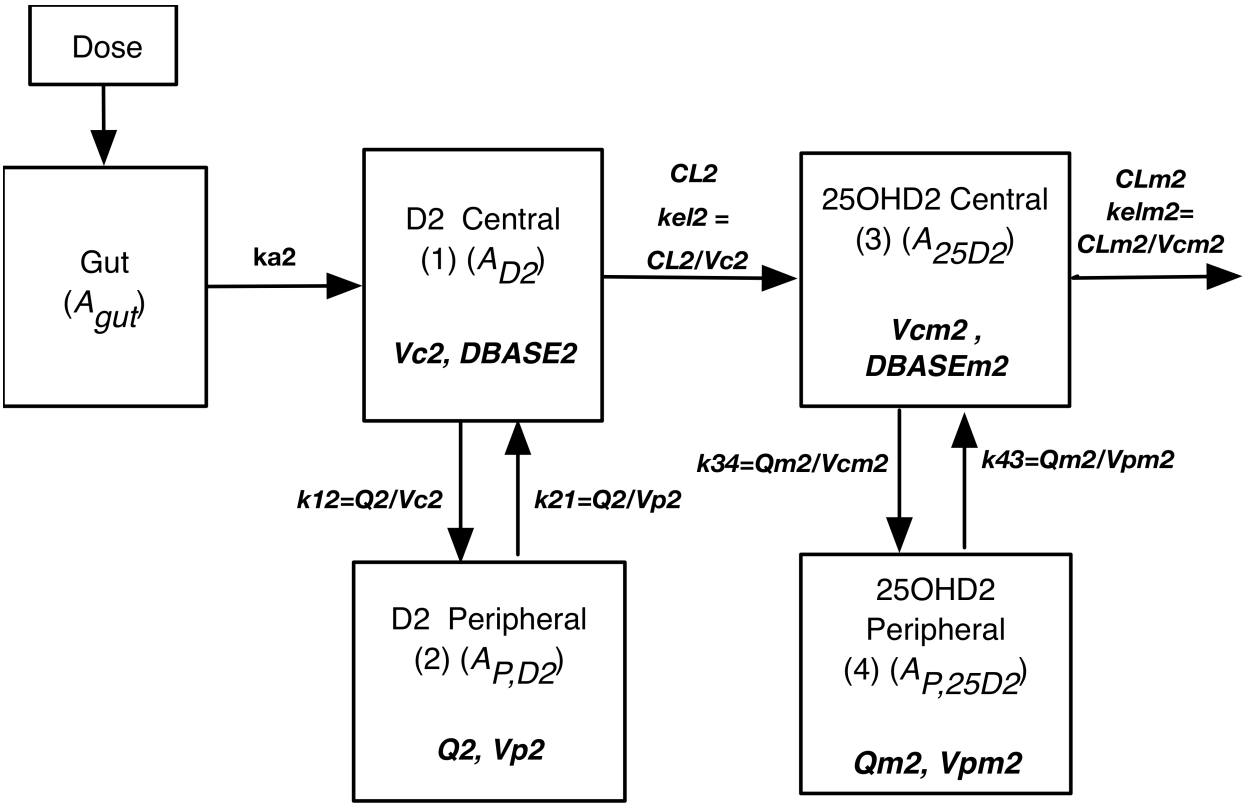


Figure 4.2: GOF diagnostics for parent-metabolite model (D2 parent a-b; 25OHD2 metabolite c-d)

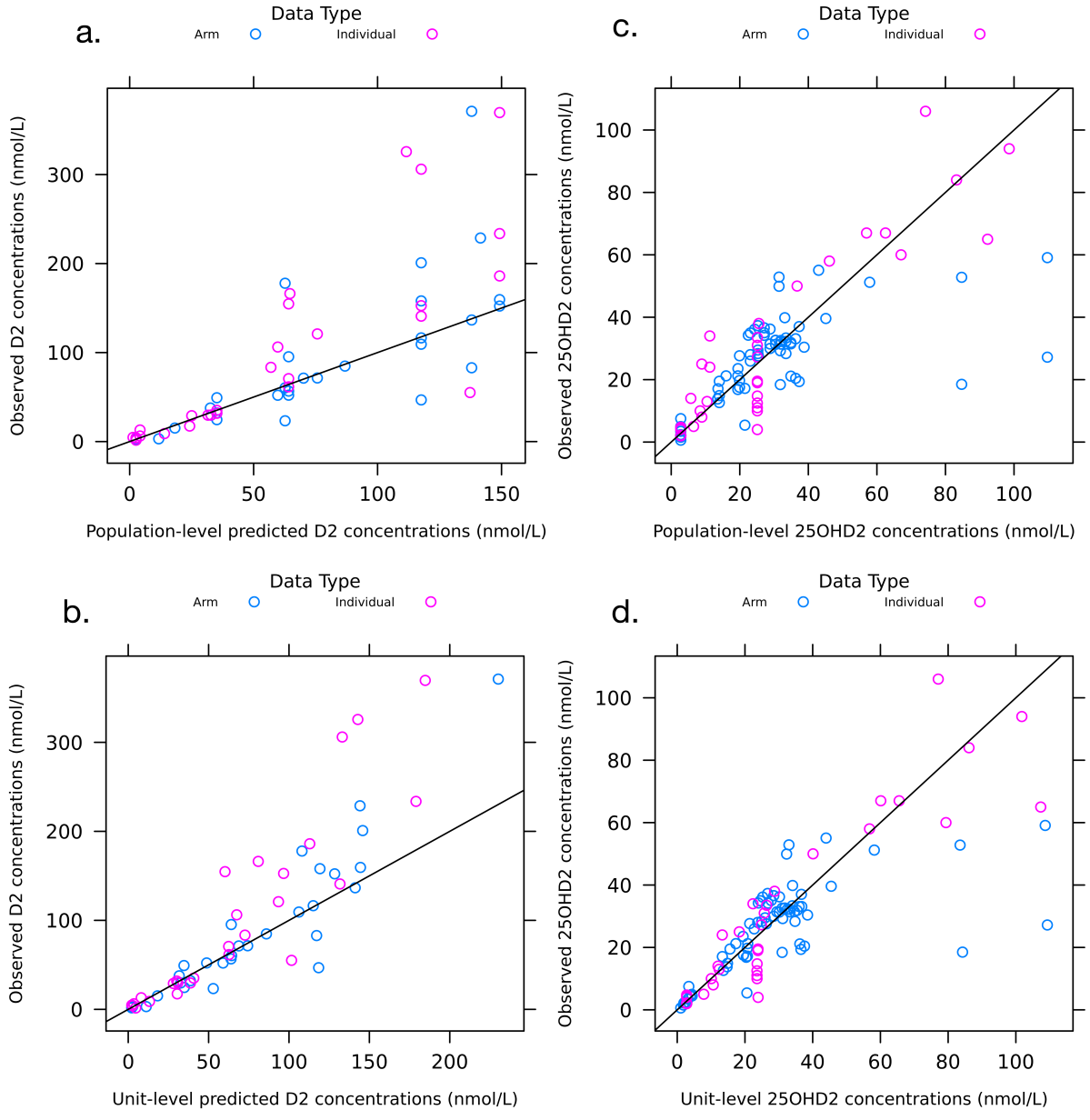


Table 4.3: D2-25OHD2 fixed effects PK parameter estimates from final parent-metabolite model (dataset: D2-25D2)[5]

Parameters	Description	Estimate (units)	SE	%RSE
Model: Parent-metabolite				
Fixed effects				
$ka2$	D2 absorption rate constant	0.323, FIX	NA	NA
$Vc2$	D2 central volume of distribution	17.1 (L)	13.7	80.1
$Vp2$	D2 peripheral volume of distribution	2320, FIX (L)	NA	NA
$Q2$	D2 inter-compartmental clearance	0.186, FIX (L/h)	NA	NA
$CL2$	D2 clearance	0.253 (L/h)	0.0682	27.0
$D1$	D2 zero-order bolus-infusion duration	5.19 (h)	4.12	79.4
$DBASE2$	D2 baseline concentration	2.58 (nmol/L)	0.584	22.6
$Vcm2$	25OHD2 central volume of distribution	15, FIX (L)	NA	NA
$Vpm2$	25OHD2 peripheral volume of distribution	72.9 (L)	58.1	80.0
$Qm2$	25OHD2 inter-compartmental clearance	0.0631 (L/h)	0.0259	41.0
$CLm2$	25OHD2 clearance	0.0264 (L/h)	0.0154	58.3
$DBASEm2$	25OHD2 baseline concentration	2.79 (nmol/L)	0.234	8.39

[5] All fixed effects estimates are relative to bioavailability.

Table 4.4: D2-25OHD2 random effects estimates from final parent-metabolite model (dataset: D2-25D2)[6]

Parameters	Description	Estimate	SE	%RSE
Model: Parent-metabolite				
Inter-unit random effects (% CV)				
ω^2_{Vc2}	Variance of inter-unit random effects on *Vc2*	0.094 (30.6)	0.127	135
ω^2_{DBASE2}	Variance of inter-unit random effects on *DBASE2*	0.167 (40.9)	0.121	72.3
$\omega^2_{DBASEm2,g}$	Variance of arm inter-unit random effects on *DBASEm2*	0.917 (95.7)	0.753	82.2
Residual effects (% CV)				
σ^2_{parent}	Variance of residual error for D2 data	0.247 (49.7)	0.095	38.5
$\sigma^2_{metabolite,i}$	Variance of residual error for individual metabolite data	0.052 (22.8)	0.020	39.2
$\sigma^2_{metabolite,g}$	Variance of residual error for arm metabolite data	0.337 (58.0)	0.049	14.7

[6] All unit-level random and residual effects for arm data are weighted by n: $\omega^2_a = \omega^2_{a,raw}$ /n and $\sigma^2_b = \sigma^2_{b,raw}$ /n, where ω^2_a is the unit-level variance for parameter a and σ^2_b is the residual variance for b (SD or MD) for median unit size (n); $\omega^2_{a,raw}$ is the un-weighted unit-level variance for parameter a and $\sigma^2_{b,raw}$ is the un-weighted residual variance for b that NONMEM reports. Random effect SE for arm data ($COV MATRIX = S) are also weighted by n: SE(ω^2_a) = SE($\omega^2_{a,raw}$/n); SE(σ^2_b) = SE($\sigma^2_{b,raw}$ /n). The median n for the collective dataset (D2-D2, D2-25D2) is 3. The covariance matrix of the estimates, as well as model code files, are included in Appendix A.

Figure 4.3: Prediction-corrected visual predictive checks for a) both arm- and individual-level 25OHD2 data (D2-25D2 dataset) b) arm-level data only c) individual-level data only. Solid red line is simulated median; red band is the simulated 80% confidence interval around the simulated median; black dots are the observed medians at a given time bin; black horizontal bars indicate time bin ranges

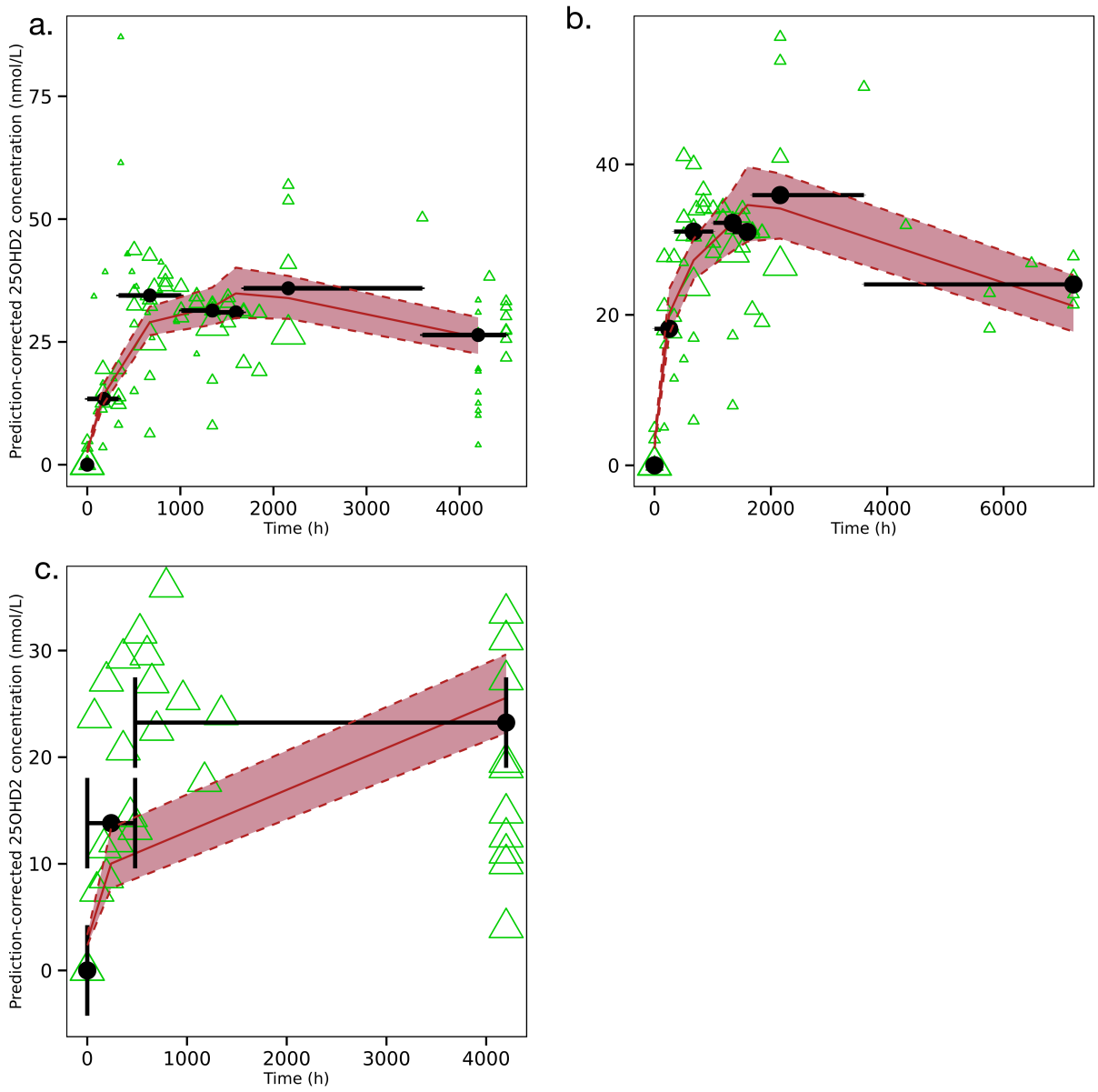


Figure 4.4: Results from the global sensitivity analysis on parameters related to D3 and 25OHD3 kinetics. The solid lines are trendiness through 2000 replicates of simulated 25OHD3 data across different parameter values, resulting from 3 months of varying doses of Vitamin D3

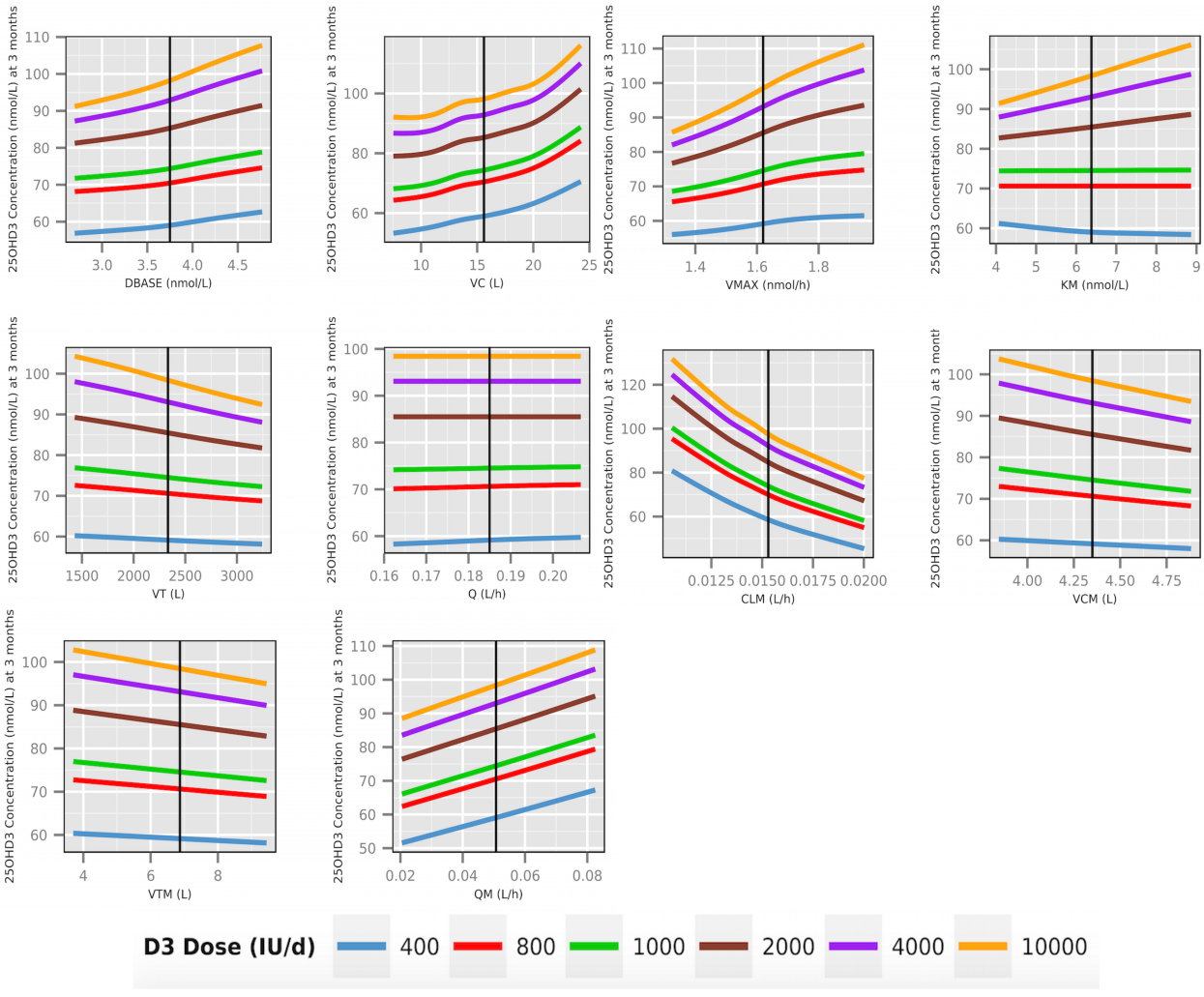


Figure 4.5: Simulated median total 25OHD concentration across baseline 25OHD (panels) after 3 months of D2 or D3 dosing; red/blue = 25OHD concentration resulting from D2/D3 dosing; error bars indicate the 5th and 95th percentiles of the simulated 25OHD median concentration, which were simulated by including uncertainty around the fixed effects parameters in the final models (25OHD3 assay = HPLC-MS); vertical lines indicate 25OHD thresholds

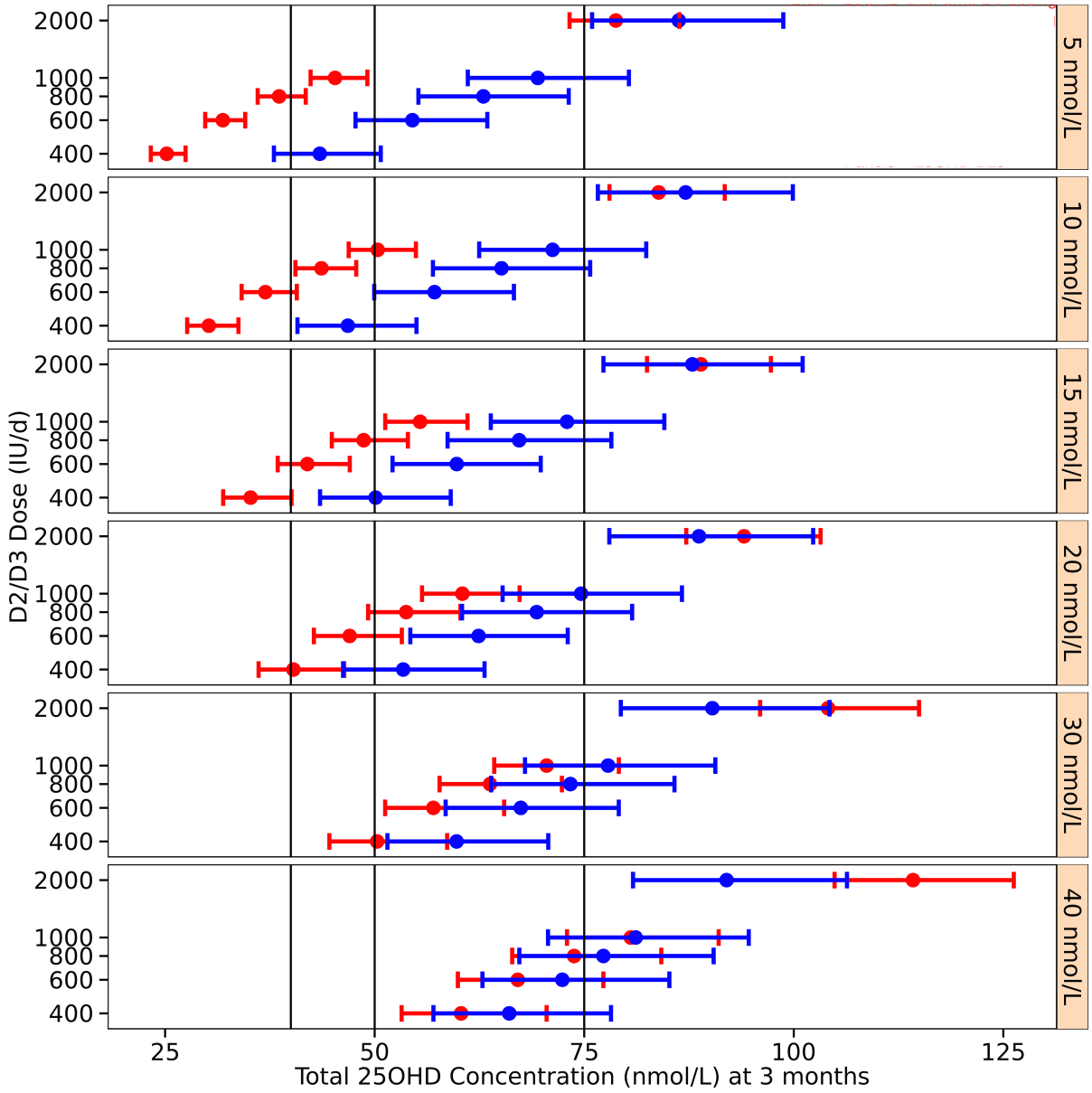


Table 4.5: 25OHD exposure following 3 months of D2 or D3 dosing[7]

D2/D3 Dose (IU/d)	25OHD BL (nmol/L)	TRT	LB (nmol/L)	Med (nmol/L)	UB (nmol/L)	%P(25OHD >=40 nmol/L)	%P(25OHD >=50 nmol/L)	%P(25OHD >=75 nmol/L)
400	5	D2	23.3	25.2	27.4	0	0	0
		D3	38.0	43.5	50.7	83.6	6.58	0
800	5	D2	36.0	38.6	41.8	21.7	0	0
		D3	55.2	63.0	73.2	100	100	2.7
2000	5	D2	73.3	78.8	86.4	100	100	86.7
		D3	76.0	86.3	98.8	100	100	97.0
400	10	D2	27.6	30.2	33.7	0	0	0
		D3	40.8	46.8	55.0	96.7	24	0
800	10	D2	40.5	43.7	47.8	97.7	0.982	0
		D3	57.0	65.1	75.7	100	94.7	5.89

Table 4.5: 25OHD exposure following 3 months of D2 or D3 dosing[7]

D2/D3 Dose (IU/d)	25OHD BL (nmol/L)	TRT	LB (nmol/L)	Med (nmol/L)	UB (nmol/L)	%P(25OHD >=40 nmol/L)	%P(25OHD >=50 nmol/L)	%P(25OHD >=75 nmol/L)
2000	10	D2	78.0	83.9	91.8	100	100	99.4
		D3	76.6	87.1	100	100	100	97.5
400	15	D2	31.9	35.2	40.1	5.21	0	0
		D3	43.5	50.1	59.1	99.4	51.1	0
800	15	D2	44.9	48.7	54.0	100	32.5	0
		D3	58.7	67.2	78.2	100	100	11.4
2000	15	D2	82.5	89.0	97.3	100	100	100
		D3	77.3	87.9	101.1	100	100	97.9
400	20	D2	36.2	40.3	46.4	53.9	0.737	0
		D3	46.2	53.4	63.1	100	76.3	0.049

Table 4.5: 25OHD exposure following 3 months of D2 or D3 dosing[7]

D2/D3 Dose (IU/d)	25OHD BL (nmol/L)	TRT	LB (nmol/L)	Med (nmol/L)	UB (nmol/L)	%P(25OHD >=40 nmol/L)	%P(25OHD >=50 nmol/L)	%P(25OHD >=75 nmol/L)
800	20	D2	49.2	53.8	60.2	100	90.6	0
		D3	60.4	69.3	80.7	100	100	18.8
2000	20	D2	87.2	94.1	103.2	100	100	100
		D3	77.3	87.9	101.1	100	100	97.9
400	30	D2	44.6	50.3	58.6	99.9	52.1	0
		D3	51.5	60.0	70.7	100	97.6	1.47
800	30	D2	57.7	63.8	72.3	100	100	1.82
		D3	63.9	73.4	85.8	100	100	39.8
2000	30	D2	96.0	104.1	115.0	100	100	100
		D3	79.4	90.3	104.3	100	100	100

Table 4.5: 25OHD exposure following 3 months of D2 or D3 dosing[7]

D2/D3 Dose (IU/d)	25OHD BL (nmol/L)	TRT	LB (nmol/L)	Med (nmol/L)	UB (nmol/L)	%P(25OHD >=40 nmol/L)	%P(25OHD >=50 nmol/L)	%P(25OHD >=75 nmol/L)
400	40	D2	53.2	60.3	70.5	100	99.3	1.13
		D3	57.0	66.1	78.2	100	99.9	10
800	40	D2	66.4	73.8	84.2	100	100	41.85
		D3	67.3	77.3	90.4	100	100	63.6
2000	40	D2	104.9	114.2	126.3	100	100	100
		D3	80.8	92.0	106.4	100	100	100

[7] TRT = Treatment; LB = simulated 25OHD lower concentration bound (5th percentile) of 90% confidence interval around median; UB= simulated 25OHD upper concentration bound (95th percentile) of 90% confidence interval around median; Med = simulated median 25OHD concentration; %P() = percent probability

Chapter 5

Vitamin D-MSPM Integration

5.1 Methods

5.1.1 Study Selection

Similar to the study selections for the model development of Vitamin D3, D2 and their 25-hydroxylated metabolites, studies were selected using public search criteria in PUBMED to identify public-source bone-marker concentration data (serum calcitriol ($1,25(OH)_2D$), serum PTH, serum calcium, serum P1NP, serum CTX, serum BSAP) and BMDLS, following Vitamin D3 or D2 administration, with or without calcium administration. The majority of studies included were those that were used in the PK analyses for Vitamin D3 and D2 and were of varying study design (randomized, non-randomized, blinded, and open label). To be included in the current analysis, studies had to include Vitamin D3 or D2 interventions and bone-marker or BMDLS measurements in healthy or osteoporotic adult populations. Studies of individuals with disorders that could affect bone and calcium metabolism, such as chronic kidney disease, were excluded. Bone-marker concentration units were converted to pmol/L (serum calcitriol), pg/mL (serum PTH, serum CTX), mmol/L (serum calcium), or μg/L (serum P1NP, serum BSAP). BMDLS measurement units were converted to g/cm^2 and measurements recorded in other relative units (e.g., T-scores) or without recorded baselines were excluded from the analysis. Principal summary measures collected included mean absolute bone-marker concentrations and bone-mineral densities or mean CFB or PCFB measurements, if absolute baselines were reported. Search terms included the following: "vitamin D3", "cholecalciferol", "25-hydroxyvitamin D3", or "25OHD3", "Vitamin D & bone mineral density", "Vitamin D & bonemarker levels", "randomized." Data were extracted from published reports using digitizing software (Arizona GraphClick, version 2.9.2). Each unit of data was digitized three times and the

average of the three trials was taken as the final values to be used in the analysis

5.1.2 Modeling Structure Development

The development of two-compartment models for Vitamin D3 and Vitamin D2 and their 25-hydroxylated metabolites has been described in Chapters 3-4. To describe the conversion of 25OHD to calcitriol, the final PK models for each of those forms of the hormone were integrated, at the level of the calcitriol compartment , with a previously-published MSPM that described calcium, PTH, and bone-remodeling [1] . This integrated model was coded using the *mrgsolve* package in R [2] and involved estimation of a scaling factor for calcitriol production and re-estimation of an exponent-gamma term, related to osteoclast bone resorption, that translated predicted changes in bone-markers to changes in BMDLS, using the *Nelder-Mead* maximum-likelihood optimization method in the *stats* R package.

The MSPM was implemented as a series of ordinary differential equations, describing mass transfer between compartments. The Vitamin D model was integrated into the systems model by considering various structures (e.g., EMAX model, EMAX model with inhibition on the Michaelis-Menten parameter, power model) to describe the conversion of 25OHD3 into calcitriol, with the latter already present in the MSPM. Potential structures were compared by simulating predictions of BMDLS and all relevant bone-markers, given a reasonable range of values for parameter(s) of interest, and overlaying the simulation with the observed data to determine the optimal structure. Parameter initial conditions during the optimization process were determined by visual inspection of these comparative simulations. A proportional residual variance was estimated for the analyzed endpoint and was inversely weighted by the sample size of the unit. GOF for each model was assessed by examining by visual inspection observed and predicted concentration vs. time plots and observed vs. predicted concentration diagnostic plots.

5.1.3 Model Qualification

The final integrated model was qualified by predicting into observed data from endpoints that were not included during the parameter optimization process. A sensitivity analysis was performed by predicting into these endpoints at the mean parameter value and point estimate +/- 50%*point estimate of the estimated parameter.

5.1.4 Simulations

Population-level simulations were implemented with the final model to evaluate the inverse relationship between calcitriol baseline (BL = 50, 70, 90, 100, 110 pmol/L) and calcitriol response over time following 1 year of supplementation of 3 different D3 doses (400, 800, 1000 IU/d). Other population-level simulations evaluated the relationship between 25OHD3 levels and changes in serum PTH and BMDLS, following Vitamin D3 dosing (0-10000 IU/d) for 1 year with our without 1000 mg/d of calcium. Another simulation was implemented by simulating the mean change in BMDLS and serum PTH, following one of two different types of dosing regimens: Vitamin D3 alone (400-5000 IU/d for 1 year) or Vitamin D3 plus calcium (400-5000 IU/d D3, 0-1000 mg/d calcium for 1 year).

5.2 Results

5.2.1 Study Selection

The scaling factor for calcitriol production was estimated using serum calcitriol concentration data following Vitamin D3 or D3CA supplementation from across 18 studies and 39 treatment arms (Table 5.1) with a median arm size of n = 32 subjects. The BMDLS gamma-parameter was re-estimated using BMDLS measurements following Vitamin D3 or D3CA supplementation across 9 studies and 13 treatment arms with a median arm size of n = 82 subjects. All of the data used to fit the calcitriol and BMDLS model was oral, multiple-dose data over a wide range of Vitamin D3 and calcium doses (357 IU/d-50000 IU/d, 250-1350 mg/d). The remainder of the observed bone-marker data (serum PTH, calcium, CTX, BSAP, P1NP) was used to validate the model by simulating into the observed concentrations after Vitamin D3 with or without calcium supplementation. This validation data was taken from across 58 different studies and 107 treatment arms with a median arm size of n = 39 subjects. As with the estimation dataset, the validation data was oral with a mix of single and multiple-dose measurements with a wide range of Vitamin D3 and calcium doses (29-300000 IU/d, 320-1500 mg/d). The calcitriol and BMDLS estimation datasets, as well as the validation dataset had a total of 106, 41, and 550 observations, respectively, and average sample sizes of of 9, 4, and 5 observations per treatment arm. Data resulting from Vitamin D2 dosing was not included in the analysis because of a paucity of data following the administration of this form of the hormone (only 1 arm measured calcitriol following Vitamin D2). Therefore, only the effect

of Vitamin D3 and 25OHD3 PK on the MSPM was evaluated. A graphical summary of observed data as well as a more detailed summary of both the estimation and validation datasets by study is available in Appendix D (Table D, Figures D.1 - D.7).

5.2.2 Model Structure Development

A power model was chosen as the optimal structure for describing the conversion between 25OHD3 and calcitriol (Equations 5.1-5.4, Figure 5.2), with the gamma exponent parameterized as a function of inverse calcitriol amount ($A_{1,25(OH)_2D3}$), reflecting the observed inverse relationship between calcitriol baseline and calcitriol change from baseline (Appendix D, Figure D.8). The parameter θ_1, as well as a residual error variance weighted by observed arm sample size, n ($\sigma^2_{calcitriol}/\sqrt{n}$), were estimated by fitting the model to calcitriol concentration data following Vitamin D3 or D3CA supplementation ($\theta_1 = 638.1$, $\sigma^2_{calcitriol} = 150.6$ for a median observed sample size n = 19) (Figure 5.1a). The model's calcitriol baseline was empirically calculated by adjusting the initial rate of 1-alpha-hydroxylase production ($AOH0$) by a factor of the observed baseline calcitriol concentration ($C_{1,25(OH)2D3,OBS}$), such that, at an observed calcitriol baseline of 90 pmol/L (typical value for healthy individuals), $AOH0 = 126$ mmol/h. This allows differences in observed calcitriol baseline to effect the rate of calcitriol production in the model through adjustment of initial 1-alpha-hydroxylase production.

$$AOH0 = \frac{A0_{1,25(OH)_2D3} * 9}{C_{1,25(OH)2D3,OBS}} \tag{5.1}$$

$$\gamma = \frac{\theta_1}{A_{1,25(OH)_2D3}} \tag{5.2}$$

$$C25D3scale = \frac{C0_{25D3}}{(\frac{T69 * A0_{1,25(OH)_2D3}}{AOH0})^{\frac{1}{\gamma}}} \tag{5.3}$$

$$\frac{d(A_{1,25(OH)_2D3})}{dt} = (\frac{C_{25D3}}{C25D3scale})^{\gamma} * AOH - T69 * A_{1,25(OH)_2D3} \tag{5.4}$$

where AOH is the alpha-hydroxylase production rate (mmol/h), $A0_{1,25(OH)_2D3}$ is the initial calctriol amount (pmol), C_{25D3} is the initial 25OHD3 concentration (63 nmol/L), T69 (0.1

h^-1) is a first-order elimination rate constant for calcitriol [1], and *C25D3scale* is a scaling factor that was solved, assuming the steady-state assumption at t=0.

BMDLS measurements following Vitamin D3 and D3CA supplementation were used to re-estimate the gamma-exponent parameter related to osteoclast bone resorption (gammaOCls = 0.038) in the differential equation describing the change in BMDLS in the original MSPM publication [1] (Figure 5.1b). As with calcitriol, a residual error variance weighted by observed arm sample size, n, was also estimated for BMDLS ($\sigma^2_{BMDLS} = 1.56$ for a median observed sample size n = 82).

5.2.3 Model Qualification

Overall the model described well the central tendency of observed data from endpoints not included during the optimization process (serum PTH, serum calcium, serum BSAP, serum CTX, serum P1NP) (Figures 5.3-5.4; Appendix D, Figures D.9-D.12). Generally the model better predicted data following Vitamin D3 administration alone than it did data following D3CA supplementation. The model predicted a larger decrease in CTX than was present in most of the observed data.

5.2.4 Simulations

Relationship Between Calcitriol Baseline and Response

Results from population-level simulations describing the relationship between calcitriol baseline and response indicated that, for a given dose and treatment time, more calcitriol is produced at lower baselines than at higher baselines (Figure 5.5). There was a linear increase in calcitriol until concentrations were >10 pmol/L, after which concentrations began to plateau.

Relationship between 25OHD3 and BMDLS and PTH

Results from population level simulations describing the relationship between changes in serum PTH/BMDLS and 25OHD3 concentration indicated, that for given 25OHD3 levels, BMDLS/serum PTH increase/decrease was greater following D3CA than following Vitamin D3 alone (Figure 5.6). Consequently, calcium regimens required less Vitamin D3 dose and metabolite levels to reach particular PTH or BMDLS targets (Tables 5.2 - 5.3). For Vitamin D3 supplementation with or without calcium, BMDLS increased by 2.5% and 2.1%, respectively, while serum PTH

concentrations dropped from 50 to 23 and 57 to 28 pg/mL (-50.9%, -54%), respectively, with increasing 25OHD3 concentrations from 43 to 102 nmol/L (Figure 5.6). For 25OHD3 >= 70 nmol/L, the rate of increase/decrease for BMDLS/serum PTH declined, due to the saturable non-linear clearance of D3 (Chapter 3) (25OHD3 40-70 nmol/L, PTH/BMDLS decreased/increased 70%/5%; 25OHD3 70-100 nmol/L, PTH/BMDLS decreased/increased 27%/3.67%). This resulted in calcium supplementation being more potent at higher 25OHD3 concentrations when Vitamin D3 is less effective at raising 25OHD3 levels. For a given 25OHD3 level (43-102 nmol/L), the difference between serum PTH/BMDLS for Vitamin D3 alone vs. D3CA (DIFF = ((value after Vitamin D3 - value after Vitamin D3 plus calcium)/value after Vitamin D3)*100) ranged from -17.8% to -7.89% for serum PTH and 0-0.4% for BMDLS, with the largest absolute differences being at higher 25OHD3 concentrations (at 25OHD3 = 50 nmol/L: PTH DIFF = -8.5%, BMDLS DIFF = 0.05%; at 25OHD3 = 102 nmol/L: PTH DIFF = -17.86%, BMDLS DIFF = 0.4%). Differences in required Vitamin D3 doses and metabolite levels with or without calcium to reach target serum PTH or BMDLS levels is also greater at higher metabolite concentrations (BMDLS target = 0.5%, 25OHD3 target with or without calcium = 50 nmol/L, Vitamin D3 dose = Vitamin D3 dose plus 1000 mg/d calcium = 300 IU/d for 1 year; BMDLS target = 2.0%, 25OHD3 target with/without calcium = 87/97% nmol/L, Vitamin D3 dose = 5000 IU/d for 1 year, Vitamin D3 plus 1000 mg/d calcium = 2000 IU/d for 1 year). The influence of assay type on the measure 25OHD3 level necessary for reaching specific BMDLS or PTH targets is summarized in Tables D.1-D.6 in Appendix D.

Effect of Vitamin D3 or Vitamin D3 plus Calcium Administration on BMDLS and Serum PTH

Results from population-level simulations, looking at the effect of different Vitamin D3/calcium regimens on BMDLS and PTH, demonstrated increasing BMDLS levels and decreasing PTH levels with increasing Vitamin D3 and calcium doses (Figure 5.7; Appendix D, Figure D.13). The change in BMDLS or serum PTH across increasing Vitamin D3 doses within a constant calcium group was greater than across increasing calcium doses within a constant Vitamin D3 dosing group (e.g., for calcium dose = 0 the change in BMDLS/serum PTH at 1 year from 400-2000 IU/d D3 was 0.685% and -18.12%, respectively ; for Vitamin D3 dose = 2000 IU/d, the change in BMDLS/serum PTH at 1 year from 0-1000 mg/d calcium was 0.534% and -3.04%, respectively). Therefore, the results

indicated that, during combination therapy, Vitamin D3 supplementation is the more effective component supplement at raising BMDLS levels and decreasing serum PTH levels.

Table 5.1: Summary of bone-marker and BMDLS studies used in MBMA with systems bone model (RT = route; REG = regimen; F = data used to fit model; V = data used to externally validate model by simulatio

Treatment	Endpoint	Doses	RT/REG	Arms	Total Subjects	Studies	Use
D3 only	serum calcitriol	357-50000 IU/d	PO/MD	33	839	13	F
D3 only	serum PTH	29-100000 IU/d	PO/MD, SD	64	1879	31	V
D3 only	serum calcium	29-200000 IU/d	PO/MD	49	1492	25	V
D3 only	serum P1NP	400, 1000 IU/d	PO/MD	2	159	1	V
D3 only	serum BSAP	400-2000 IU/d	PO/MD	8	148	4	V
D3 only	BMDLS	400-3571 IU/d	PO/MD	5	403	3	F
D3 + Ca	serum calcitriol	400-2000 IU/d; 600-1300 mg/d	PO/MD	6	388	5	F
D3 + Ca	serum PTH	400-300000 IU/d; 320-1500 mg/d	PO/MD, SD	24	3092	18	V
D3 + Ca	serum calcium	400-5000 IU/d; 320-1350 mg/d	PO/MD	13	2821	13	V
D3 + Ca	serum P1NP	400 IU/d; 800 mg/d	PO/MD	1	32	1	V
D3 + Ca	serum BSAP	400-1000 IU/d; 500-1500 mg/d	PO/MD	5	532	4	V
D3 + Ca	BMDLS	400-5000 IU/d; 250-1350 mg/d	PO/MD	8	646	6	F

Figure 5.1: GOF diagnostics for a) serum calcitriol and b) BMDLS resulting from Vitamin D3 or D3CA supplementation)

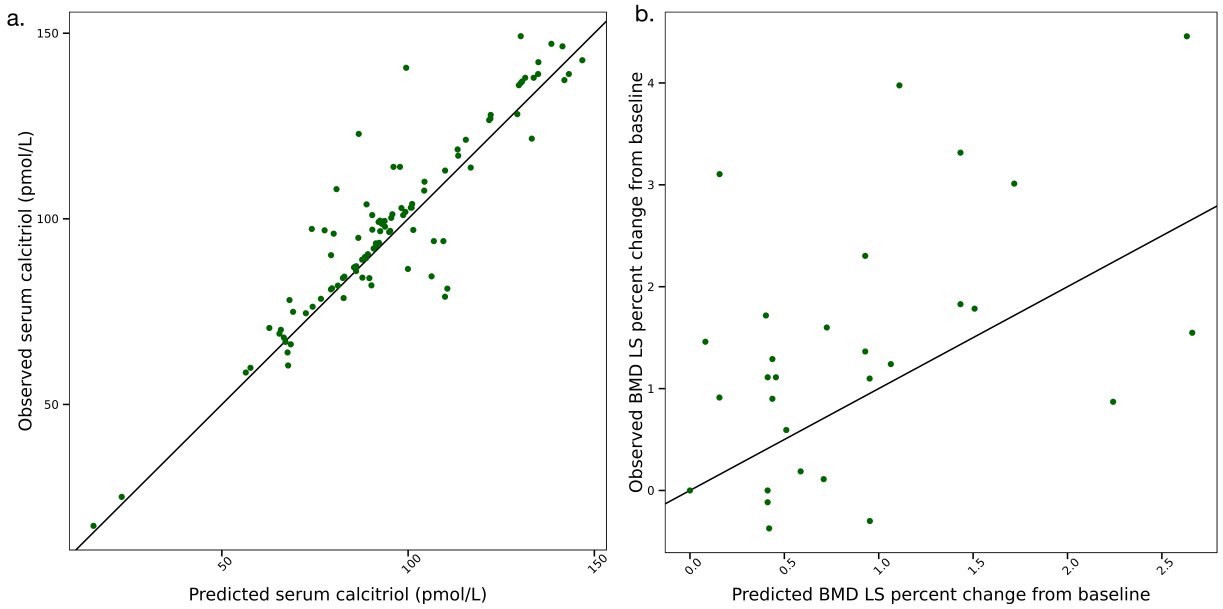


Figure 5.2: Integrating the D3-25OHD3 model with the MSPM [1]

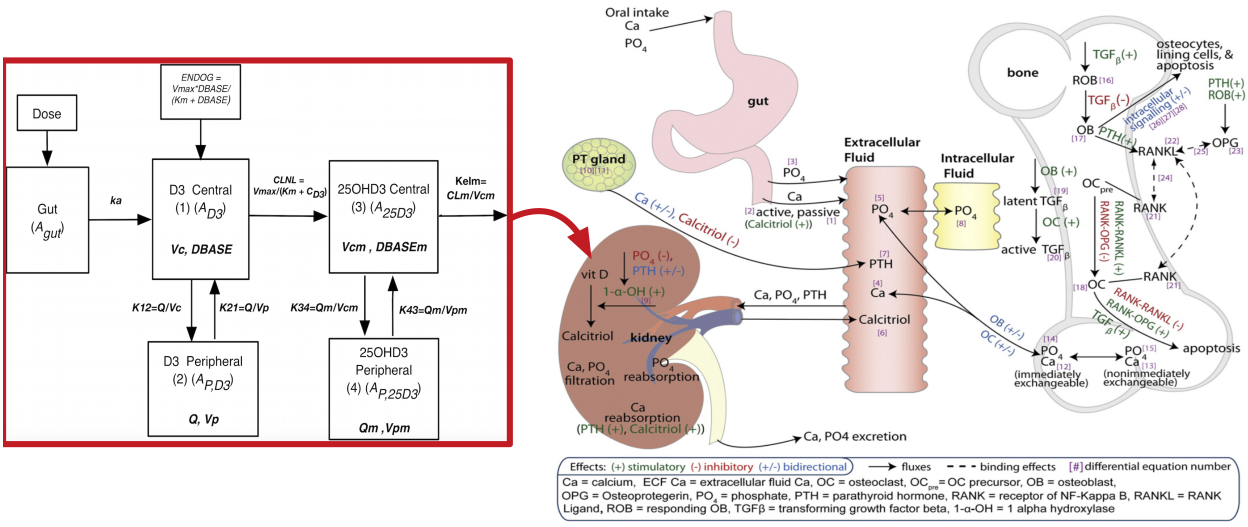


Figure 5.3: Sample of final model predictions into observed data not included in the optimization process for a) serum PTH following Vitamin D3 dosing alone b) serum PTH following D3CA dosing c) serum calcium following Vitamin D3 dosing alone d) serum calcium following D3CA dosing. Peach horizontal strip indicates Vitamin D3 dose, calcium dose, and arm ID, respectively. Peach vertical strip indicates dependent variable and status of calcium administration (CaTrt = 0, no calcium dosing; CaTrt = 1, calcium dosing)

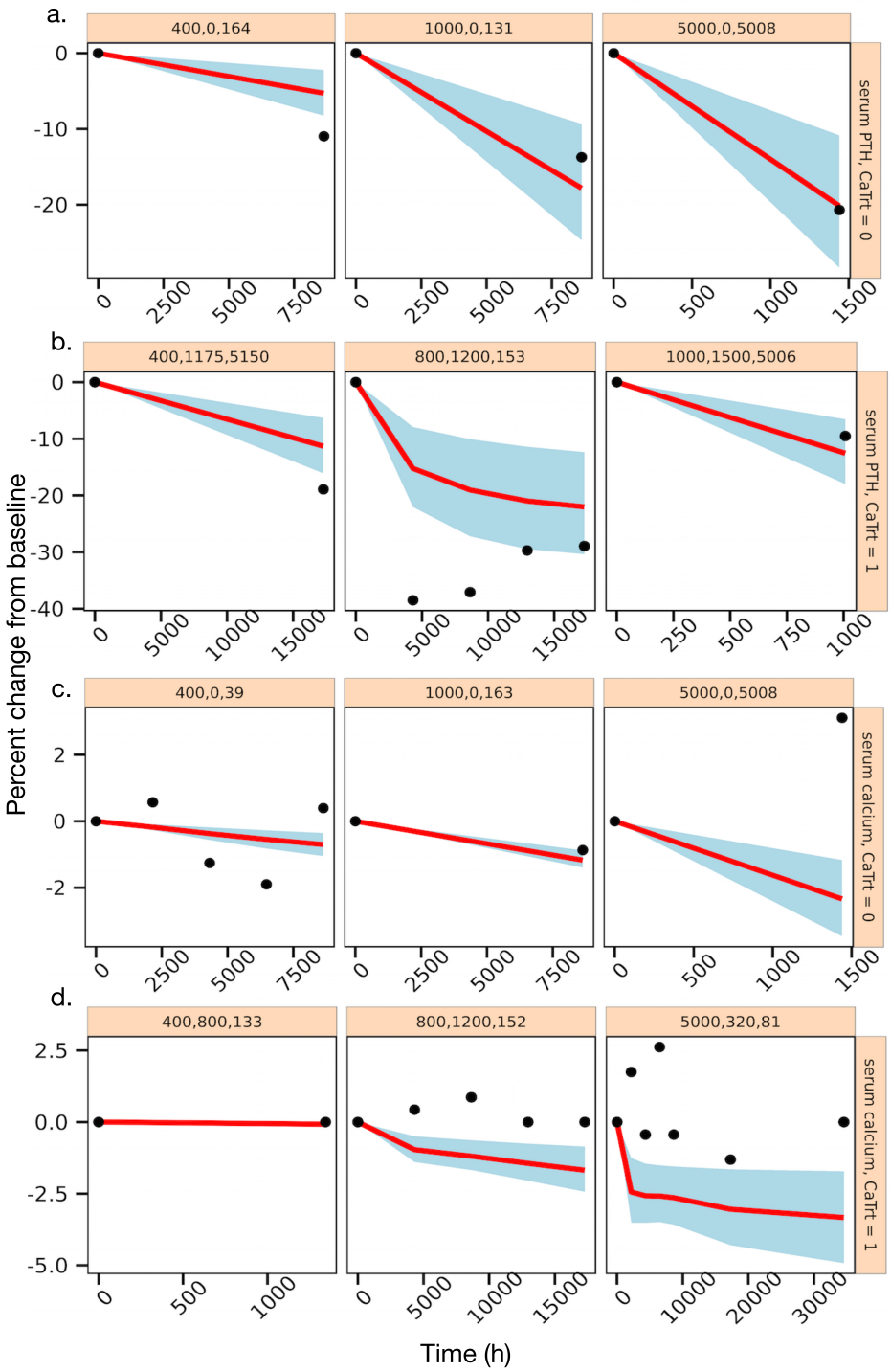


Figure 5.4: Sample of final model predictions into observed data not included in the optimization process for a) serum CTX following Vitamin D3 dosing alone b) serum CTX following D3CA dosing c) serum BSAP following Vitamin D3 dosing alone d) serum BSAP following D3CA dosing e) serum P1NP. Peach horizontal strip indicates Vitamin D3 dose, calcium dose, and arm ID, respectively. Peach vertical strip indicates dependent variable and status of calcium administration (CaTrt = 0, no calcium dosing; CaTrt = 1, calcium dosing)

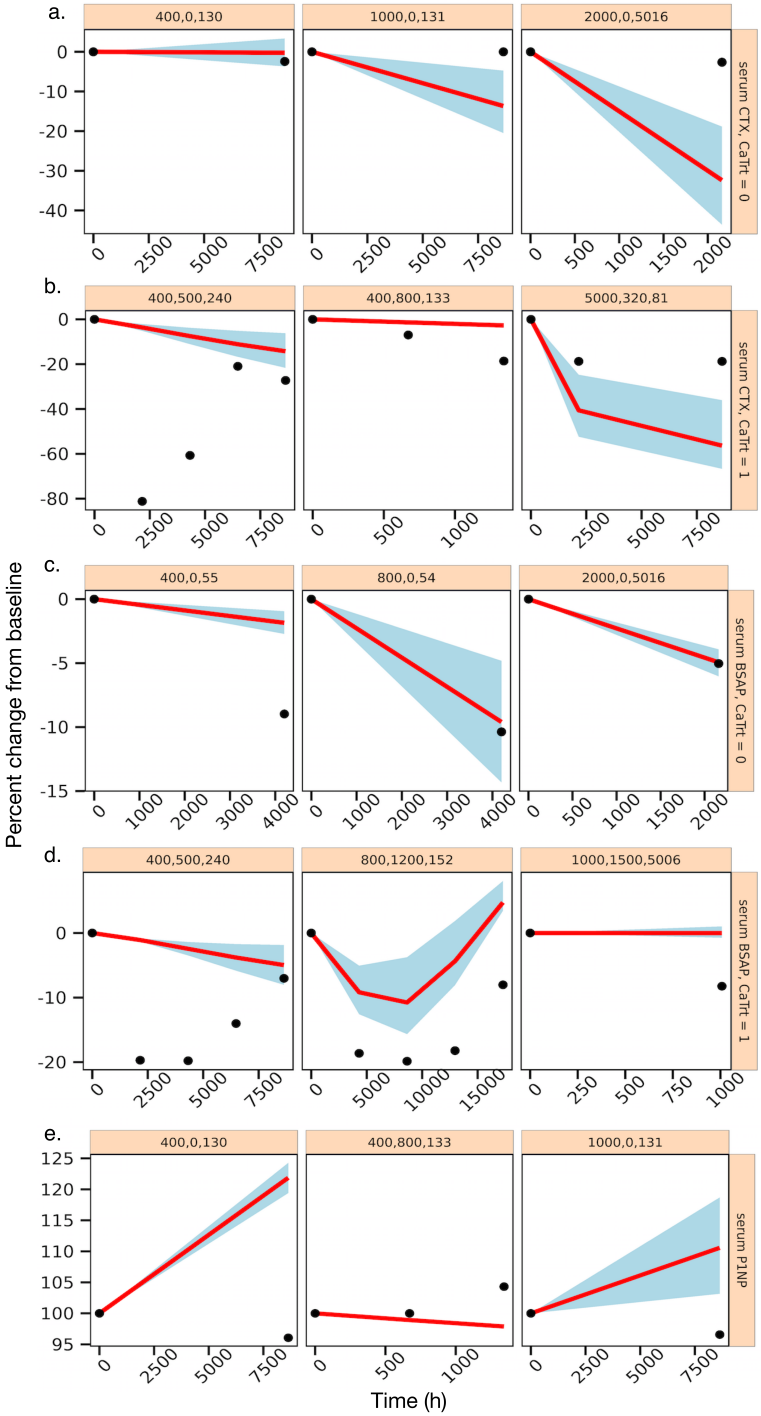


Figure 5.5: Simulated relationship between calcitriol baseline and response to 1 year of D3 supplementation a) paneled by D3 dose b) paneled by calcitriol baseline

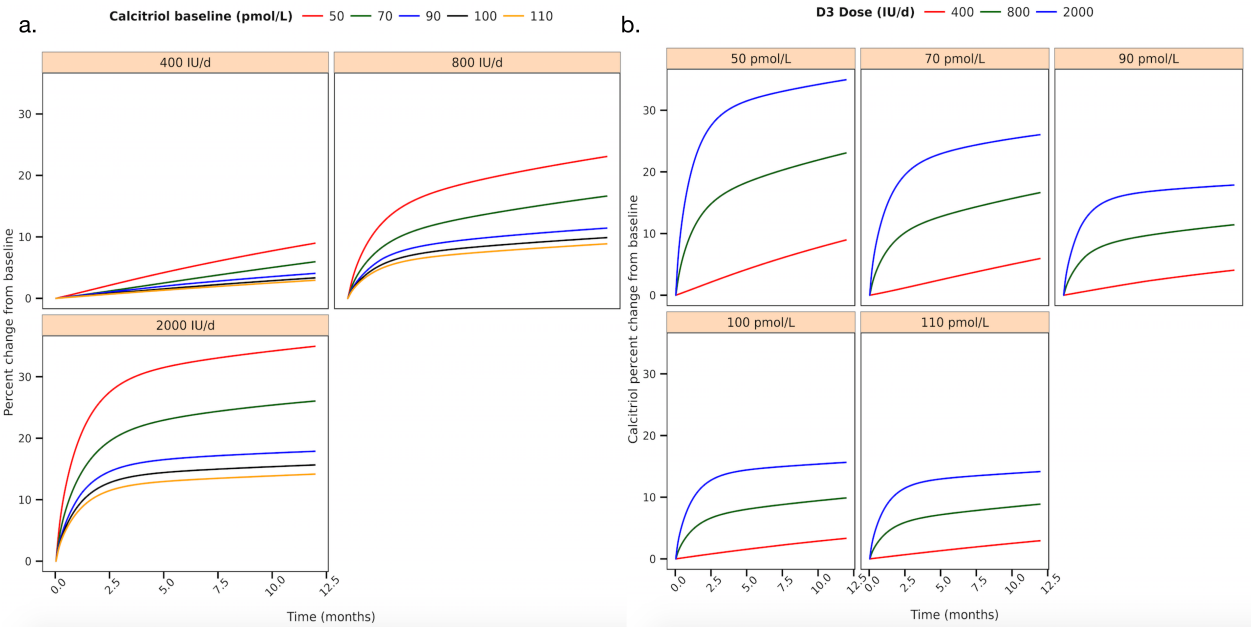


Figure 5.6: Simulated relationship between 25OHD concentration and a) serum PTH and b) percent change in BMDLS, following 1 year of Vitamin D3 without or without 1000 mg/d of calcium therapy

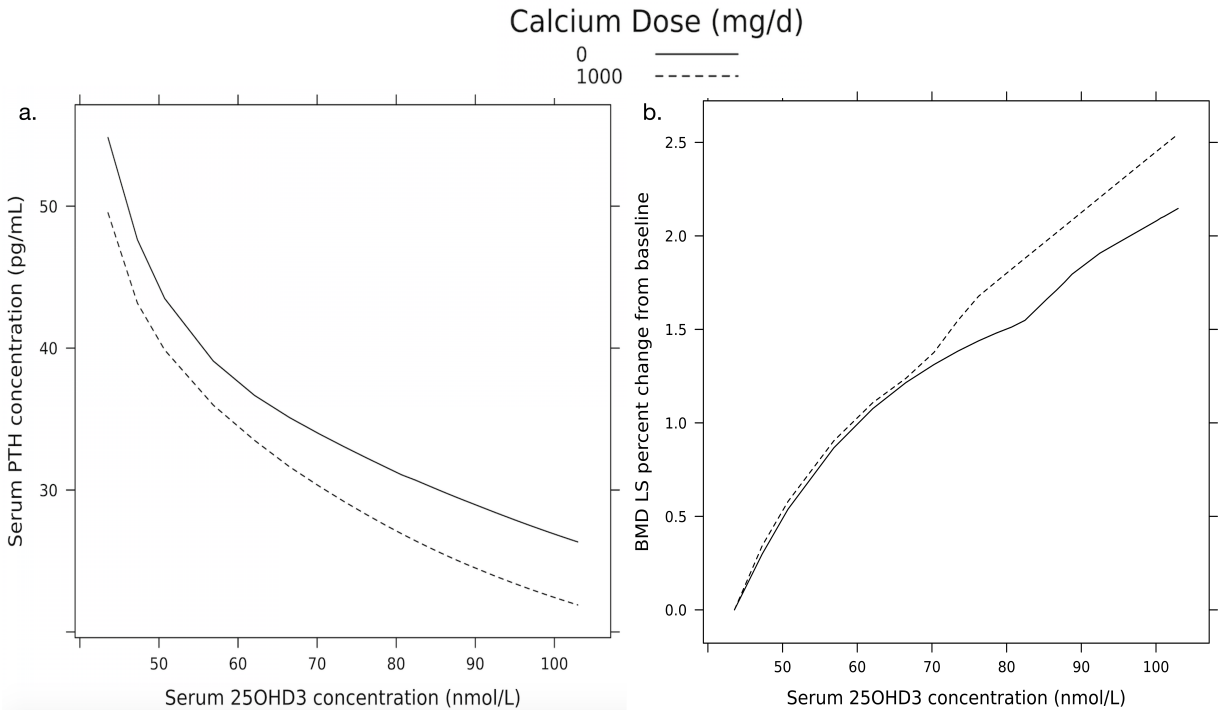


Figure 5.7: Simulated change in a) serum PTH and b) BMDLS following administration of different Vitamin D3 and calcium regimens for 1 year

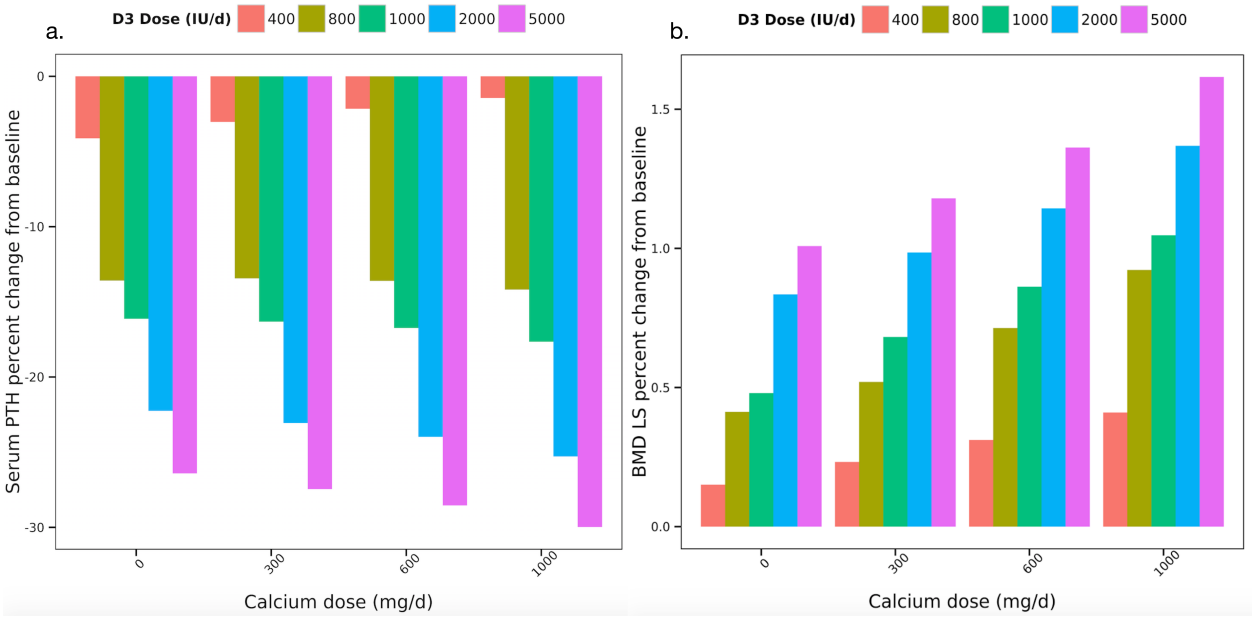


Table 5.2: Vitamin D3 doses with or without 1000 mg/d calcium and 25OHD3 levels (assay = HPLC-MS) necessary for reaching BMDLS percent increase targets at 1 year; 25OHD3 BL = 30 nmol/L; PTH BL 50-60 pg/mL

Target BMDLS % Increase	D3 Dose (IU/d)	D3CA Dose (IU/d)	25OHD3 after D3 (nmol/L)	25OHD3 after D3CA (nmol/L)	PTH after D3 (pg/mL)	PTH after D3CA (pg/mL)
0.5	300	300	50	50	46	42
1.0	400	400	61	60	38	35
1.5	1000	700	80	73	32	30
2.0	3100	2000	97	87	28	26
2.5	>5000	5000	>100	100	<27	23

Table 5.3: Vitamin D3 doses with or without 1000 mg/d calcium and 25OHD3 levels (assay = HPLC-MS) necessary for reaching serum PTH targets at 1 year; 25OHD3 BL = 30 nmol/L; PTH BL 50-60 pg/mL

Target PTH (pg/mL)	D3 Dose (IU/d)	D3CA Dose (IU/d)	25OHD3 after D3 (nmol/L)	25OHD3 after D3CA (nmol/L)	BMDLS % after D3	BMDLS % after D3CA
>= 50	200	200	44	44	0	0
40	300-400	300	57	51	0.7	0.6
30	1500	700	85	70	1.7	1.6
25	>5000	2000	>102	88	>2.1	2.0

Chapter 6

Discussion and Conclusion

6.1 D3-25OHD3 PK Model Discussion

6.1.1 Data Limitations

There were several limitations to the model development, including shortcomings of the data itself. Very few studies collect serum parent concentration data because it is difficult to measure [65]. There is a similar shortage of data measuring 25OHD3 after dosing metabolite. Only 12 arms of parent data (D3-D3) from 7 different studies were found during the literature search. Eleven individuals and 7 arms of metabolite data (25D3-25D3) were found from across 5 different studies. This paucity of parent data made it especially difficult to develop the parent model on its own in a piece-wise fashion, particularly the estimation of its non-linear parameters. Even though the 25D3-25D3 dataset was very limited as well, the metabolite model was better informed by its data than the parent model because of a combination of individual and arm-level data as well as IV data. Another issue with the parent (D3-D3) and metabolite (25D3-25D3) data was sparse sampling. The first samples began >= 20 hours post-baseline in almost every study, making the estimation of ka very difficult. It was for this reason that ka was fixed to an estimate from a D2 parent model.

The single dose data in both the D3-D3 and D3-25D3 datasets were not well described by the model. There was a tremendous amount of variability in the single dose data. Vieth et al [66] excluded all single dose data from their review of several Vitamin D3 studies because of similar large variability present in their metabolite data. The single dose data in the dataset was retained, but there was no concern about being unable to fit it as well as the multiple dose data because large bolus doses (e.g., >= 10,000 IU/d) are not typical clinical regimens in the United States [66]. The large variability may have been due to the data?s sparse sampling (especially

during the absorption phase) or a failure to control D3 intake external to the study dose. In Ilahi et al [31] the maximum D3 concentration for an arm (n = 30) given a single 100,000 IU/d dose of D3 (baseline D3 concentration = 5 nmol/L) was 532 nmol/L at 24 hours post dose. In Armas et al [67] the maximum D3 concentration for a single D3 dose of 50,000 IU/d (n =10) was only 38 nmol/L at 24 hours. Both arms had similar parent baselines. The population predicted maximum concentration for this latter dose was 168 nmol/L. It's possible that the peak concentration in the Armas et al study was missed during the 24 hour period between the bolus dose and the first post-baseline measurement.

The single dose, individual-level data in the D3-25D3 dataset from the Jetter et al [68] study was also suspect. Metabolite concentrations continued to rise after the initial peak, leading to suspicion that additional amounts of D3 were being ingested or large amounts of endogenous D3 were being produced. The study was conducted between January and July 2008 in Zurich, Switzerland. If the bolus doses were given in the spring or summer months then this might explain the secondary large rise in concentration >= 48 hours post-dose.

There is a general under-prediction of the metabolite data, especially at higher concentrations (Figure 3.2). This is because of an asymmetrical distribution of observed metabolite baselines for different subsections of the data. The 11 arms of data that have both parent and metabolite concentration measurements (PM1) have observed parent and metabolite baselines between 2-10 nmol/L and 60-85 nmol/L, respectively. The average observed metabolite baseline in PM1 is relatively high at 71 nmol/L. The 106 IDs in the D3-25D3 dataset that have only metabolite measurements (PM0) have 25OHD3 baselines between 7-90 nmol/L with a much lower average baseline of 42 nmol/L. The typical value estimate of *DBASE* is 3.75 nmol/L, making the average metabolite baseline estimate ∼40 nmol/L. Therefore, the model is favoring the majority of the data (PM0), which has a typical observed metabolite baseline of 42 nmol/L, making the data with higher baselines (and subsequently higher post-baseline concentrations) slightly under-predicted. As a result, the present model's use is more reliable for metabolite concentrations between 20-50 nmol/L, as that is where the majority of the data lie. This is also in the range of typical clinical concentrations of 25OHD3. In order to better characterize the kinetics of D3 and 25OHD3 at higher concentrations, consideration of data with higher average baselines and subsequent concentrations is necessary.

6.1.2 Modeling Inferences

One of the objectives of this work was to integrate knowledge of Vitamin D3 pharmacokinetics and explain apparent discrepancies across a diverse group of published clinical studies. The resulting model-based meta-analysis quantitatively characterized the kinetics of D3 and 25OHD3 using individual and arm-level data across a wide range of conditions. The major finding of this analysis was the non-linear kinetics governing parent conversion to metabolite, which was described using a single Michaelis Menten-type non-linear elimination for D3 (non-linear clearance (CLNL)), where the D3 elimination rate was equal to the input rate for 25OHD3. These findings were in agreement with the results from the 2011 IOM DRI meta-analysis [13], which found that there was a steeper rise in serum 25OHD3 when studies dosed <1000 IU/d and a slower, more flattened response was observed when doses >= 1000 IU/d were administered. One study found that there was a biphasic relationship between D3 and 25OHD3 concentrations, with a steep rise in 25OHD3 for near steady-state D3 concentrations <= 15 nmol/L (corresponds to $\sim$ 1000 IU/d of D3 supplementation). A much slower rise was seen in 25OHD3 concentrations for D3 levels above this cut-point, which resulted in 25OHD3 concentrations between 80-100 nmol/L. They note that this is "consistent with standard enzyme kinetics, involving a first-order reaction at low substrate concentrations and then a zero-order reaction at higher concentrations" [69]. The results from the current model are consistent with those earlier reported and now provide a quantification of these earlier qualitative observations, as the rate in the rise of 25OHD3 begins to decrease starting at D3 doses of $\sim$ 2000 IU/d, which results in 25OHD3 concentrations of $\sim$ 90 nmol/L, for all 25OHD3 baselines after 12 months of daily oral dosing (Figure 3.4). In addition, the final model's estimate for Km was 6.38 nmol/L of D3 (SE = +/- 0.718), which means that the D3 clearance is half-maximal at that value of Km. This estimate is reasonable, given the cited study's findings that the rate of formation of 25OHD3 slowed after 15 nmol/L of D3 [69].

In another previously conducted study [27], a linear relationship was found between the change in 25OHD3 concentration and D3 input; however, the D3 doses studied were few and relatively large (1000 IU/d, 5000 IU/d and 10,000 IU/d) and the average metabolite baseline for all subjects (n = 67) was 70 nmol/L. Given the evidence from the current analysis and the other two studies discussed above, these patients' metabolite concentrations were likely too high to observe the dose-response non-linearity. The current analysis' results may be more reliable for

determining at what parent concentration the change in formation rate occurs, since the current investigation included regimens dosing below, as well as well above 1000 IU/d. Of note, the data that had previously shown the linear relationship [27] were included in the analysis dataset and were described by the model, which is evidence that the model can describe this apparent linearity under that study's conditions (i.e. - at higher doses), while describing the nonlinearity at lower baselines and a broader dose range.

Multiple non-linear D3 elimination pathways were considered; the available parent and metabolite data, however, seemed to support a single non-linear elimination. Physiologically, D3 can be metabolized by the P450scc (CYP11A1) enzyme into several poorly characterized metabolites, including 20OHD3, 20,23-dihydroxyvitamin D3 and 17alpha,20,23-trihydroxyvitamin D3 [70]. There is also evidence that Vitamin D2 can be 24-hydroxylated directly to form 24-hydroxylated Vitamin D2 (24OHD2), but the difference in side-chain structure between D2 and D3 prevents this direct hydroxylation in Vitamin D3 [53], [55]. Therefore, although a single expression describing D3 elimination was consistent with the available data it might be possible to include additional elimination pathways to both the D3 and 25OHD3 models if sufficient data for other metabolites become available. An additional elimination pathway to the 25OHD3 model is the 24,25(OH)2D3 pathway, as mentioned in the Introduction (Chapter 1).

By virtue of the nonlinear clearance, the model demonstrated an inverse relationship between 25OHD3 baseline and 25OHD3 response to D3 dosing (Figure 3.4). The maximum simulated CFB across all doses (400-10,000 IU/d) for someone with an average 25OHD3 baseline of 10 nmol/L or 80 nmol/L is 97 nmol/L and $\sim$ 20 nmol/L, respectively. Depending on how much metabolite is already present in the body, a large increase in D3 dose does not necessarily translate to a large increase in 25OHD3 levels due to the non-linear kinetics of the system; therefore, D3 dosage can be adjusted based on metabolite baseline and parent endogenous production. Similar inverse relationships between D3 baseline and 25OHD3 production were demonstrated by Trang et al [71] and Harris et al [72]. The current model provides a quantification of this relationship and a platform to predict expected changes in 25OHD3 for a given baseline and dose.

Along this same line, the results were used to evaluate average dose recommendations necessary to meet certain 25OHD3 thresholds for maintaining bone health (Figures 3.4a-3.4b). The IOM recommends 25OHD3 plasma levels between 40-50 nmol/L (16-20 ng/mL) for people

1-70 years of age to maintain BMD and reduce risk of fracture. In order to reach this threshold, IOM recommended 400 IU/d D3 Estimated Average Requirement (EAR) for 50% of the population to reach >= 40 nmol/L and 600 IU/d D3 RDA for 97.5% of the population to reach >= 50 nmol/L under conditions of limited sunlight. The model agrees with the IOM's EAR recommendation for those receiving limited sunlight (i.e., 25OHD3 baselines of 10 or 30 nmol/L, endogenous D3 production $\sim$ 56 or 169 IU/d to maintain these baselines). Figures 3.4a-3.4b indicate that 400 IU/d of D3 for 1 year would be sufficient for 50% of those with metabolite baselines at or below the Vitamin D3 deficiency cutoff (25OHD3 concentrations <= 30 nmol/L, 12 ng/mL) to reach >40 nmol/L of 25OHD3. The figure also demonstrates that, on average, 600 IU/d of daily D3 for 1 year would be sufficient for getting those <= 30 nmol/L at baseline to $\sim$ 60 nmol/L by 1 year. It was not possible to fully evaluate the IOM's RDA recommendation since the model was only evaluated as a population-level model, which is based on a goal achievement for a specific fraction (50%) of the population.

As demonstrated in Equation 3.4, there is a constant endogenous D3 ($ENDOG$) input to the central parent compartment, which is a function of the parent baseline and D3 elimination parameters ($DBASE$, Km, $Vmax$) (Equation 3.8). $ENDOG$ is a constant, zero-order input rate that is defined based on the assumption of steady-state at $t = 0$. Therefore, the input rate for D3 ($ENDOG$) is equal to the output rate at $t = 0$ or whenever there is no external supplementation ($ENDOG$=*D3 rate of elimination*=$\frac{(Vmax*C_{D3})}{(Km+C_{D3})}$, where $C_{D3} = DBASE$ at $t = 0$). When this is true, the parent and metabolite concentrations are always equal to $DBASE$ and $DBASEm$, respectively. In this model, the relationship between $ENDOG$, $DBASE$, $Vmax$, and Km is empirical and was not described by real data. For the higher baselines (e.g. 80 nmol/L), this endogenous production is quite high ($\sim$ 450 IU/d) and may cause an over-prediction for a given oral D3 dose. However, since the non-linear kinetics of the system have the effect of plateauing 25OHD3 concentrations as dose increases, the over-prediction may be tempered. Also, in reality, endogenous productions fluctuate due to season and lifestyle. An improvement to the model would be to model the endogenous production over time using data from studies that measure 25OHD3 production after UV exposure. Currently, sufficient data of this longitudinal nature were not available.

The high peripheral volume of distribution for the parent (Vp/F = 2333 L, SE = +/- 27.9 L) was consistent with evidence that shows that a large fraction of Vitamin D3 is stored in

the fat, especially when parent concentrations are high, as is the case in the D3-D3 dataset. An analysis of Vitamin D3 distribution in rachitic animals given ^{14}C-labeled Vitamin D3 daily for 2 weeks demonstrated that the largest amount of D3 ($\sim$ 10%) appeared in the body fat and was slowly released over time [73]. It is also known that serum D3 and 25OHD3 levels are lower in obese subjects than in normal subjects [74], [75], [34]. For these, an initial goal of this model development was to investigate body weight or BMI as a covariate. Unfortunately, literature reports inconsistently reported either of these body size measurements and so further consideration of this covariate effect will need to await more robust data. Furthermore, the weight and BMI data that were available represented a range without extremes in body size; therefore this investigation regrettably could not appropriately evaluate this covariate effect.

The data did not support the estimation of inter-unit random effects on every parameter. Therefore, inter-unit random effects were chosen based on which parameters were best supported by the data (*DBASEm* and *CLm* for the metabolite model; *Vmax* and *DBASE* for the parent-metabolite model). For the metabolite model (25D3-25D3), 16/18 profiles resulted from multiple dosing (some out to steady-state), with the majority of samples taken well after the first dose; therefore, the data supported the estimation of *CLm* but there was little absorption information to support *kam* or *Vcm*. Inter-unit random effects for *Vpm* and *Qm* were not estimated because the data did not consistently support a two-compartment model. Each unit had an observed metabolite baseline so an inter-unit random effect was estimated for *DBASEm*. Inter-unit random effects were included in the combined model based on similar reasoning. Most of the data for the combined model measured 25OHD3 resulting from multiple D3 administration; therefore an inter-unit random effect was estimated for the parent-to-metabolite conversion parameter *Vmax* but none were estimated for the D3 parameters (only 12 D3-D3 profiles available). There was not enough data resulting from lower D3 doses to estimate a random effect for *Km*. Since every unit had an observed parent baseline a random effect was estimated for *DBASE*. The sparsity of the data may have lead to high %RSE's on ω^2_{CLM} (458%) and $\omega^2_{DBASE-Vmax,g}$ (164%).

The dosing regimens, sampling intervals/ranges, assay types and other covariates varied greatly across individuals and treatment arms; therefore, the pcVPC was used to remove variability in a time bin due to variations in the independent variables by normalizing the observed and simulated concentrations by the typical population prediction. Based on the pcVPC results for

25OHD3, the model predicted the central tendency of the data (Figure 3.3) but it over-predicted the variability in the arm-level data (Appendix B, Figure B.10). This over-prediction may be due to the sparse metabolite concentration data available in the literature. The model did a better job simulating the variability in the individual-level data (Figure 3.3c); however, since there were only 14 individual-level metabolite concentration profiles, there was high eta-shrinkage on the individual variance parameter for $Vmax$ (70%) as well as a high %RSE (70.5%). Therefore, the model was not fully evaluated for use in individual-level inferences. As stated in the Introduction (Chapter 1), the main application of this model is to describe the mean 25OHD3 response to D3 supplementation.

Some of the variability in the data was explained by incorporating assay type as a covariate on the 25OHD prediction, estimating proportional and additive biases and precisions for each type of assay, relative to HPLC-MS. Alternative models estimating only proportional assay biases were considered, but the model with both the additive and proportional assay biases was chosen because it provided an improved fit of the data, especially at lower concentration values (not shown). This was seen as an advantage even though two of the additive assay bias parameters were not well estimated (AAB_1: %RSE = 127%; AAB_3: %RSE = 140%). In the final model, RIA was found to be almost equivalent to HPLC-MS. Based on point estimates for their additive and proportional biases, 25OHD3 measurements made by CPBA and CHEMI differed the most from HPLC-MS, suggesting that these assays over-predict the true measurement and are less trust-worthy than HPLC-MS and RIA. However, because of the large uncertainty around the assay parameters for CHEMI, the model cannot definitively detect differences between HPLC-MS and CHEMI (Figure 3.5). This uncertainty resulted because there was less observed data measured by CPBA and CHEMI (26 units, 15 units, respectively) than for RIA and HPLC-MS (40 units, 32 units, respectively). More observed data measured by CHEMI would reduce this uncertainty. A similar simulation, as presented in Figure 3.5, may be conducted where the dose is fixed and the effect of assay is compared across different metabolite baselines. However, similar results are expected under these conditions, since the assay biases enter at the level of the 25OHD3 prediction (Equations 3.17-3.20), acting as scaling factors; therefore, the results are expected to be independent of metabolite baseline.

Other studies also demonstrate variability in 25OHD3 between different assays. Lips et al [76] measured 25OHD3 samples (n = 104) from a population of subjects taking Vitamin D3 supplementation with three different assay types. Metabolite concentrations measured by CPBA

or RIA were 80% and 40% higher, respectively, than those measured by HPLC-MS. In another study [41], samples from 172 hip fracture patients were analyzed for 25OHD3 concentration by DiaSorin RIA, IDS RIA, Nichols Advantage-automated protein binding assay (NA-CLPBA), and HPLC-MS. A persistent, proportional positive bias was found for all three assay types, relative to HPLC-MS. Linear regression analysis demonstrated the following Y-intercepts and slopes relative to HPLC-MS for DiaSorin RIA, IDS RIA, and NA-CLPBA: 12.8 nmol/L, 12.8 nmol/L, 6.5 nmol/L, 0.97, 0.64, 0.97. Finally Snellman et al [77] also showed high inter-assay disagreement between HPLC-MS, RIA and CHEMI. Their results indicated that RIA and CHEMI under-predicted assay concentrations relative to HPLC-MS; however, similar to the current model's results, CHEMI was found to be most dissimilar to HPLC-MS with a correlation coefficient of only r = 0.4 compared to r = 0.5 for RIA.

6.2 D2-25OHD2 PK Model Discussion

6.2.1 Data Limitations

Given a limited number of parent PK profiles (9 units across 6 studies) and sampling times past 30 hours, two-compartment disposition was not apparent in most of the D2 SD data. Since it is unlikely that the metabolite would have a larger distribution than the parent, both D2 and 25OHD2 were described using a two-compartment model, with *Q2* and *Vp2* fixed to the final D3 estimates [78]. The local sensitivity analysis results on these fixed parameters indicated that 25OHD2 exposure was not sensitive to changes in these parameters, except for *Vcm2*, for which increasing values caused a large decrease in 25OHD2 (Appendix C, Figure C.7) at 25OHD2 concentration values outside the typical clinically observed range (>120 nmol/L). Including IV 25OHD2 data would make *Vcm2* identifiable, but this is not currently available in the literature.

Dose-normalized D2 and 25OHD2 observations showed little to no indication of nonlinear kinetics; therefore, inclusion of a non-linear clearance/formation rate for D2/25OHD2 was not feasible, as was expected, given the D3-25OHD3 model results [78]. As with the large D3 bolus data, there was no indication of non-linearity in the D2 parent data (doses >10000 IU/d). Vieth et al [66] excluded all single dose data from their review of several Vitamin D3 studies because of large variability present in their metabolite data, suggesting PK differences between SD and MD

regimens. The D3 dataset included several smaller multiple dose data profiles (400-5000 IU/d) where non-linearity was detectable. The current analysis would benefit from including smaller, multiple-dose D2 data; however, none were available in the literature. Differences in enzymes that metabolize parent into metabolite may also contribute to elimination discrepancies between the two forms [50], [51], [52].

As with D3, maximum D2 concentrations (Cmax) were under-predicted by the model, due to considerable within-dose variability, resulting from sparse sampling. For example, three different profiles following a D2 bolus dose of 50000 IU/d had D2 Cmax values of 37.1, 369.6, 228.7, and 46.8 nmol/L at 8, 12, 16, and 24 hours (Tmax) [79], [65], [80], [67]. The latter two samples had a sampling gap of 8 and 24 hours between peak time and the previous time point, whereas the former two had more frequent sampling, with gaps of 4 and 6 hours. For comparison, one study found that D3 concentrations, following oral SD supplementation in healthy adults, peaked between 8-10 hours post-baseline [81].

The D2-25OHD2 model had a simpler random effects structure than the D3-25OHD3 model because of the former's smaller dataset (33 total units over 17 studies vs. 136 total units over 57 studies). Separate random effects for 25OHD2 arms and individuals (12 of 24 25OHD2 profiles were individual-level) were included for the metabolite baseline ($\omega^2_{DBASEM2,i}$, $\omega^2_{DBASEM2,g}$), as well as for the residual error ($\sigma^2_{metabolite,i}$, $\sigma^2_{metabolite,g}$); however, estimation of separate inter-unit random effects for D2 was not possible with only 3 individual-level parent profiles. The inter-unit random effect for the group metabolite baseline had high eta-shrinkage (46.3%), since the total metabolite dataset was small (total units = 24). Only a single random effect was estimated per parameter for *CLm2*, *Vpm2*, and *Qm2*, since variance estimates for the separate unit types were similar for these parameters.

As was the case for D3 and 25OHD3, the model predicted the central tendency of the data but it over- or under-predicted the variability in both the arm and individual level data in different time bins. Therefore, the model was not fully evaluated for use in individual-level inferences. As stated in the Introduction (Chapter 1), the main application of this model is to describe the mean 25OHD2 response to D2 supplementation.

External validation simulations of total 25OHD, following D2 administration resulted in gross over-prediction of the median observed 25OHD data (Appendix C, Figure C.3), which

may indicate an interaction between 25OHD2 and 25OHD3 not accounted for in the model or that the assumption of a constant endogenous 25OHD3 input to the system (40 nmol/L) is overly simplistic. Realistically, that endogenous amount would fluctuate with time. Any additional D2 supplementation would superimpose 25OHD2 on top of this constant 25OHD3 endogenous input. Non-linear PK was evident from dose-normalizing observed 25OHD concentration data, indicating that subjects may have been exposed to more sunlight than expected (not shown). The linear kinetics of the D2 model could not accommodate the apparent non-linearity in the 25OHD data.

6.2.2 D2 vs. D3 inferences

The differences in metabolite concentration, resulting from doses of D2 or D3, diminished with increasing dose and baseline (BL = 5 nmol/L, median difference in 25OHD after 3 months D3 vs. D2 dosing at 400 IU/d (90% CI): 18.3 nmol/L (14.7, 23.3); BL = 40 nmol/L, 25OHD difference after 3 months of 400 IU/d (90% CI): 3.79 nmol/L (5.74, 7.68)), until D2 eventually became more effective then D3 in raising 25OHD levels (BL = 5 nmol/L, median 25OHD difference after 3 months of D3 vs. D2 dosing at 4000 IU/d (90% CI): -49.4 (-51.5, -49.4); BL = 40 nmol/L, median 25OHD difference after 3 months of 2000 IU/d (90% CI): -22.2 (-24, -19.9)). This was a consequence of the D3 non-linear clearance, which limited the amount of D3 converted to 25OHD3,with increasing D3 concentration. Both D2 and 25OHD2 were described entirely by linear kinetics, causing 25OHD to change proportionally to the dose. Therefore, the model suggested that for very Vitamin D deficient individuals (<= 20 nmol/L), D3 doses $<\sim$ 2000 IU/d were more beneficial for restoring 25OHD levels than equivalent D2 doses. There was less added benefit from taking D3 as you increased in dose and/or baseline.

With a larger dataset (D3: 136 total units over 57 studies vs. D2: 33 total units over 17 studies), the estimated D3 and 25OHD3 parameter standard errors were smaller than those for D2 and 25OHD2 (Tables 3.1, 4.1). The uncertainty associated with the former's parameters, however, had a larger overall effect on 25OHD exposure at 3 months because of the latter model's 4 fixed parameters. It is expected that if more D2 and 25OHD2 PK data become available for inclusion,D2/25OHD2 parameter estimate SEs would decrease, but overall uncertainty on 25OHD exposure would increase, possibly narrowing the gap in D2 and D3's ability to maintain 25OHD at lower concentrations.

The clinical significance of dose-dependent increase in D3/25OHD3 parameter uncertainty depends on the 25OHD concentration range of the effect. Effect of uncertainty associated with *DBASE*, *Vmax*, and *Km* was greatest for 25OHD concentrations well above nutritional adequacy (Figure 4.4) However, the uncertainty associated with *CLm* was substantial, even at lower doses and concentrations; therefore, if the true value of *CLm* were much lower or higher than the point estimate this would make D3 more or less effective than D2 at raising 25OHD levels (Figure 4.4). Metabolite data with increased sampling at steady-state would decrease uncertainty around *CLm*.

Previously published studies comparing D2 and D3's effectiveness at raising 25OHD levels have given variable results across studies, with most comparative studies showing that D3 is more effective than D2 [67], [82], [83], [84], [85], [86], [87]. The current analysis is consistent with these findings for doses <2000 IU/d and 25OHD baselines <20 nmol/L; however, most of the studies showing non-equivalence have 25OHD baselines >30 nmol/L. In one study, after D2 (n = 19, 25OHD BL = 53.2 nmol/L) or D3 (n = 20, 25OHD BL = 51.8 nmol/L) dosing of 400 IU/d for 3 months, 25OHD concentrations changed by 7.8 and 15.9 nmol/L (D2 vs. D3, p = 0.08), respectively [86]. Another study dosed 2000 IU/d of D2 (n = 46, 25OHD BL = 37.6 nmol/L) or D3 (n = 42, 25OHD BL = 43.7 nmol/L) for 2 months resulting in 25OHD concentration changes of 30.2 and 45.5 nmol/L (D2 vs. D3, p = 0.001) [85]. A third study dosed 4000 IU/d of D2 (n = 9, 25OHD BL = 74.2 nmol/L) or D3 (n = 9, 25OHD BL = 77.5 nmol/L) for 2 months, causing 25OHD concentrations to change by 12.9 and 32.8 nmol/L [87]. A final study dosed 1000 IU/d of D2 (n = 48, 25OHD BL = 37.2 nmol/L) or D3 (n = 47, 25OHD BL = 42.4 nmol/L) for 3 months, resulting in 25OHD concentration changes of 20.5 and 36.7 nmol/L (D2 vs. D3 p <0.001) [84]. Most of these studies attributed their non-equivalence findings to differences in enzyme metabolism for D2 and D3, as well as differences in binding affinities for DBP and VDR, as mentioned in the Introduction (Chapter 1). An additional consideration, however, is to focus on the stability of the oral dosage forms. In particular, ex vivo D2 degrades much faster than D3 [88], suggesting that, without dose-validation prior to study, subjects may ingest less D2 than expected, making appear D2 less effective than D3 in raising 25OHD levels.

Studies concluding D2 and D3 equivalence have met with criticism. One such study dosed 1000 IU/d of D2 (n = 17, 25OHD BL = 39.5 nmol/L) or D3 (n = 18, 25OHD BL = 44.75 nmol/L) for 11 weeks, resulting in statistically non-significant differences in 25OHD AUCs (698 vs. 769

nmol*wk/mL, p >0.05) [89]. A second study by Holick et al [90] provided the same daily dose and regimen (D2 n = 16, 25OHD BL = 42.25 nmol/L; D3 n = 20, 25OHD BL = 49 nmol/L), again finding no statistically significant difference in 25OHD concentration change between the two groups (24.75 vs. 23.2 nmol/L, p = 0.027). Potential confounding factors within these studies, however, include obese (>30 kg/m^2) populations and a high proportion of blacks participants. Both factors could alter the PK of 25OHD, leading to an underestimation of 25OHD3 [91]. Both studies also had a high limit of detection for 25OHD2 (10 nmol/L), calculating its value for observations below this limit by taking the difference between total 25OHD and 25OHD3, possibly leading to overestimation of 25OHD2. Despite these limitations, the current model bridged these results to support these study findings that D2 and D3 are equivalent at or around 1000 IU/d, given the reported 25OHD baselines.

In addition to supporting previous investigations, the model also provided new evidence that D2 became relatively more effective than D3 above a certain concentration range. The clinical evidence instead indicates that D3 continues to be more effective than D2 at higher concentrations [82], [92], [71]. The current analysis showed that D3 is more effective than D2 in raising 25OHD levels for doses $<\sim$ 2000 IU/d at metabolite baselines <20 nmol/L. Only a few studies have compared D2 and D3 at low doses (some of which are listed above) and none have looked at equivalence for patients with very low baselines. Studies comparing D2 and D3 under these conditions would be helpful in validating, or refuting, the model's results.

Another reason for the discrepancy may be that D2 PK was not realistically described by the model because of limited available data, especially for the parent. While differences in enzyme metabolism and DBP binding affinity may have account for differences in D2 and D3 elimination, it was also plausible that the non-linear clearance of D2 was not observable given the small dataset and limited dose range. If D2, as with D3, was actually eliminated by a saturable, non-linear clearance, then at higher doses and BLs, D2 and D3 might be more comparable in maintaining 25OHD levels and D3 might have continued to outpace D2 at higher concentrations. Trang et al [71] provided evidence for D2 being described by non-linear clearance, demonstrating that total 25OHD (25OHD3 + 25OHD2) change from baseline after D2 administration was inversely proportional to metabolite baseline. This data could not be included in the analysis since 25OHD2 serum concentration was not recorded in the study.

A further reason that might explain the discrepancy between the model and clinical results is that there was no interaction between 25OHD2 and 25OHD3 kinetics in the model, which would require data resulting from co-dosing of D2 and D3 and/or varied D3 baseline. Most studies are not designed accordingly. Given the current data, 25OHD2 and 25OHD3 baselines were plotted against each other to observe trends that would indicate an interaction but none were found (results not shown). Therefore, the two forms were modeled independently so that 25OHD = 25OHD2 + 25OHD3. Some studies have reported non-equivalence between D2 and D3,with a decrease in 25OHD3 concentrations in arms that were administered D2 [84], [85], [86], [87], suggesting that D2 may inhibit D3 hydroxylation. However, decreasing 25OHD3 may be explained by decreasing sun exposure, especially since several comparative studies have high 25OHD baselines. A further analysis improvement would be to simultaneously model total 25OHD, 25OHD2 and 25OHD3 to capture any interaction between the forms. However, data supplying all three endpoints are limited.

6.3 Vitamin D-MSPM Integration Discussion

6.3.1 Data and Modeling Limitations

As with the previous modeling work in Chapters 3-4, the condition of the data was a limiting factor in this portion of the analysis, involving the MSPM. Due to limited, sparse, and highly variable bone-marker data, even within a Vitamin D3/calcium dose group (e.g., D3 dose = 1000 IU/d, serum PTH CFB = 14.1 pg/mL [84] and 3.3 pg/mL [93] for concomitant calcium doses = 600 mg/d or 1500 mg/d, respectively for 2 or 3 months), optimizing parameters related to the conversion of 25OHD3 was done using only a single endpoint (e.g., calcitriol concentrations, following Vitamin D3 or D3CA administration). Optimizing the integrated Vitamin D PK and systems model against multiple endpoints, including serum PTH, calcium, and BMDLS led to convergence problems, even when using the gradient-free optimization methods available in the *minqa* package in R. As model-validation proof, the model predicted into most of the observed bone-marker data reasonably well, with predicted values moving in the correct direction, relative to baseline, and remaining within a similar range of the observed data. However, the model showed a large over-predictive bias relative to observed BMDLS data, which was the main clinical endpoint of interest. To remedy this, a gamma-exponent parameter, related to osteoclast resorption, was refit using the BMDLS

data alone, following Vitamin D3 or D3CA supplementation. Refitting some of the parameters related to changes in BMDLS has been done in previous analyses for different therapies due to differences in the condition of the bone, depending on the mechanism of the treatment [14], [18], [94]. Therefore, while simultaneously optimizing against more than one endpoint would improve the model's predictive ability across different markers following D3CA supplementation, the current model gives a reasonable depiction of the broader physiological response of the system to Vitamin D3 and calcium therapy.

Another limitation was the lack of bone-marker and BMDLS data resulting from administration of Vitamin D2. As a result, this analysis' efficacy comparisons for D2 and D3 are limited to comparing increases in 25OHD following administration of either form of the hormone (Chapter 4). Given the PK results discussed in Chapter 4, it's plausible that BMDLS increases following large doses of D2 (>2000 IU/d) would surpass those following D3 due to D3's saturable non-linear kinetics. The model would benefit from more bone-marker and BMDLS data resulting from D2 administration to validate or reject this conjecture.

The model is more accurate at predicting response to Vitamin D3 alone than D3CA supplementation (Figures 5.3-5.4; Appendix D, Figures D.9-D.12), although model simulations show that the response is more pronounced following duel therapy (Figures 5.6-5.7), as is consistent with evidence in the literature [95], [96], [97], [98]. A solution to improve the model's prediction of response to duel therapy is to model the response to oral calcium supplementation alone by estimating an absorption rate constant from a calcium depot compartment. Currently, the model's calcium dose is set by adjusting a constant input rate ($OralCa$, mmol/h) into calcium's central compartment, based on the reported dose. Therefore, while the current model's results may give an accurate idea of the system's overall response to calcium therapy, specific calcium dose recommendations for reaching targeted levels of bone-marker or BMDLS should be reserved for analyses following future PK modeling of oral calcium data, which was beyond the scope of the current project.

Since the conversion of Vitamin D to 25OHD to calcitriol was not simultaneously modeled, there was a disconnect between the baselines for the first two forms of the hormone and calcitriol. As reported in Chapter 3, the 25OHD3 metabolite baseline is a function of the parent baseline (Equation 3.16). A similar relationship was not established for calcitriol since it was not included

in the original PK models (Chapters 3-4). The observed data also demonstrated an inverse relationship between baseline calcitriol and response to D3 supplementation (Appendix D, Figure D.8). The model's positive feedback between PTH and calcitriol [1] was not enough to capture the magnitude of this inverse effect. Therefore, the model's calcitriol baseline was empirically calculated by adjusting the initial 1-alpha-hydroxylase enzyme input rate ($AOH0$)to the calcitriol equation (Equation 5.4) by a factor of the observed calcitriol baseline (Equation 5.1). Large observed calcitriol baselines decreased $AOH0$, thereby slowing the initial production of calcitriol. This baseline adjustment, along with parameterizing the gamma-exponent related to the calcitriol scaling factor as a function of inverse calcitriol amount (Equation 5.2), allowed the hormone to control its own formation, as is consistent with the known biology [99].The power structure in Equation 5.4 was chosen as the final calcitriol model because more mechanistic and complex models (e.g. EMAX, inhibition EMAX) for describing calcitriol formation and inhibition were not mathematically identifiable, especially given the calcitriol PK data that was available.

6.3.2 Modeling Inferences

The final model integrated the Vitamin D PK models described in Chapters 3-4 with a previously published MSPM that described calcium, PTH, and bone-remodeling [1]. The model's resulting physiological response to Vitamin D input is consistent with known biology [23], [100], [26]. An increase in calcitriol, following Vitamin D3 administration, caused inhibition of PTH and a decrease in serum calcium, which also decreased osteoclast bone resorption (reduced serum CTX levels), leading to increases in BMDLS. Concomitant calcium doses reduced PTH more than D3 alone, thereby causing an even greater increase in BMDLS.

Overall, the model's simulation results indicated that the effect of Vitamin D3 with or without calcium on serum calcitriol (median PCFB = 2.64%), serum calcium (PCFB range: -3 - -0.8%), and BMDLS (PCFB range: 0-2.5%) is minimal (simulated PCFB ranges:-3 - -0.8%, 0-2.5%, respectively). This is consistent with observed effects on these same endpoints in the literature (Appendix D, Table D) with concomitant calcium supplementation resulting in larger increases/decreases in BMDLS and serum PTH compared to Vitamin D3 alone groups. Studies administering Vitamin D3 alone (400-3500 IU/d for 1-2 years, sample sizes (n) 75-84) resulted in BMDLS PCFB -0.37-0.19%/ [101], [102], [103]. Comparatively, studies administering Vitamin D3

(400-5000 IU/d, sample sizes (n) 39-124) plus calcium (250-1350 mg/d) for a similar treatment period [95], [96], [104], [105], [106], resulted in larger BMDLS responses (-0.1 - 3.1%, with the majority >1%). A similar pattern was seen for serum PTH, with treatment arms receiving D3CA [97], [98], [86] having larger decreases in PTH (D3 doses: 400-800 IU/d; calcium doses: 800-1175 mg/d for 2 months-2 years; serum PTH PCFB: -47.7% - -10.5% for sample sizes (n) 8-32) than those receiving Vitamin D3 alone for a similar time period (D3 doses: 400-800 IU/d D3; serum PTH PCFB: -9.57% - 4.2%, with the majority <0%) [107], [101], [108], [109].

In general, evidence for the relationship between 25OHD levels and PTH has yielded discordant results. The current analysis results are consistent with results from the majority of studies showing an inverse relationship between 25OHD and PTH [13], [110], [111], [112], [113]. In one recent study, serum 25OHD concentration was negatively correlated with serum PTH concentration at lower and higher calcium takes (r = -0.606 and -0.483, respectively, p ¡0.01) [114]. However, the exact level of 25OHD necessary to result in a specific level of PTH varies between studies because PTH is dependent upon several factors, including calcium and calcitriol levels. Consequently, results have varied from studies that recommend 25OHD thresholds beyond which serum PTH decline begins to plateau . One 2014 study done in Syrian women (n = 372) found that PTH started to increase below 25 nmol/L 25OHD [115], whereas another study conducted in Chinese males found serum PTH plateaued for 25OHD values >= 50 nmol/L [112]. The IOM's meta-analysis [13] found a wide range of 25OHD values beyond which PTH plateaus (30-125 nmol/L) [13]. The current model's PTH values do not plateau [13], [116], and [117] at a particular threshold but continue to decline more slowly after 25OHD = 70 nmol/L (Figure 5.6). The assay used to measure 25OHDmay also contribute to variability in the 25OHD-PTH relationship [118], as evidenced by simulations of 25OHD across assay types (Figure 3.5; Appendix D, Tables D.4-D.6).

The current model's simulations of the PTH-25OHD relationship (Figure 5.6) are also consistent with results which indicated that for given 25OHD levels, PTH decreased more in groups with higher calcium supplementation, in addition to their dietary intake of Vitamin D. In a 2015 study in healthy Indian adolescents, subjects with calcium intakes >520 mg/d had lower serum PTH values for a given 25OHD concentration compared to those with intakes <520 mg/d [114]. In another earlier study, at both low (<25 nmol/L) and high (>45 nmol/L) 25OHD levels there was a significant difference in serum PTH between those taking 800 and 1200 mg/d of calcium (p =

0.04) [113]. This latter study also suggested that calcium supplementation may be unnecessary if 25OHD levels are sufficient for maintaining PTH within a health range.

As with PTH, evidence for the relationship between 25OHD and BMD has yielded discrepancies. As the IOM concluded in their 2010 DRI, "there is fair evidence to support an association between serum 25OHD concentration and BMD or changes in BMD at the femoral neck" but not at BMDLS [13]. Some more recent studies since the DRI continue to yield discrepancies between results [112], [111], [119], [120]. Since the change in BMDLS due to Vitamin D3 or calcium is small (0.5-2%), the weak signal may not register as statistically significant relative to changes in 25OHD during statistical analyses, which generally assess statistical correlation between 25OHD and BMDLS. BMD changes, however, may still be clinically relevant, if not statistically significant. For example, the expected decrease in BMDLS and BMD at the femoral neck for post-menopausal women is 2% and 1.4% per year [121], respectively. Therefore, the model results (Figure 5.7) suggest that changes in BMDLS following Vitamin D3 with or without calcium may be adequate for offsetting BMDLS decline for post-menopausal women, if BMDLS continues to increase at the same rate.

Lastly, the integrated model agrees with the IOM EAR for Vitamin D3 that 400 IU/d D3 is more than adequate for increasing 25OHD3 levels to >= 40 nmol/L (Figure 3.4). However, the current analysis indicates that much more than 40 nmol/L of 25OHD is necessary for increasing BMDLS values to >= 1% (Table 5.2). The 25OHD threshold recommendation from this analysis to increase BMDLS by 1.5% would be 70-80 nmol/L, depending on concomitant calcium administration, which would also adjust the necessary dose for reaching this target.

6.4 Discussion on Completion of Specific Aims

Some of the Specific Aims set forth in Chapter 2 were not feasible, given the limitations of the data. Because of incomplete reporting between studies, only differences in 25OHD3 assay type were included during the PK covariate analysis. Weight, BMI, dietary Vitamin D and calcium intake were not consistently reported enough for inclusion to be informative to the analysis (Specific Aim 1d). As mentioned above, Vitamin D2's effect on pharmacodynamic endpoints such as bone-markers and BMD were not analyzed because little information on these relationships were found in the literature (Specific Aim 2a). Finally, the complete integrated model of Vitamin D PK and the MSPM was not

used to run clinical trial simulations to inform design of osteoporosis drug trials involving Vitamin D as a background drug (Specific Aim 4). Firstly, the data previously used to model teriparatide and denosumab's effects on BMD and bone-marker response in the MSPM [1] already included the influence of Vitamin D as background therapy. Therefore, either the Vitamin D effect would have to be subtracted out or an entirely new therapy would have to be modeled without previous Vitamin D effect included, but this was beyond the scope of this project. Secondly, a clinical trial simulation would require the use of more reliable inter-individual random effects to describe Vitamin D PK than was currently estimated (Tables 3.2-3.5, 4.3-4.4), given the paucity of individual-level Vitamin D PK data. The MSPM is a mean model, so realistic inter-individual variances would have to be assigned to all relevant BMD and bone-marker parameters to make individual-level inferences. There is not enough individual-level bone-marker and BMD data available in the literature to reliably estimate those variances. Nevertheless, the results from this analysis established the basis for comparing the concomitant effects of D3 or D3CA administration during clinical trials of other anti-osteoporosis therapeutics and also can be investigated to quantify D3 effects in other disease states such as CKD-MBD.

6.5 Conclusion

The MBMA presented here is the first analysis to simultaneously integrate D3, D2, and 25OHD3, and 25OHD2 individual and summary-level data across studies, to describe the kinetics of this parent-metabolite system and then integrate it with a MSPM [1] to quantitatively evaluate the effect of D3CA administration on bone-health endpoints as mediated through calcium and PTH homeostatic mechanisms. Vitamin D3, D2 and their 25-hydroxylated metabolites were described by 2 compartment models with first-order absorption. Vitamin D3 and 25OHD3 clearance and formation, respectively, were non-linear, which resulted in an inverse relationship between metabolite baseline and response, suggesting that baseline should be considered when recommending dosage, as increasing dose does not necessarily increase metabolite levels. Inclusion of 25OHD3 assay type as a covariate on metabolite prediction indicated that HPLC-MS and RIA produced similar results, whereas RIA and CHEMI were over-predictive, relative to HPLC-MS, confirming that assay type is a source of variability that should be considered when comparing 25OHD3 measurements across studies. Vitamin D2 did not demonstrate non-linear kinetics, resulting in D2's more effective

maintenance of total 25OHD levels at doses >2000 IU/d. The Vitamin D model was integrated with the MSPM using a power model to describe the conversion of 25OHD to calcitriol. A scaling factor related to this conversion was estimated with a related gamma-exponent parameterized in terms of inverse calcitriol amount, reflecting the apparent self-inhibition of calcitriol on its own production. Consistent with known biology, simulation results after 1 year of Vitamin D3 supplementation with or without calcium indicated small increases in BMDLS and substantial decreases in serum PTH, with larger changes following concomitant calcium dosing. Overall, the model results supported the IOM 2011 DRI [13] findings that 400-600 IU/d D3 was sufficient for raising 25OHD3 levels to 40-50 nmol/L for those with limited sunlight. However, simulations involving the MSPM suggested that 25OHD3 levels between 70-80 nmol/L were necessary for increasing BMDLS by >= 1%. The current analysis would benefit from the inclusion of more PK data for Vitamin D and 25OHD, especially for the D2 form of the hormone, allowing for estimation of the several PK parameters that were fixed in the current analysis. Also a PK analysis of serum calcium, following oral calcium supplementation would improve BMDLS and serum PTH predictions, resulting from dual therapy of Vitamin D3 and calcium and also improve the reliability of calcium dose recommendations. Overall these model results may be useful when comparing the concomitant effects of D3 or D3CA administration during clinical trials of other anti-osteoporosis therapeutics and also can be investigated to quantify D3 effects in other disease states such as CKD-MBD.

Chapter 7

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Appendix A

NONMEM Control Stream Files for Final Vitamin D Models

```
$PROB FINAL 25D3-25D3 MODEL

$INPUT C SID ID TREATMENT2 TRTn MTRT nTOTAL nARM nOBS DOSEorig
DOSEIU D2DoseOrig D3DoseOrig DOSEnmol TIME CMT DVTYPE2 DVN MULTDV DV
ADDL RATE SS II AMT EVID BASEval BASE0 NUM GTYPE REG2 RT2 RR

$DATA ../../../../../../data/set2/derived/meta_manip_data_25D-25D_NO_ORALSD2.csv IGNORE=C

$SUB ADVAN13 TOL = 6

$MODEL NCOMPARTMENT = 3

$PK
TYPE = DVTYPE2
REGI2 = REG2

TVKA = THETA(1)
KA = TVKA*EXP(ETA(1)/SQRT(nOBS)) ; absorption ka for 25D3 (h^-1)

TVCL = THETA(2)
CL = TVCL*EXP(ETA(2)/SQRT(nOBS)) ; LINEAR CLEARANCE (L/h)

TVV = THETA(3)
VC = TVV*EXP(ETA(3)/SQRT(nOBS)) ; central volume for 25D3 (L)

TVVT = THETA(4)
VT = TVVT*EXP(ETA(4)/SQRT(nOBS)) ; PERIPHERAL VOLUME FOR 25D3 (L)

TVQ = THETA(5)
Q = TVQ*EXP(ETA(5)/SQRT(nOBS)) ; peripheral clearance (L/h)

D25BASE = THETA(6)*EXP(ETA(6)/SQRT(nOBS)) ; baseline 25D3 concentration (nmol/L)

F1 = THETA(7)*EXP(ETA(7)/SQRT(nOBS)) ; ORAL BA OF 25D3

F2 = THETA(8)*EXP(ETA(8)/SQRT(nOBS)) ; IV BA OF 25D3

A_0(2) = D25BASE*VC ; INTIALIZE CENTRAL 25D3 CMT WITH 25D3 AMT (nmol)

A_0(3) = D25BASE*VT ; INITIAL IZE PERIPHERAL 25D3 CMT WITH 25D3 AMT (nmol)

$DES
DADT(1) = -KA*A(1) ; 25DD3 GUT CMT

C2 = A(2)/VC ; CONCENTRATION OF CENTRAL COMPARTMENT

C3 = A(3)/VT; CONCENTRATION OF PERIPHERAL COMPARTMENT

ENDOG25 = CL*D25BASE ; ENDOG (nmol/h)

DADT(2) = ENDOG25 + KA*A(1) - (CL + Q)*C2 + Q*C3 ; 25D3 CENTRAL CMT

DADT(3) = Q*C2 - Q*C3 ;25D3 PERIPHERAL CMT

$ERROR
IND2 = 0

CC = A(2)/VC ; 25D3 CENTRAL CONCENTRATION

IF(REGI2.EQ.1) Y25D3 = CC*EXP(EPS(1)/SQRT(nOBS)) ; single dose

IF (REGI2.EQ.2) Y25D3 = CC*EXP(EPS(2)/SQRT(nOBS)) ; multi dose

IF (TYPE.EQ.6) IND2 = 1
Y = YD3*IND2

$THETA
0.323, FIX ; KA (h^-1)
(0, 0.1) ; CL (L/h)
(0, 10); VC (L)
(0, 100); VT (L)
(0, 0.2); Q (L/H)
(0, 3); D25BASE (NMOL/L)
(0, 6) ; F1
1, FIX; F2

$OMEGA
0, FIX ; KA
0.09 ; CL
0, FIX ; VC
0, FIX ; VT
0, FIX ; Q
0.09 ; D25BASE
0, FIX; F1
0, FIX; F2

$SIGMA
0.04 ;20% proportional residual error
0.04

$ESTIMATION MAXEVAL=9999 PRINT=5 METHOD = 1 INT MSFO=./964.msf
$COV PRINT=E

#TERM:
0MINIMIZATION SUCCESSFUL
NO. OF FUNCTION EVALUATIONS USED: 531
NO. OF SIG. DIGITS IN FINAL EST.: 3.3

ETABAR IS THE ARITHMETIC MEAN OF THE ETA-ESTIMATES,
AND THE P-VALUE IS GIVEN FOR THE NULL HYPOTHESIS THAT THE TRUE MEAN IS 0.
ETABAR: 0.0000E+00 4.8434E-03 0.0000E+00 0.0000E+00 0.0000E+00 2.5706E-02 0.0000E+00 0.0000E+00
SE: 0.0000E+00 5.8932E-03 0.0000E+00 0.0000E+00 0.0000E+00 9.7610E-02 0.0000E+00 0.0000E+00
P VAL.: 1.0000E+00 4.1115E-01 1.0000E+00 1.0000E+00 1.0000E+00 7.9228E-01 1.0000E+00 1.0000E+00

ETAshrink(%): 1.0000E+02 5.3807E+01 1.0000E+02 1.0000E+02 1.0000E+02 6.4204E+00 1.0000E+02 1.0000E+02
EPSshrink(%): 4.5204E+00 7.4903E+00

#TERE:
Elapsed estimation time in seconds: 64.78
```

Elapsed covariance time in seconds: 64.64
1
****
******************** ********************
******************** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION **********
#OBJT:********** MINIMUM VALUE OF OBJECTIVE FUNCTION **********
******************** ********************
****
#OBJV:** 922.977 **
1
****
******************** ********************
******************** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION **********
******************** FINAL PARAMETER ESTIMATE **********
******************** ********************
****
THETA - VECTOR OF FIXED EFFECTS PARAMETERS *********
TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8
3.23E-01 1.53E-02 4.35E+00 6.87E+00 5.07E-02 3.66E+01 9.98E-01 1.00E+00
OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS ********
ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8
ETA1
+ 0.00E+00
ETA2
+ 0.00E+00 3.10E-03
ETA3
+ 0.00E+00 0.00E+00 0.00E+00
ETA4
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ETA5
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ETA6
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 2.07E-01
ETA7
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ETA8
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS ****
EPS1 EPS2
EPS1
+ 8.56E-03
EPS2
+ 0.00E+00 4.81E-02
1
OMEGA - CORR MATRIX FOR RANDOM EFFECTS - ETAS *******
ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8
ETA1
+ 0.00E+00
ETA2
+ 0.00E+00 5.57E-02
ETA3
+ 0.00E+00 0.00E+00 0.00E+00
ETA4
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ETA5
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ETA6
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 4.55E-01
ETA7
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ETA8
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
SIGMA - CORR MATRIX FOR RANDOM EFFECTS - EPSILONS ***
EPS1 EPS2
EPS1
+ 9.25E-02
EPS2
+ 0.00E+00 2.19E-01
1
****
******************** ********************
******************** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION **********
******************** STANDARD ERROR OF ESTIMATE **********
******************** ********************
****
THETA - VECTOR OF FIXED EFFECTS PARAMETERS *********
TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8
..... 1.40E-03 1.45E-01 8.65E-01 9.47E-03 3.19E+00 6.69E-02 .....
OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS ********
ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8
ETA1
+ .....
ETA2
+ 1.42E-02
ETA3
+ ....
ETA4
+ ....
ETA5
+ ....
ETA6
+ 8.45E-02
ETA7
+ ....
ETA8
+ ....
SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS ****
EPS1 EPS2
EPS1
+ 2.95E-03
EPS2
+ 2.17E-02
1
OMEGA - CORR MATRIX FOR RANDOM EFFECTS - ETAS *******
ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8
ETA1
+ .....
ETA2
+ 1.28E-01
ETA3

+ ....
ETA4
+ ....
ETA5
+ ....
ETA6
+ 9.28E-02
ETA7
+ ....
ETA8
+ ....
SIGMA - CORR MATRIX FOR RANDOM EFFECTS - EPSILONS ***
EPS1 EPS2
EPS1
+ 1.59E-02
EPS2
+ 4.94E-02
1
****
******************** ********************
******************** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION **********
******************** COVARIANCE MATRIX OF ESTIMATE **********
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TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 OM11 OM12 OM13 OM14
OM15 OM16 OM17 OM18 OM22 OM23 OM24 OM25 OM26 OM27 OM28 OM33
OM34 OM35 OM36 OM37 OM38 OM44 OM45 OM46 OM47 OM48 OM55 OM56
OM57 OM58 OM66 OM67 OM68 OM77 OM78 OM88 SG11 SG12 SG22
TH 1
+ ....
TH 2
+ 1.96E-06
TH 3
+ 1.88E-05 2.09E-02
TH 4
+ .... -1.69E-05 7.92E-02 7.48E-01
TH 5
+ .... -4.40E-06 6.37E-04 2.24E-05 8.96E-05
TH 6
+ .... -1.77E-05 6.92E-02 6.65E-02 1.15E-02 1.02E+01
TH 7
+ 7.72E-05 5.25E-03 1.31E-02 5.19E-05 2.64E-02 4.48E-03
TH 8
+ ....
OM11
+ ....
OM12
+ ....
OM13
+ ....
OM14
+ ....
OM15
+ ....
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OM16
+ ....
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OM17
+ ....
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OM18
+ ....
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OM22
+ .... -1.07E-05 1.00E-03 1.79E-05 1.11E-04 1.54E-02 -6.74E-05 ....
.... 2.03E-04
OM23
+ ....
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TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 OM11 OM12 OM13 OM14
OM15 OM16 OM17 OM18 OM22 OM23 OM24 OM25 OM26 OM27 OM28 OM33
OM34 OM35 OM36 OM37 OM38 OM44 OM45 OM46 OM47 OM48 OM55 OM56
OM57 OM58 OM66 OM67 OM68 OM77 OM78 OM88 SG11 SG12 SG22
OM24
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OM44
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OM46
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TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 OM11 OM12 OM13 OM14
OM15 OM16 OM17 OM18 OM22 OM23 OM24 OM25 OM26 OM27 OM28 OM33
OM34 OM35 OM36 OM37 OM38 OM44 OM45 OM46 OM47 OM48 OM55 OM56
OM57 OM58 OM66 OM67 OM68 OM77 OM78 OM88 SG11 SG12 SG22
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OM58
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OM66
+ .... 6.32E-05 -1.80E-03 -6.07E-03 8.15E-06 1.28E-01 1.95E-03 ....
.... -3.55E-04 ....
....
.... 7.15E-03
OM67
+ ....
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OM68
+ ....
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OM77
+ ....
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TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 OM11 OM12 OM13 OM14
OM15 OM16 OM17 OM18 OM22 OM23 OM24 OM25 OM26 OM27 OM28 OM33
OM34 OM35 OM36 OM37 OM38 OM44 OM45 OM46 OM47 OM48 OM55 OM56
OM57 OM58 OM66 OM67 OM68 OM77 OM78 OM88 SG11 SG12 SG22
OM78
+ ....
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OM88
+ ....
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SG11
+ .... 8.74E-08 3.03E-04 1.15E-03 1.69E-05 8.07E-04 8.23E-05 ....
.... 1.65E-05 ....
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.... 2.40E-05 .... 8.71E-06
SG12
+ ....
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SG22
+ .... 3.46E-06 -4.88E-04 7.68E-03 -1.08E-04 -2.66E-02 -2.01E-04 ....
.... -1.88E-04 ....
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.... 3.12E-04 .... -7.49E-06 .... 4.70E-04
1
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********** **********
********** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION **********
********** CORRELATION MATRIX OF ESTIMATE **********
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TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 OM11 OM12 OM13 OM14
OM15 OM16 OM17 OM18 OM22 OM23 OM24 OM25 OM26 OM27 OM28 OM33
OM34 OM35 OM36 OM37 OM38 OM44 OM45 OM46 OM47 OM48 OM55 OM56
OM57 OM58 OM66 OM67 OM68 OM77 OM78 OM88 SG11 SG12 SG22
TH 1
+ ....
TH 2

+ 1.40E-03
TH 3
+ 9.30E-02 1.45E-01
TH 4
+ ..... -1.40E-02 6.33E-01 8.65E-01
TH 5
+ ..... -3.32E-01 4.66E-01 2.73E-03 9.47E-03
TH 6
+ ..... -3.96E-03 1.50E-01 2.41E-02 3.80E-01 3.19E+00
TH 7
+ 8.25E-01 5.43E-01 2.26E-01 8.19E-02 1.23E-01 6.69E-02
TH 8
+ ....
OM11
+ ....
OM12
+ ....
OM13
+ ....
OM14
+ ....
OM15
+ ....
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OM16
+ ....
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OM17
+ ....
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OM18
+ ....
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OM22
+ ..... -5.38E-01 4.87E-01 1.45E-03 8.26E-01 3.39E-01 -7.07E-02 ....
.... 1.42E-02
OM23
+ ....
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TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 OM11 OM12 OM13 OM14
OM15 OM16 OM17 OM18 OM22 OM23 OM24 OM25 OM26 OM27 OM28 OM33
OM34 OM35 OM36 OM37 OM38 OM44 OM45 OM46 OM47 OM48 OM55 OM56
OM57 OM58 OM66 OM67 OM68 OM77 OM78 OM88 SG11 SG12 SG22
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TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 OM11 OM12 OM13 OM14
OM15 OM16 OM17 OM18 OM22 OM23 OM24 OM25 OM26 OM27 OM28 OM33
OM34 OM35 OM36 OM37 OM38 OM44 OM45 OM46 OM47 OM48 OM55 OM56
OM57 OM58 OM66 OM67 OM68 OM77 OM78 OM88 SG11 SG12 SG22
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.... 8.45E-02
OM67
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OM68
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OM77
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TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 OM11 OM12 OM13 OM14
OM15 OM16 OM17 OM18 OM22 OM23 OM24 OM25 OM26 OM27 OM28 OM33
OM34 OM35 OM36 OM37 OM38 OM44 OM45 OM46 OM47 OM48 OM55 OM56
OM57 OM58 OM66 OM67 OM68 OM77 OM78 OM88 SG11 SG12 SG22
OM78
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SG11
+ ..... 2.12E-02 7.11E-01 4.50E-01 6.05E-01 8.56E-02 4.17E-01 ....
.... 3.92E-01 ....
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.... 9.62E-02 .... 2.95E-03
SG12
+ ....
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SG22
+ ..... 1.14E-01 -1.56E-01 4.09E-01 -5.25E-01 -3.84E-01 -1.39E-01 ....
.... -6.10E-01 ....
....
.... 1.70E-01 .... -1.17E-01 ..... 2.17E-02
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********** **********
********** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION **********
********** INVERSE COVARIANCE MATRIX OF ESTIMATE **********
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TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 OM11 OM12 OM13 OM14
OM15 OM16 OM17 OM18 OM22 OM23 OM24 OM25 OM26 OM27 OM28 OM33
OM34 OM35 OM36 OM37 OM38 OM44 OM45 OM46 OM47 OM48 OM55 OM56
OM57 OM58 OM66 OM67 OM68 OM77 OM78 OM88 SG11 SG12 SG22
TH 1
+ .....
TH 2
+ ..... 2.08E+07
TH 3
+ ..... -3.99E+04 4.57E+02
TH 4
+ ..... 6.32E+03 -3.39E+01 7.41E+00
TH 5
+ ..... 1.38E+03 3.56E+02 7.22E+01 6.80E+04
TH 6
+ ..... 6.74E+02 -2.90E+00 -3.76E-01 -4.96E+00 3.55E-01
TH 7
+ ..... -3.20E+05 2.14E+02 -8.94E+01 4.07E+02 -5.65E+00 5.80E+03
TH 8
+ ....
OM11
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OM13
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OM14
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OM22
+ ..... 8.85E+05 -3.41E+03 3.41E+02 -3.24E+04 1.28E+01 -1.16E+04 ....
.... 7.37E+04
OM23
+ ....
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TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 OM11 OM12 OM13 OM14
OM15 OM16 OM17 OM18 OM22 OM23 OM24 OM25 OM26 OM27 OM28 OM33
OM34 OM35 OM36 OM37 OM38 OM44 OM45 OM46 OM47 OM48 OM55 OM56
OM57 OM58 OM66 OM67 OM68 OM77 OM78 OM88 SG11 SG12 SG22
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TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 OM11 OM12 OM13 OM14
OM15 OM16 OM17 OM18 OM22 OM23 OM24 OM25 OM26 OM27 OM28 OM33
OM34 OM35 OM36 OM37 OM38 OM44 OM45 OM46 OM47 OM48 OM55 OM56
OM57 OM58 OM66 OM67 OM68 OM77 OM78 OM88 SG11 SG12 SG22
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OM58
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OM66
+ ..... -7.72E+04 3.07E+02 -6.18E+00 -1.31E+03 -1.33E+01 8.16E+02 ....
.... -2.37E+03 ....
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.... 8.99E+02
OM67
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OM68
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OM77

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TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 OM11 OM12 OM13 OM14
OM15 OM16 OM17 OM18 OM22 OM23 OM24 OM25 OM26 OM27 OM28 OM33
OM34 OM35 OM36 OM37 OM38 OM44 OM45 OM46 OM47 OM48 OM55 OM56
OM57 OM58 OM66 OM67 OM68 OM77 OM78 OM88 SG11 SG12 SG22
OM78
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OM88
+ ....
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SG11
+ ..... 1.88E+06 -8.21E+03 1.64E+02 -9.14E+04 2.14E+02 -2.73E+04 ....
.... 1.07E+05 ....
....
.... -1.23E+04 .... 6.19E+05
SG12
+ ....
....
....
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SG22
+ ..... 3.84E+04 -3.64E+02 -1.03E+02 1.14E+03 3.21E+01 7.04E+02 ....
.... 5.51E+03 ....
....
.... -1.46E+03 .... 1.53E+04 ..... 8.94E+03
1
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******************** ********************
********** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION **********
******************** EIGENVALUES OF COR MATRIX OF ESTIMATE ********************
******************** ********************
****
1 2 3 4 5 6 7 8 9 10
1.27E-02 6.44E-02 8.79E-02 1.11E-01 2.32E-01 6.36E-01 1.07E+00 1.83E+00 2.59E+00 3.37E+00
Stop Time:
Fri Jul 11 20:19:48 UTC 2014

```
$PROB FINAL D3-25D3 MODEL

$INPUT C SID ID NUM TREATMENT2 nOBS DOSEnmol
TIME CMT DVTYPE2 DVN ADDL RATE SS II AMT BASEval
BASE0 REG2 GTYPE RR KAMI CLMI VCMI VTMI QMI D25BASEMI
F4I F5I KAI CLI VCI VTI QI DBASEI VMAXI KMI FIT DV EVID
ASSAYN BMI2 WEIGHT BMIind WTind WTCAT BMICAT ASSAYN2

$DATA ../../../../../data/set2/derived/data-cov.csv IGNORE=C WIDE

$SUB ADVAN13 TOL = 6

$MODEL NCOMPARTMENT = 6

$PK
TYPE = DVTYPE2
REGI2 = REG2
FITS = FIT
ASSAY = ASSAYN2

;FIX METABOLITE PARAMETERS (FROM 964.CTL)
KAM = KAMI; ABSORPTION RATE CONSTANT FOR 25D3

CLM = CLMI; LINEAR TOTAL CLEARANCE FOR 25D3

VCM = VCMI ; central volume for 25D3

VTM = VTMI; PERIPHERAL VOLUME FOR 25D3

QM = QMI ; INTERCOMPARTMENTAL CL FOR 25D3

F4 = F4I ; BA ORAL 25D3

F5 = F5I ; IV BA OF 25D3 = 1

;ESTIMATE D3-25D3 PK PARAMETERS AND ALSO PARENT DBASE (MIX OF GROUPS AND INDIVIDUALS)
;SEPARATE ETAS FOR INDIV AND GROUPS
INDDAT = 0
IF(nOBS.EQ.1) INDDAT = 1
ETAVMAX = ETA(1)*INDDAT + ETA(4)*(1-INDDAT) ; ETA1 IS INDIVIDUAL LEVEL ETA

ETAKM = ETA(3)*INDDAT + ETA(6)*(1-INDDAT); ETA3 IS INDIVIDUAL LEVEL ETA

ETADBASE = ETA(2)*INDDAT + ETA(5)*(1-INDDAT) ; ETA2 IS INDIVIDUAL LEVEL ETA

VMAX = THETA(1)*EXP(ETAVMAX/SQRT(nOBS)) ; RATE OF METABOLISM (NMOL/H)

KM = THETA(2)*EXP(ETAKM/SQRT(nOBS)) ; AT THIS PARENT CONCENTRATION, HALF OF THE MAXIMUM RATE OF METABOLISM IS ACHIEVED

DBASE = THETA(3)*EXP(ETADBASE/SQRT(nOBS))

;ESTIMATE D3 PK PARAMETERS (ONLY GROUP DATA)
KA = THETA(4)*EXP(ETA(7)/SQRT(nOBS)) ; absorption ka FOR D3

VC = THETA(5)*EXP(ETA(8)*(1-INDDAT)/SQRT(nOBS)) ; central volume for D3 (L)

VT = THETA(6)*EXP(ETA(9)/SQRT(nOBS)) ; PERIPHERAL VOLUME FOR D3 (L) ; ETA ALLOWED FOR GROUPS ONLY

Q = THETA(7)*EXP(ETA(10)/SQRT(nOBS)); INTERCOMPARTMENTAL CL FOR D3 (L/H)

CL = THETA(8)*EXP(ETA(11)/SQRT(nOBS)) ; LINEAR CLEARANCE FOR D3 (L/H) = 0

;SET INITIAL CMT AMOUNTS, ENDOG, AND DBASEM
A_0(2) = DBASE*VC ; INTIALIZE CENTRAL D3 CMT WITH D3 AMT (nmol)
A_0(3) = DBASE*VT ; INITIALIZE PERIPHERAL D3 CMT

ENDOG = VMAX*DBASE/(KM + DBASE) ; endogenous rate of Vit D3 (nmol/h)

CLTM0 = VMAX/(KM + DBASE)

A_0(5) = CLTM0*(DBASE*VCM/CLM); INTIALIZE CENTRAL 25D3 CMT WITH 25D3 AMT (nmol)
A_0(6) = CLTM0*(DBASE*VTM/CLM); INITIALIZE PERIPHERAL 25D3 CMT

;SET METABOLITE BASELINE TO METABOLITE ESTIMATE FROM 25D3-25D3 MODEL FOR 25D3-25D3 DATASET UNITS
IF(FITS.EQ.1) DBASEM = D25BASEMI

;ESTIMATE METABOLITE BASELINE FOR D3-25D3 DATASET UNITS
IF(FITS.EQ.0) DBASEM = (CLTM0*DBASE)/CLM; BASELINE CONCENTRATION FOR 25D3 (NMOL/L)

$DES
DADT(1) = -KA*A(1) ; D3 GUT CMT

C2 = A(2)/VC ; CONCENTRATION OF CENTRAL COMPARTMENT FOR D3
C3 = A(3)/VT; CONCENTRATION OF PERIPHERAL COMPARTMENT FOR D3

RATE2 = VMAX*C2/(KM + C2) ; RATE OF METABOLISM (NMOL/H)
CLNL = RATE2/C2 ; NON-LINEAR CLEARANCE (L/H)
CLTM = CLNL ; CLEARANCE OF D3 INTO METABOLTE (L/H)

DADT(2) = ENDOG + KA*A(1) - (CLNL + CL + Q)*C2 + Q*C3 ; D3 CENTRAL CMT

DADT(3) = Q*C2 - Q*C3 ; D3 PERIPHERAL CMT

DADT(4) = -KAM*A(4) ; 25D3 GUT CMT

C5 = A(5)/VCM ; CONCENTRATION OF CENTRAL COMPARTMENT FOR 25D3
C6 = A(6)/VTM; CONCENTRATION OF PERIPHERAL COMPARTMENT FOR 25D3

DADT(5) = KAM*A(4) + CLTM*C2 - CLM*C5 - QM*C5 + QM*C6 ; 25D3 CENTRAL CMT

DADT(6) = QM*C5 - QM*C6 ; 25D3 PERIPHERAL CMT

$ERROR
IND2 = 0
IND3 = 0
CP = A(2)/VC ; D3 CENTRAL CONCENTRATION
CM = A(5)/VCM ; 25D3 CENTRAL CONCENTRATION

IF (TYPE.EQ.6) IND2 = 1 ; 25D3
IF (TYPE.EQ.3) IND3 = 1 ; D3
```

```
;INDIVDIUALS — METABOLITE ENDPT
IF(TYPE.EQ.6.AND.nOBS.EQ.1) Y25D3 = CM*EXP(EPS(2)/SQRT(nOBS)) ; individuals METABOLITE ENDPT
; no assay distinction because only measured with HPlC (STANDARD ASSAY)

; GROUPS-- METABOLITE ENDPT
IF(TYPE.EQ.6.AND.nOBS.GT.1.AND.ASSAY.EQ.3) Y25D3 = CM*EXP(EPS(1)/SQRT(nOBS)) ; REF ASSAY = 3 HPLC
IF(TYPE.EQ.6.AND.nOBS.GT.1.AND.ASSAY.EQ.1) Y25D3 = THETA(9)*CM*EXP(THETA(12)*EPS(1)/SQRT(nOBS)) + THETA(15) ; ASSAY = 1 RIA
IF(TYPE.EQ.6.AND.nOBS.GT.1.AND.ASSAY.EQ.2) Y25D3 = THETA(10)*CM*EXP(THETA(13)*EPS(1)/SQRT(nOBS)) + THETA(16) ; ASSAY = 2 CPBA
IF(TYPE.EQ.6.AND.nOBS.GT.1.AND.ASSAY.EQ.4) Y25D3 = THETA(11)*CM*EXP(THETA(14)*EPS(1)/SQRT(nOBS)) + THETA(17) ; ASSAY = 4 CHEMILUM

; D3 — BROKEN UP BY SD OR MD REGIMENS
IF(TYPE.EQ.3.AND.REGI2.EQ.1) YD3 = CP*EXP(EPS(3)/SQRT(nOBS)) ; SD--PARENT ENDPT; no assay distinction because only one type of
assay
IF(TYPE.EQ.3.AND.REGI2.EQ.2) YD3 = CP*EXP(EPS(4)/SQRT(nOBS)) ; MD--PARENT ENDPT ; two subjects each with different assays so no
assay distinction because picked up in iiv

Y = Y25D3*IND2 + YD3*IND3 ; MODEL PREDICTION

$THETA
(0, 3) ; VMAX
(0, 12) ; KM
(0, 5) ;DBASE
0.323, FIX ; KA
(0, 11) ;VC
(0, 1800) ;VT
(0, 0.2) ;Q
0, FIX ;CL
(0, 0.8); PROP BIAS FOR ASSAY 1
(0, 1); PROP BIAS FOR ASSAY 2
(0, 1); PROP BIAS FOR ASSAY 4
(0, 1); PRECISION FOR ASSAY 1
(0, 1); PREC FOR ASSAY 2
(0, 1); PREC FOR ASSAY 4
1; ADD BIAS FOR ASSAY 1
-20; ADD BIAS FOR ASSAY 2
1; ADD BIAS FOR ASSAY 4

$OMEGA
0.3 ;VMAX INDIV
0,FIX ; DBASE INDIV

$OMEGA
0, FIX ; KM INDIV

$OMEGA BLOCK (2)
3; VMAX2 GROUP
-1 8 ; DBASE GROUP

$OMEGA
0, FIX ; KM GROUP
0, FIX; KA
0, FIX ; VC
0, FIX ; VT
0, FIX ; Q
0, FIX; CL

$SIGMA
0.02 ; INDIV METAB ENDPT
1.5 ; GROUPS METAB ENDPT
6.9; SD PARENT ENDPT
0.29 ; MD PARENT ENDPT

$ESTIMATION MAXEVAL=9999 PRINT=5 METHOD = 1 INT SIGDIGITS = 2 MSFO=./1784.msf
$COV PRINT=E MATRIX = S

#TERM:
0MINIMIZATION SUCCESSFUL
NO. OF FUNCTION EVALUATIONS USED: 1557
NO. OF SIG. DIGITS IN FINAL EST.: 2.4

ETABAR IS THE ARITHMETIC MEAN OF THE ETA-ESTIMATES,
AND THE P-VALUE IS GIVEN FOR THE NULL HYPOTHESIS THAT THE TRUE MEAN IS 0.
ETABAR: -3.5973E-03 0.0000E+00 0.0000E+00 5.9053E-02 2.2645E-01 0.0000E+00 0.0000E+00 0.0000E+00 0.0000E+00
0.0000E+00
0.0000E+00
SE: 3.3416E-03 0.0000E+00 0.0000E+00 1.0204E-01 1.9621E-01 0.0000E+00 0.0000E+00 0.0000E+00 0.0000E+00
0.0000E+00
0.0000E+00
P VAL.: 2.8170E-01 1.0000E+00 1.0000E+00 5.6279E-01 2.4844E-01 1.0000E+00 1.0000E+00 1.0000E+00 1.0000E+00
1.0000E+00
1.0000E+00
ETAshrink(%): 7.0099E+01 1.0000E+02 1.0000E+02 1.9151E+01 2.2989E+01 1.0000E+02 1.0000E+02 1.0000E+02 1.0000E+02
1.0000E+02
1.0000E+02
EPSshrink(%): 1.7425E+00 8.0006E+00 3.7401E+00 2.4321E+00
#TERE:

Elapsed estimation time in seconds: 10680.71
Elapsed covariance time in seconds: 1003.11
1
********************************************************************************************************************************
******************** ********************
******************** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION ********************
#OBJT:*************** MINIMUM VALUE OF OBJECTIVE FUNCTION ********************
******************** ********************
********************************************************************************************************************************
#OBJV:****************************************** 4009.529 **************************************************
1
********************************************************************************************************************************
******************** ********************
******************** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION ********************
******************** FINAL PARAMETER ESTIMATE ********************
******************** ********************
********************************************************************************************************************************
THETA - VECTOR OF FIXED EFFECTS PARAMETERS *********
TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 TH 9 TH10 TH11 TH12
TH13 TH14 TH15 TH16 TH17
1.62E+00 6.38E+00 3.75E+00 3.23E-01 1.56E+01 2.33E+03 1.85E-01 0.00E+00 1.01E+00 1.50E+00 1.37E+00 1.45E+00
1.51E+00 1.16E+00 2.49E+00 -2.21E+01 -1.01E+01
OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS ********
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ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8 ETA9 ET10 ET11
ETA1
+ 1.69E-02
ETA2
+ 0.00E+00 0.00E+00
ETA3
+ 0.00E+00 0.00E+00 0.00E+00
ETA4
+ 0.00E+00 0.00E+00 0.00E+00 2.15E+00
ETA5
+ 0.00E+00 0.00E+00 0.00E+00 -5.23E-01 8.76E+00
ETA6
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ETA7
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ETA8
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ETA9
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ET10
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ET11
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS ****
EPS1 EPS2 EPS3 EPS4
EPS1
+ 1.85E-01
EPS2
+ 0.00E+00 2.81E-02
1
EPS1 EPS2 EPS3 EPS4
EPS3
+ 0.00E+00 0.00E+00 5.50E+00
EPS4
+ 0.00E+00 0.00E+00 0.00E+00 2.99E-01
1
OMEGA - CORR MATRIX FOR RANDOM EFFECTS - ETAS *******
ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8 ETA9 ET10 ET11
ETA1
+ 1.30E-01
ETA2
+ 0.00E+00 0.00E+00
ETA3
+ 0.00E+00 0.00E+00 0.00E+00
ETA4
+ 0.00E+00 0.00E+00 0.00E+00 1.47E+00
ETA5
+ 0.00E+00 0.00E+00 0.00E+00 -1.20E-01 2.96E+00
ETA6
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ETA7
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ETA8
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ETA9
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ET10
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
ET11
+ 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
SIGMA - CORR MATRIX FOR RANDOM EFFECTS - EPSILONS ***
EPS1 EPS2 EPS3 EPS4
EPS1
+ 4.31E-01
EPS2
+ 0.00E+00 1.68E-01
EPS3
+ 0.00E+00 0.00E+00 2.35E+00
EPS4
+ 0.00E+00 0.00E+00 0.00E+00 5.46E-01
1
****
******************** ********************
******************** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION ********************
******************** STANDARD ERROR OF ESTIMATE ********************
******************** ********************
****
THETA - VECTOR OF FIXED EFFECTS PARAMETERS *********
TH 1 TH 2 TH 3 TH 4 TH 5 TH 6 TH 7 TH 8 TH 9 TH10 TH11 TH12
TH13 TH14 TH15 TH16 TH17
8.72E-02 7.18E-01 2.88E-01 .... 2.49E+00 2.79E+02 5.99E-03 .... 8.02E-02 8.52E-02 5.26E-01 1.27E-01
1.64E-01 2.28E-01 3.16E+00 4.79E+00 1.40E+01
OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS ********
ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8 ETA9 ET10 ET11
ETA1
+ 1.17E-02
ETA2
+ ....
ETA3
+ ....
ETA4
+ 4.40E-01
ETA5
+ .... 8.79E-01 1.91E+00
ETA6
+ ....
ETA7
+ ....
ETA8
+ ....
ETA9
+ ....
ET10
+ ....
ET11
+ ....
SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS ****
EPS1 EPS2 EPS3 EPS4
EPS1
+ 1.55E-02

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EPS2
+ ......... 2.22E-03
1
EPS1 EPS2 EPS3 EPS4
EPS3
+ ......... ......... 4.62E+01
EPS4
+ ......... ......... ......... 1.02E-01
1
OMEGA - CORR MATRIX FOR RANDOM EFFECTS - ETAS *******
ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8 ETA9 ET10 ET11
ETA1
+ 4.49E-02
ETA2
+ ......... .........
ETA3
+ ......... ......... .........
ETA4
+ ......... ......... ......... 1.50E-01
ETA5
+ ......... ......... ......... 1.94E-01 3.22E-01
ETA6
+ ......... ......... ......... ......... ......... .........
ETA7
+ ......... ......... ......... ......... ......... ......... .........
ETA8
+ ......... ......... ......... ......... ......... ......... ......... .........
ETA9
+ ......... ......... ......... ......... ......... ......... ......... ......... .........
ET10
+ ......... ......... ......... ......... ......... ......... ......... ......... ......... .........
ET11
+ ......... ......... ......... ......... ......... ......... ......... ......... ......... ......... .........
SIGMA - CORR MATRIX FOR RANDOM EFFECTS - EPSILONS ***
EPS1 EPS2 EPS3 EPS4
EPS1
+ 1.80E-02
EPS2
+ ......... 6.64E-03
EPS3
+ ......... ......... 9.85E+00
EPS4
+ ......... ......... ......... 9.33E-02
1
********************************************************************************************************************************
******************** ********************
******************** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION ********************
******************** COVARIANCE MATRIX OF ESTIMATE ********************
******************** ********************
********************************************************************************************************************************
TH 1 | TH 1 TH 2 | TH 1 TH 2 | TH 2 TH 3 | TH 1 TH 3 | TH 2 TH 3 | TH 3 TH 5 | TH 1
7.60E-03 3.26E-02 5.15E-01 4.57E-03 1.47E-01 8.30E-02 -1.03E-02
TH 5 | TH 2 TH 5 | TH 3 TH 5 | TH 5 TH 6 | TH 1 TH 6 | TH 2 TH 6 | TH 3 TH 6 | TH 5
-5.55E-01 -1.54E-01 6.22E+00 -9.26E+00 -8.14E+01 -1.91E+01 2.44E+01
TH 6 | TH 6 TH 7 | TH 1 TH 7 | TH 2 TH 7 | TH 3 TH 7 | TH 5 TH 7 | TH 6 TH 7 | TH 7
7.78E+04 -5.26E-05 -1.13E-03 -4.46E-04 7.30E-03 -3.56E-01 3.59E-05
TH 9 | TH 1 TH 9 | TH 2 TH 9 | TH 3 TH 9 | TH 5 TH 9 | TH 6 TH 9 | TH 7 TH 9 | TH 9
-4.72E-03 -1.30E-02 3.00E-03 -1.93E-02 1.01E+01 -4.56E-05 6.43E-03
TH10 | TH 1 TH10 | TH 2 TH10 | TH 3 TH10 | TH 5 TH10 | TH 6 TH10 | TH 7 TH10 | TH 9
-4.64E-03 -5.97E-03 5.41E-03 -2.30E-02 4.45E+00 7.52E-05 3.17E-03
TH10 | TH10 TH11 | TH 1 TH11 | TH 2 TH11 | TH 3 TH11 | TH 5 TH11 | TH 6 TH11 | TH 7
7.26E-03 -3.46E-03 2.44E-03 -1.64E-03 -3.91E-02 -2.25E+00 5.86E-05
TH11 | TH 9 TH11 | TH10 TH11 | TH11 TH12 | TH 1 TH12 | TH 2 TH12 | TH 3 TH12 | TH 5
1.58E-03 3.15E-03 2.77E-01 3.94E-03 4.05E-02 1.94E-03 -4.30E-02
TH12 | TH 6 TH12 | TH 7 TH12 | TH 9 TH12 | TH10 TH12 | TH11 TH12 | TH12 TH13 | TH 1
4.12E+00 -2.79E-04 -1.92E-03 -4.22E-03 -1.49E-03 1.62E-02 2.99E-03
TH13 | TH 2 TH13 | TH 3 TH13 | TH 5 TH13 | TH 6 TH13 | TH 7 TH13 | TH 9 TH13 | TH10
4.83E-02 9.50E-03 1.69E-02 -1.45E+01 -5.37E-05 -1.40E-03 -7.29E-03
TH13 | TH11 TH13 | TH12 TH13 | TH13 TH14 | TH 1 TH14 | TH 2 TH14 | TH 3 TH14 | TH 5
-1.10E-03 8.63E-03 2.70E-02 3.01E-03 2.51E-02 -7.17E-04 2.00E-02
TH14 | TH 6 TH14 | TH 7 TH14 | TH 9 TH14 | TH10 TH14 | TH11 TH14 | TH12 TH14 | TH13
-1.18E+01 2.91E-05 -2.84E-03 -3.49E-03 -5.61E-02 5.35E-03 8.40E-03
TH14 | TH14 TH15 | TH 1 TH15 | TH 2 TH15 | TH 3 TH15 | TH 5 TH15 | TH 6 TH15 | TH 7
5.18E-02 7.63E-02 7.53E-01 -1.22E-01 1.48E-01 -1.67E+02 -2.41E-04
TH15 | TH 9 TH15 | TH10 TH15 | TH11 TH15 | TH12 TH15 | TH13 TH15 | TH14 TH15 | TH15
-9.17E-02 -7.08E-02 3.69E-02 2.56E-01 1.28E-01 1.14E-01 9.98E+00
TH16 | TH 1 TH16 | TH 2 TH16 | TH 3 TH16 | TH 5 TH16 | TH 6 TH16 | TH 7 TH16 | TH 9
-1.16E-02 2.61E-01 -2.57E-01 1.14E+00 -5.43E+00 -3.34E-03 -2.21E-03
TH16 | TH10 TH16 | TH11 TH16 | TH12 TH16 | TH13 TH16 | TH14 TH16 | TH15 TH16 | TH16
-2.58E-01 -9.53E-03 2.47E-01 5.83E-01 1.94E-01 3.84E+00 2.30E+01
TH17 | TH 1 TH17 | TH 2 TH17 | TH 3 TH17 | TH 5 TH17 | TH 6 TH17 | TH 7 TH17 | TH 9
8.15E-02 5.87E-01 -1.28E-01 2.73E+00 -1.56E+02 1.59E-03 -9.05E-02
TH17 | TH10 TH17 | TH11 TH17 | TH12 TH17 | TH13 TH17 | TH14 TH17 | TH15 TH17 | TH16
-8.92E-02 -4.68E+00 1.49E-01 1.63E-01 3.01E+00 3.79E+00 5.14E+00
TH17 | TH17 OM0101 | TH 1 OM0101 | TH 2 OM0101 | TH 3 OM0101 | TH 5 OM0101 | TH 6 OM0101 | TH 7
1.97E+02 4.90E-04 -3.92E-04 -2.96E-04 4.50E-03 -3.08E-01 -1.16E-06
OM0101 | TH 9 OM0101 | TH10 OM0101 | TH11 OM0101 | TH12 OM0101 | TH13 OM0101 | TH14 OM0101 | TH15
-3.33E-04 -3.91E-04 -3.90E-04 6.99E-05 -3.54E-05 8.64E-05 4.00E-04
OM0101 | TH16 OM0101 | TH17 OM0101 | OM0101 OM0404 | TH 1 OM0404 | TH 2 OM0404 | TH 3 OM0404 | TH 5
-3.63E-03 3.21E-03 1.36E-04 -3.56E-03 3.42E-04 -8.71E-03 9.35E-02
OM0404 | TH 6 OM0404 | TH 7 OM0404 | TH 9 OM0404 | TH10 OM0404 | TH11 OM0404 | TH12 OM0404 | TH13
1.72E+00 5.50E-05 7.75E-03 -1.17E-02 -1.73E-03 1.70E-02 3.48E-02
OM0404 | TH14 OM0404 | TH15 OM0404 | TH16 OM0404 | TH17 OM0404 | OM0101 OM0404 | OM0404 OM0405 | TH 1
1.44E-02 2.74E-01 1.12E+00 1.44E-01 -3.61E-04 1.94E-01 -5.01E-03
OM0405 | TH 2 OM0405 | TH 3 OM0405 | TH 5 OM0405 | TH 6 OM0405 | TH 7 OM0405 | TH 9 OM0405 | TH10
-1.70E-01 -6.95E-02 4.78E-01 -2.34E+01 5.67E-04 -1.94E-02 -2.93E-03
OM0405 | TH11 OM0405 | TH12 OM0405 | TH13 OM0405 | TH14 OM0405 | TH15 OM0405 | TH16 OM0405 | TH17
-9.58E-03 -9.47E-03 -6.00E-03 1.25E-02 -4.45E-01 3.47E-01 6.71E-01
OM0405 | OM0101 OM0405 | OM0404 OM0405 | OM0405 OM0505 | TH 1 OM0505 | TH 2 OM0505 | TH 3 OM0505 | TH 5
7.24E-04 -1.73E-01 7.73E-01 4.88E-02 5.02E-01 5.59E-03 -1.02E+00
OM0505 | TH 6 OM0505 | TH 7 OM0505 | TH 9 OM0505 | TH10 OM0505 | TH11 OM0505 | TH12 OM0505 | TH13
-1.06E+02 -7.82E-05 -4.16E-02 -1.57E-02 4.37E-02 6.35E-02 1.19E-02
OM0505 | TH14 OM0505 | TH15 OM0505 | TH16 OM0505 | TH17 OM0505 | OM0101 OM0505 | OM0404 OM0505 | OM0405
5.21E-02 2.61E+00 1.38E-02 1.30E+00 4.41E-06 7.59E-02 -4.80E-01
OM0505 | OM0505 SG0101 | TH 1 SG0101 | TH 2 SG0101 | TH 3 SG0101 | TH 5 SG0101 | TH 6 SG0101 | TH 7
3.63E+00 -4.56E-04 -3.97E-03 -5.02E-04 -3.53E-03 1.17E+00 8.10E-06
SG0101 | TH 9 SG0101 | TH10 SG0101 | TH11 SG0101 | TH12 SG0101 | TH13 SG0101 | TH14 SG0101 | TH15
2.53E-04 6.74E-04 2.99E-04 -1.18E-03 -1.77E-03 -1.06E-03 -1.41E-02
SG0101 | TH16 SG0101 | TH17 SG0101 | OM0101 SG0101 | OM0404 SG0101 | OM0405 SG0101 | OM0505 SG0101 | SG0101
-4.02E-02 -1.96E-02 -1.72E-05 -4.26E-03 -1.67E-04 -4.70E-03 2.41E-04
```

SG0202 | TH 1 SG0202 | TH 2 SG0202 | TH 3 SG0202 | TH 5 SG0202 | TH 6 SG0202 | TH 7 SG0202 | TH 9
-9.96E-06 1.72E-04 -1.20E-04 -5.12E-04 2.34E-02 -1.01E-06 -3.68E-06
SG0202 | TH10 SG0202 | TH11 SG0202 | TH12 SG0202 | TH13 SG0202 | TH14 SG0202 | TH15 SG0202 | TH16
-6.40E-06 3.81E-05 6.22E-05 3.59E-05 3.32E-05 1.73E-03 2.15E-03
SG0202 | TH17 SG0202 | OM0101 SG0202 | OM0404 SG0202 | OM0405 SG0202 | OM0505 SG0202 | SG0101 SG0202 | SG0202
1.33E-03 -1.13E-06 7.46E-05 -1.34E-05 6.96E-04 -2.91E-06 4.95E-06
SG0303 | TH 1 SG0303 | TH 2 SG0303 | TH 3 SG0303 | TH 5 SG0303 | TH 6 SG0303 | TH 7 SG0303 | TH 9
-2.67E-01 -9.42E+00 -2.55E+00 8.63E+01 -2.51E+03 1.25E-01 -6.35E-01
SG0303 | TH10 SG0303 | TH11 SG0303 | TH12 SG0303 | TH13 SG0303 | TH14 SG0303 | TH15 SG0303 | TH16
-1.15E-01 -2.21E-01 -1.64E+00 -9.20E-02 3.28E-01 -3.53E+00 -8.35E-01
SG0303 | TH17 SG0303 | OM0101 SG0303 | OM0404 SG0303 | OM0405 SG0303 | OM0505 SG0303 | SG0101 SG0303 | SG0202
3.42E+01 6.16E-02 -1.21E+00 1.15E+01 -1.41E+01 6.20E-03 -9.81E-03
SG0303 | SG0303 SG0404 | TH 1 SG0404 | TH 2 SG0404 | TH 3 SG0404 | TH 5 SG0404 | TH 6 SG0404 | TH 7
2.13E+03 1.65E-04 3.81E-03 -1.48E-04 3.19E-02 7.25E-01 2.11E-04
SG0404 | TH 9 SG0404 | TH10 SG0404 | TH11 SG0404 | TH12 SG0404 | TH13 SG0404 | TH14 SG0404 | TH15
-2.71E-05 3.97E-04 2.35E-04 1.39E-03 2.31E-03 1.35E-03 1.41E-02
SG0404 | TH16 SG0404 | TH17 SG0404 | OM0101 SG0404 | OM0404 SG0404 | OM0405 SG0404 | OM0505 SG0404 | SG0101
1.15E-02 2.90E-02 -4.55E-05 -2.93E-04 3.15E-03 1.07E-02 -3.08E-04
SG0404 | SG0202 SG0404 | SG0303 SG0404 | SG0404
2.59E-06 3.14E-01 1.04E-02
1

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********** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION **********
********** CORRELATION MATRIX OF ESTIMATE **********
********** **********

TH 1 | TH 1 TH 2 | TH 1 TH 2 | TH 2 TH 3 | TH 1 TH 3 | TH 2 TH 3 | TH 3 TH 5 | TH 1
8.72E-02 5.20E-01 7.18E-01 1.82E-01 7.12E-01 2.88E-01 -4.75E-02
TH 5 | TH 2 TH 5 | TH 3 TH 5 | TH 5 TH 6 | TH 1 TH 6 | TH 2 TH 6 | TH 3 TH 6 | TH 5
-3.10E-01 -2.15E-01 2.49E+00 -3.81E-01 -4.07E-01 -2.38E-01 3.51E-02
TH 6 | TH 6 TH 7 | TH 1 TH 7 | TH 2 TH 7 | TH 3 TH 7 | TH 5 TH 7 | TH 6 TH 7 | TH 7
2.79E+02 -1.01E-01 -2.62E-01 -2.58E-01 4.88E-01 -2.13E-01 5.99E-03
TH 9 | TH 1 TH 9 | TH 2 TH 9 | TH 3 TH 9 | TH 5 TH 9 | TH 6 TH 9 | TH 7 TH 9 | TH 9
-6.75E-01 -2.26E-01 1.30E-01 -9.66E-02 4.51E-01 -9.50E-02 8.02E-02
TH10 | TH 1 TH10 | TH 2 TH10 | TH 3 TH10 | TH 5 TH10 | TH 6 TH10 | TH 7 TH10 | TH 9
-6.24E-01 -9.76E-02 2.20E-01 -1.08E-01 1.87E-01 1.47E-01 4.64E-01
TH10 | TH10 TH11 | TH 1 TH11 | TH 2 TH11 | TH 3 TH11 | TH 5 TH11 | TH 6 TH11 | TH 7
8.52E-02 -7.55E-02 6.46E-03 -1.08E-02 -2.98E-02 -1.53E-02 1.86E-02
TH11 | TH 9 TH11 | TH10 TH11 | TH11 TH12 | TH 1 TH12 | TH 2 TH12 | TH 3 TH12 | TH 5
3.74E-02 7.03E-02 5.26E-01 3.55E-01 4.42E-01 5.29E-02 -1.35E-01
TH12 | TH 6 TH12 | TH 7 TH12 | TH 9 TH12 | TH10 TH12 | TH11 TH12 | TH12 TH13 | TH 1
1.16E-01 -3.65E-01 -1.88E-01 -3.89E-01 -2.23E-02 1.27E-01 2.09E-01
TH13 | TH 2 TH13 | TH 3 TH13 | TH 5 TH13 | TH 6 TH13 | TH 7 TH13 | TH 9 TH13 | TH10
4.09E-01 2.01E-01 4.12E-02 -3.16E-01 -5.46E-02 -1.06E-01 -5.21E-01
TH13 | TH11 TH13 | TH12 TH13 | TH13 TH14 | TH 1 TH14 | TH 2 TH14 | TH 3 TH14 | TH 5
-1.27E-02 4.13E-01 1.64E-01 1.52E-01 1.53E-01 -1.09E-02 3.52E-02
TH14 | TH 6 TH14 | TH 7 TH14 | TH 9 TH14 | TH10 TH14 | TH11 TH14 | TH12 TH14 | TH13
-1.86E-01 2.13E-02 -1.56E-01 -1.80E-01 -4.69E-01 1.84E-01 2.25E-01
TH14 | TH14 TH15 | TH 1 TH15 | TH 2 TH15 | TH 3 TH15 | TH 5 TH15 | TH 6 TH15 | TH 7
2.28E-01 2.77E-01 3.32E-01 -1.34E-01 1.88E-02 -1.90E-01 -1.27E-02
TH15 | TH 9 TH15 | TH10 TH15 | TH11 TH15 | TH12 TH15 | TH13 TH15 | TH14 TH15 | TH15
-3.62E-01 -2.63E-01 2.22E-02 6.37E-01 2.46E-01 1.59E-01 3.16E+00
TH16 | TH 1 TH16 | TH 2 TH16 | TH 3 TH16 | TH 5 TH16 | TH 6 TH16 | TH 7 TH16 | TH 9
-2.78E-02 7.58E-02 -1.86E-01 9.58E-02 -4.06E-03 -1.16E-01 -5.76E-03
TH16 | TH10 TH16 | TH11 TH16 | TH12 TH16 | TH13 TH16 | TH14 TH16 | TH15 TH16 | TH16
-6.30E-01 -3.78E-03 4.05E-01 7.41E-01 1.78E-01 2.54E-01 4.79E+00
TH17 | TH 1 TH17 | TH 2 TH17 | TH 3 TH17 | TH 5 TH17 | TH 6 TH17 | TH 7 TH17 | TH 9
6.66E-02 5.83E-02 -3.16E-02 7.81E-02 -3.98E-02 1.89E-02 -8.04E-02
TH17 | TH10 TH17 | TH11 TH17 | TH12 TH17 | TH13 TH17 | TH14 TH17 | TH15 TH17 | TH16
-7.46E-02 -6.34E-01 8.31E-02 7.08E-02 9.43E-01 8.55E-02 7.63E-02
TH17 | TH17 OM0101 | TH 1 OM0101 | TH 2 OM0101 | TH 3 OM0101 | TH 5 OM0101 | TH 6 OM0101 | TH 7
1.40E+01 4.82E-01 -4.68E-02 -8.82E-02 1.55E-01 -9.48E-02 -1.66E-02
OM0101 | TH 9 OM0101 | TH10 OM0101 | TH11 OM0101 | TH12 OM0101 | TH13 OM0101 | TH14 OM0101 | TH15
-3.56E-01 -3.94E-01 -6.36E-02 4.71E-02 -1.85E-02 3.26E-02 1.08E-02
OM0101 | TH16 OM0101 | TH17 OM0101 | OM0101 OM0404 | TH 1 OM0404 | TH 2 OM0404 | TH 3 OM0404 | TH 5
-6.49E-02 1.96E-02 1.17E-02 -9.28E-02 1.08E-03 -6.87E-02 8.51E-02
OM0404 | TH 6 OM0404 | TH 7 OM0404 | TH 9 OM0404 | TH10 OM0404 | TH11 OM0404 | TH12 OM0404 | TH13
1.40E-02 2.08E-02 2.20E-01 -3.13E-01 -7.45E-03 3.03E-01 4.81E-01
OM0404 | TH14 OM0404 | TH15 OM0404 | TH16 OM0404 | TH17 OM0404 | OM0101 OM0404 | OM0404 OM0405 | TH 1
1.43E-01 1.97E-01 5.29E-01 2.32E-02 -7.03E-02 4.40E-01 -6.54E-02
OM0405 | TH 2 OM0405 | TH 3 OM0405 | TH 5 OM0405 | TH 6 OM0405 | TH 7 OM0405 | TH 9 OM0405 | TH10
-2.70E-01 -2.74E-01 2.18E-01 -9.55E-02 1.08E-01 -2.75E-01 -3.91E-02
OM0405 | TH11 OM0405 | TH12 OM0405 | TH13 OM0405 | TH14 OM0405 | TH15 OM0405 | TH16 OM0405 | TH17
-2.07E-02 -8.45E-02 -4.15E-02 6.25E-02 -1.60E-01 8.22E-02 5.44E-02
OM0405 | OM0101 OM0405 | OM0404 OM0405 | OM0405 OM0505 | TH 1 OM0505 | TH 2 OM0505 | TH 3 OM0505 | TH 5
7.06E-02 -4.48E-01 8.79E-01 2.94E-01 3.67E-01 1.02E-02 -2.14E-01
OM0505 | TH 6 OM0505 | TH 7 OM0505 | TH 9 OM0505 | TH10 OM0505 | TH11 OM0505 | TH12 OM0505 | TH13
-1.99E-01 -6.84E-03 -2.72E-01 -9.69E-02 4.36E-02 2.62E-01 3.82E-02
OM0505 | TH14 OM0505 | TH15 OM0505 | TH16 OM0505 | TH17 OM0505 | OM0101 OM0505 | OM0404 OM0505 | OM0405
1.20E-01 4.33E-01 1.51E-03 4.85E-02 1.98E-04 9.05E-02 -2.86E-01
OM0505 | OM0505 SG0101 | TH 1 SG0101 | TH 2 SG0101 | TH 3 SG0101 | TH 5 SG0101 | TH 6 SG0101 | TH 7
1.91E+00 -3.37E-01 -3.57E-01 -1.12E-01 -9.12E-02 2.70E-01 8.71E-02
SG0101 | TH 9 SG0101 | TH10 SG0101 | TH11 SG0101 | TH12 SG0101 | TH13 SG0101 | TH14 SG0101 | TH15
2.03E-01 5.10E-01 3.67E-02 -5.96E-01 -6.97E-01 -3.01E-01 -2.87E-01
SG0101 | TH16 SG0101 | TH17 SG0101 | OM0101 SG0101 | OM0404 SG0101 | OM0405 SG0101 | OM0505 SG0101 | SG0101
-5.41E-01 -9.00E-02 -9.53E-02 -6.24E-01 -1.22E-02 -1.59E-01 1.55E-02
SG0202 | TH 1 SG0202 | TH 2 SG0202 | TH 3 SG0202 | TH 5 SG0202 | TH 6 SG0202 | TH 7 SG0202 | TH 9
-5.14E-02 1.08E-01 -1.87E-01 -9.23E-02 3.77E-02 -7.57E-02 -2.07E-02
SG0202 | TH10 SG0202 | TH11 SG0202 | TH12 SG0202 | TH13 SG0202 | TH14 SG0202 | TH15 SG0202 | TH16
-3.38E-02 3.25E-02 2.20E-01 9.83E-02 6.56E-02 2.46E-01 2.01E-01
SG0202 | TH17 SG0202 | OM0101 SG0202 | OM0404 SG0202 | OM0405 SG0202 | OM0505 SG0202 | SG0101 SG0202 | SG0202
4.25E-02 -4.36E-02 7.62E-02 -6.87E-03 1.64E-01 -8.45E-02 2.22E-03
SG0303 | TH 1 SG0303 | TH 2 SG0303 | TH 3 SG0303 | TH 5 SG0303 | TH 6 SG0303 | TH 7 SG0303 | TH 9
-6.64E-02 -2.84E-01 -1.91E-01 7.49E-01 -1.95E-01 4.52E-01 -1.72E-01
SG0303 | TH10 SG0303 | TH11 SG0303 | TH12 SG0303 | TH13 SG0303 | TH14 SG0303 | TH15 SG0303 | TH16
-2.92E-02 -9.08E-03 -2.78E-01 -1.21E-02 3.12E-02 -2.42E-02 -3.77E-03
SG0303 | TH17 SG0303 | OM0101 SG0303 | OM0404 SG0303 | OM0405 SG0303 | OM0505 SG0303 | SG0101 SG0303 | SG0202
5.27E-02 1.14E-01 -5.97E-02 2.84E-01 -1.60E-01 8.65E-03 -9.55E-02
SG0303 | SG0303 SG0404 | TH 1 SG0404 | TH 2 SG0404 | TH 3 SG0404 | TH 5 SG0404 | TH 6 SG0404 | TH 7
4.62E+01 1.86E-02 5.21E-02 -5.05E-03 1.25E-01 2.55E-02 3.45E-01
SG0404 | TH 9 SG0404 | TH10 SG0404 | TH11 SG0404 | TH12 SG0404 | TH13 SG0404 | TH14 SG0404 | TH15
-3.31E-03 4.57E-02 4.39E-03 1.07E-01 1.38E-01 5.83E-02 4.39E-02
SG0404 | TH16 SG0404 | TH17 SG0404 | OM0101 SG0404 | OM0404 SG0404 | OM0405 SG0404 | OM0505 SG0404 | SG0101
2.36E-02 2.03E-02 -3.82E-02 -6.53E-03 3.51E-02 5.49E-02 -1.95E-01
SG0404 | SG0202 SG0404 | SG0303 SG0404 | SG0404
1.14E-02 6.66E-02 1.02E-01
1

********** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION **********
********** INVERSE COVARIANCE MATRIX OF ESTIMATE **********
********** **********
****
TH 1 | TH 1 TH 2 | TH 1 TH 2 | TH 2 TH 3 | TH 1 TH 3 | TH 2 TH 3 | TH 3 TH 5 | TH 1
2.31E+03 -1.65E+02 2.65E+01 9.07E+01 -2.78E+01 6.59E+01 -1.90E+00
TH 5 | TH 2 TH 5 | TH 3 TH 5 | TH 5 TH 6 | TH 1 TH 6 | TH 2 TH 6 | TH 3 TH 6 | TH 5
3.60E-01 -1.01E+00 6.00E-01 -5.13E-02 1.17E-02 -7.15E-04 -1.31E-03
TH 6 | TH 6 TH 7 | TH 1 TH 7 | TH 2 TH 7 | TH 3 TH 7 | TH 5 TH 7 | TH 6 TH 7 | TH 7
4.96E-05 -3.30E+03 1.18E+02 4.06E+02 -7.75E+01 3.50E-01 7.57E+04
TH 9 | TH 1 TH 9 | TH 2 TH 9 | TH 3 TH 9 | TH 5 TH 9 | TH 6 TH 9 | TH 7 TH 9 | TH 9
2.81E+02 -2.93E+00 -1.83E+01 -2.65E-01 -1.86E-02 4.60E+01 4.97E+02
TH10 | TH 1 TH10 | TH 2 TH10 | TH 3 TH10 | TH 5 TH10 | TH 6 TH10 | TH 7 TH10 | TH 9
2.05E+03 -1.54E+02 4.34E+01 9.30E-01 -7.78E-02 -3.97E+03 0.00E+00
TH10 | TH10 TH11 | TH 1 TH11 | TH 2 TH11 | TH 3 TH11 | TH 5 TH11 | TH 6 TH11 | TH 7
2.45E+03 8.48E+00 -2.25E+00 2.09E+00 -6.42E-01 -4.73E-03 7.79E+01
TH11 | TH 9 TH11 | TH10 TH11 | TH11 TH12 | TH 1 TH12 | TH 2 TH12 | TH 3 TH12 | TH 5
0.00E+00 0.00E+00 1.28E+01 -7.96E+01 -1.54E+01 9.60E+00 1.53E+00
TH12 | TH 6 TH12 | TH 7 TH12 | TH 9 TH12 | TH10 TH12 | TH11 TH12 | TH12 TH13 | TH 1
-7.17E-02 1.01E+03 -4.17E+01 0.00E+00 0.00E+00 3.76E+02 -3.63E+01
TH13 | TH 2 TH13 | TH 3 TH13 | TH 5 TH13 | TH 6 TH13 | TH 7 TH13 | TH 9 TH13 | TH10
1.38E+00 -1.96E+01 3.39E-01 1.91E-02 -4.50E+02 0.00E+00 -4.43E+01
TH13 | TH11 TH13 | TH12 TH13 | TH13 TH14 | TH 1 TH14 | TH 2 TH14 | TH 3 TH14 | TH 5
0.00E+00 0.00E+00 1.81E+02 -3.26E+01 1.37E+01 -2.96E+00 6.19E+00
TH14 | TH 6 TH14 | TH 7 TH14 | TH 9 TH14 | TH10 TH14 | TH11 TH14 | TH12 TH14 | TH13
5.74E-02 -8.26E+02 0.00E+00 0.00E+00 -7.21E+01 0.00E+00 0.00E+00
TH14 | TH14 TH15 | TH 1 TH15 | TH 2 TH15 | TH 3 TH15 | TH 5 TH15 | TH 6 TH15 | TH 7
7.99E+02 3.70E+00 -3.63E-01 1.21E+00 -9.04E-02 1.59E-03 -9.55E+00
TH15 | TH 9 TH15 | TH10 TH15 | TH11 TH15 | TH12 TH15 | TH13 TH15 | TH14 TH15 | TH15
3.28E+00 0.00E+00 0.00E+00 -8.02E+00 0.00E+00 0.00E+00 3.70E-01
TH16 | TH 1 TH16 | TH 2 TH16 | TH 3 TH16 | TH 5 TH16 | TH 6 TH16 | TH 7 TH16 | TH 9
2.75E+01 -2.38E+00 1.78E+00 -1.85E-02 -1.48E-03 -2.30E+01 0.00E+00
TH16 | TH10 TH16 | TH11 TH16 | TH12 TH16 | TH13 TH16 | TH14 TH16 | TH15 TH16 | TH16
3.19E+01 0.00E+00 0.00E+00 -4.81E+00 0.00E+00 0.00E+00 6.11E-01
TH17 | TH 1 TH17 | TH 2 TH17 | TH 3 TH17 | TH 5 TH17 | TH 6 TH17 | TH 7 TH17 | TH 9
6.97E-01 -2.80E-01 1.27E-01 -1.12E-01 -9.79E-04 1.46E+01 0.00E+00
TH17 | TH10 TH17 | TH11 TH17 | TH12 TH17 | TH13 TH17 | TH14 TH17 | TH15 TH17 | TH16
0.00E+00 1.39E+00 0.00E+00 0.00E+00 -1.37E+01 0.00E+00 0.00E+00
TH17 | TH17 OM0101 | TH 1 OM0101 | TH 2 OM0101 | TH 3 OM0101 | TH 5 OM0101 | TH 6 OM0101 | TH 7
2.45E-01 -1.19E+03 1.18E+02 -1.06E+02 -5.94E+00 1.70E-02 2.22E+03
OM0101 | TH 9 OM0101 | TH10 OM0101 | TH11 OM0101 | TH12 OM0101 | TH13 OM0101 | TH14 OM0101 | TH15
0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
OM0101 | TH16 OM0101 | TH17 OM0101 | OM0101 OM0404 | TH 1 OM0404 | TH 2 OM0404 | TH 3 OM0404 | TH 5
0.00E+00 0.00E+00 1.20E+04 1.80E+01 6.82E+00 -1.44E+00 1.32E-02
OM0404 | TH 6 OM0404 | TH 7 OM0404 | TH 9 OM0404 | TH10 OM0404 | TH11 OM0404 | TH12 OM0404 | TH13
2.19E-03 -3.78E+02 -1.04E+01 -1.28E+01 6.48E-02 5.08E+00 1.20E+01
OM0404 | TH14 OM0404 | TH15 OM0404 | TH16 OM0404 | TH17 OM0404 | OM0101 OM0404 | OM0404 OM0405 | TH 1
1.14E+00 -2.22E-01 -1.13E+00 -2.17E-02 0.00E+00 3.52E+01 -3.97E+00
OM0405 | TH 2 OM0405 | TH 3 OM0405 | TH 5 OM0405 | TH 6 OM0405 | TH 7 OM0405 | TH 9 OM0405 | TH10
3.22E+00 6.68E-01 -8.31E-02 3.68E-03 -5.02E+01 1.09E+01 -2.57E+01
OM0405 | TH11 OM0405 | TH12 OM0405 | TH13 OM0405 | TH14 OM0405 | TH15 OM0405 | TH16 OM0405 | TH17
5.44E-03 -6.10E+00 6.37E+00 -4.16E-01 2.62E-01 -6.69E-01 1.36E-04
OM0405 | OM0101 OM0405 | OM0404 OM0405 | OM0405 OM0505 | TH 1 OM0505 | TH 2 OM0505 | TH 3 OM0505 | TH 5
0.00E+00 9.95E+00 5.30E+00 5.42E-02 -5.93E-01 1.04E+00 6.76E-02
OM0505 | TH 6 OM0505 | TH 7 OM0505 | TH 9 OM0505 | TH10 OM0505 | TH11 OM0505 | TH12 OM0505 | TH13
-1.54E-04 -1.24E+01 1.80E+00 -1.06E+00 -3.27E-02 1.41E+00 1.77E+00
OM0505 | TH14 OM0505 | TH15 OM0505 | TH16 OM0505 | TH17 OM0505 | OM0101 OM0505 | OM0404 OM0505 | OM0405
-5.92E-01 -7.03E-02 -2.99E-02 7.21E-03 0.00E+00 3.07E-01 3.53E-01
OM0505 | OM0505 SG0101 | TH 1 SG0101 | TH 2 SG0101 | TH 3 SG0101 | TH 5 SG0101 | TH 6 SG0101 | TH 7
4.74E-01 4.79E+02 1.15E+02 -7.78E+00 3.87E+01 -1.05E-01 -1.16E+04
SG0101 | TH 9 SG0101 | TH10 SG0101 | TH11 SG0101 | TH12 SG0101 | TH13 SG0101 | TH14 SG0101 | TH15
-1.62E+02 -1.81E+02 -2.26E+02 1.46E+03 7.38E+02 2.50E+03 -3.12E+01
SG0101 | TH16 SG0101 | TH17 SG0101 | OM0101 SG0101 | OM0404 SG0101 | OM0405 SG0101 | OM0505 SG0101 | SG0101
-1.96E+01 -4.30E+01 0.00E+00 7.74E+02 2.02E+02 1.95E+01 3.95E+04
SG0202 | TH 1 SG0202 | TH 2 SG0202 | TH 3 SG0202 | TH 5 SG0202 | TH 6 SG0202 | TH 7 SG0202 | TH 9
2.72E+03 -6.73E+02 1.41E+03 -1.64E+00 -1.06E-01 6.96E+03 0.00E+00
SG0202 | TH10 SG0202 | TH11 SG0202 | TH12 SG0202 | TH13 SG0202 | TH14 SG0202 | TH15 SG0202 | TH16
0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00 0.00E+00
SG0202 | TH17 SG0202 | OM0101 SG0202 | OM0404 SG0202 | OM0405 SG0202 | OM0505 SG0202 | SG0101 SG0202 | SG0202
0.00E+00 -6.57E+03 0.00E+00 0.00E+00 0.00E+00 0.00E+00 2.65E+05
SG0303 | TH 1 SG0303 | TH 2 SG0303 | TH 3 SG0303 | TH 5 SG0303 | TH 6 SG0303 | TH 7 SG0303 | TH 9
9.63E-02 1.04E-02 1.56E-04 -1.85E-02 6.14E-05 3.19E-01 5.73E-02
SG0303 | TH10 SG0303 | TH11 SG0303 | TH12 SG0303 | TH13 SG0303 | TH14 SG0303 | TH15 SG0303 | TH16
0.00E+00 0.00E+00 3.27E-02 0.00E+00 0.00E+00 -1.09E-04 0.00E+00
SG0303 | TH17 SG0303 | OM0101 SG0303 | OM0404 SG0303 | OM0405 SG0303 | OM0505 SG0303 | SG0101 SG0303 | SG0202
0.00E+00 0.00E+00 1.75E-02 8.35E-04 -7.19E-04 1.29E-01 0.00E+00
SG0303 | SG0303 SG0404 | TH 1 SG0404 | TH 2 SG0404 | TH 3 SG0404 | TH 5 SG0404 | TH 6 SG0404 | TH 7
1.37E-03 1.67E+01 1.61E+00 -8.69E-03 6.91E-01 -1.26E-02 -1.42E+03
SG0404 | TH 9 SG0404 | TH10 SG0404 | TH11 SG0404 | TH12 SG0404 | TH13 SG0404 | TH14 SG0404 | TH15
-1.20E+01 -7.81E+00 0.00E+00 -9.76E+00 -8.18E+00 0.00E+00 9.63E-02
SG0404 | TH16 SG0404 | TH17 SG0404 | OM0101 SG0404 | OM0404 SG0404 | OM0405 SG0404 | OM0505 SG0404 | SG0101
-1.17E-01 0.00E+00 0.00E+00 2.33E+01 5.04E+00 -4.93E-02 6.93E+02
SG0404 | SG0202 SG0404 | SG0303 SG0404 | SG0404
0.00E+00 0.00E+00 1.46E+02
1
****
********** **********
********** FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION **********
********** EIGENVALUES OF COR MATRIX OF ESTIMATE **********
********** **********
****
1 2 3 4 5 6 7 8 9 10 11 12
13 14 15 16 17 18 19 20 21 22 23
1.06E-02 1.93E-02 5.98E-02 8.39E-02 9.54E-02 1.49E-01 1.76E-01 2.24E-01 3.46E-01 4.17E-01 4.85E-01 5.19E-01
5.72E-01 7.88E-01 9.07E-01 1.07E+00 1.24E+00 1.58E+00 1.76E+00 2.21E+00 2.52E+00 2.99E+00 4.78E+00
1THERE ARE ERROR MESSAGES IN FILE PRDERR
Stop Time:
Thu Jan 15 22:00:09 UTC 2015

```
$SIZES LVR = 200 LTH =100 LPAR = 300
$PROB FINAL D2-25D2 MODEL

$INPUT C NUM AUTHOR=DROP SID ID nOBS DOSEIU DOSEnmol
CDOSE DOSEN TIME CMT DVTYPE2 MULTDV ADDL RATE SS II
AMT BASEval REG2 GTYPE DV EVID KA2I CL2I VC2I D1I
DBASE2I VPM2I Q2I BL BQL

$DATA  ../../../../../data/set2/derived/meta_manip_data-PMD225D2-trunc9-rate9.csv IGNORE=C WIDE

$SUB ADVAN13 TOL = 6

$MODEL NCOMPARTMENT = 5

$PK
TYPE = DVTYPE2
REGI2 = REG2
DOSE = DOSEnmol
CONC = DV
STUDY = SID

;D2 PK PARAMETERS
KA2 = THETA(6)*EXP(ETA(7)/SQRT(nOBS)) ; ABSORPTION RATE CONSTANT FOR D2 (h^-1)

VC2 = THETA(7)*EXP(ETA(8)/SQRT(nOBS))  ; CENTRAL VOLUME FOR D2 (L)

VP2 = THETA(8)*EXP(ETA(9)/SQRT(nOBS)) ; PERIPHERAL VOLUME FOR D2 (L) ; ETA ALLOWED FOR GROUPS ONLY

Q2 = THETA(9)*EXP(ETA(10)/SQRT(nOBS)) ; INTERCOMPARTMENTAL CL FOR D2 (L/h)

D1 = THETA(10)*EXP(ETA(11)/SQRT(nOBS)) ; DELAY (h)

DBASE2 = THETA(11)*EXP(ETA(12)/SQRT(nOBS)) ; BASELINE CONC FOR D2 (nmol/L)

CL2 = THETA(12)*EXP(ETA(13)/SQRT(nOBS))  ; D2 CL (L/h)

;25OHD2 METABOLITE PARAMETERS
CLM2 = THETA(1)*EXP(ETA(1)/SQRT(nOBS))  ; 25OHD2 CLEARANCE (L/h)

VCM2 = THETA(2)*EXP(ETA(2)/SQRT(nOBS))  ; CENTRAL VOLUME FOR 25OHD2 (L/h)

VPM2 = THETA(3)*EXP(ETA(3)/SQRT(nOBS)); PERIPHERAL VOLUME FOR 25OHD2 (L/h)

QM2 = THETA(4)*EXP(ETA(4)/SQRT(nOBS)) ; INTERCOMPARTMENTAL CL FOR 25OHD2 (L/h)

INDDAT = 0
IF(nOBS.EQ.1) INDDAT = 1

; SEPARATE ETAS FOR INDIVIDUAL AND ARM LEVEL DATA FOR DBASEM2
ETADBASEM2 =  ETA(5)*INDDAT + ETA(6)*(1-INDDAT) ; ETA5 IS INDIVIDUAL LEVEL ETA
DBASEM2 = THETA(5)*EXP(ETADBASEM2/SQRT(nOBS)) ; 25OHD2 BASELINE CONC (nmol/L)

;SET INITIAL CMT AMOUNTS
A_0(2) = DBASE2*VC2 ; INTIALIZE CENTRAL D2 CMT WITH D2 AMT (nmol)
A_0(3) = DBASE2*VP2 ; INITIALIZE PERIPHERAL D2 CMT (nmol)

A_0(4) = DBASEM2*VCM2 ; INTIALIZE CENTRAL 25OHD2 CMT WITH 25OHD2 AMT (nmol)
A_0(5) = DBASEM2*VPM2 ; INITIALIZE PERIPHERAL 2OH5D2 CMT (nmol)

$DES
DADT(1) = -KA2*A(1)   ; D2 GUT CMT

C2 = A(2)/VC2 ; CONCENTRATION OF CENTRAL COMPARTMENT FOR D2
C3 = A(3)/VP2; CONCENTRATION OF PERIPHERAL COMPARTMENT FOR D2

CLTM2 = CL2 ; CLEARANCE OF D2 INTO METABOLTE (L/h)

DADT(2) = KA2*A(1) - (CL2 + Q2)*C2 + Q2*C3    ; D2 CENTRAL CMT
DADT(3) = Q2*C2 - Q2*C3 ; D2 PERIPHERAL CMT

C4 = A(4)/VCM2 ; CONCENTRATION OF CENTRAL COMPARTMENT FOR 25OHD2
C5 = A(5)/VPM2; CONCENTRATION OF PERIPHERAL COMPARTMENT FOR 25OHD2

DADT(4) = CLTM2*C2 - CLM2*C4 - QM2*C4 + QM2*C5    ; 25OHD2 CENTRAL CMT
DADT(5) = QM2*C4 - QM2*C5 ; 25OHD2 PERIPHERAL CMT

$ERROR
IND1 = 0
IND2 = 0
CP = A(2)/VC2 ; D2 CENTRAL CONCENTRATION
CM = A(4)/VCM2 ; 25D2 CENTRAL CONCENTRATION

IF (TYPE.EQ.2) IND1 = 1 ; D2 endpoint
IF (TYPE.EQ.5) IND2 = 1 ; 25OHD2 endpoint

;SEPARATE RESIDUAL ERRORS FOR DIFFERENT ENDPOINTS
IF(TYPE.EQ.2) YD2 = CP*EXP(EPS(1)/SQRT(nOBS)) ; D2 endpoint
IF(TYPE.EQ.5.AND.nOBS.EQ.1) Y25D2 = CM*EXP(EPS(2)/SQRT(nOBS)); 25OHD2 ENDPOINT (INDIVIDUALS)
IF(TYPE.EQ.5.AND.nOBS.GT.1) Y25D2 = CM*EXP(EPS(3)/SQRT(nOBS)) ; 25OHD2 ENDPOINT (ARMS)

Y = YD2*IND1 + Y25D2*IND2    ; THIS IS PREDICTION WITH IIV AND RESIDUAL VARIABILITY

$THETA ; initialize thetas
(0, 0.015); CLM2
15, FIX ; VCM2
(0, 99) ; VPM2
(0, 0.08) ; QM2
(0, 2.25) ; DBASEM2
0.323, FIX; KA2
(0, 12); VC2
2321.572, FIX; VP2
0.186, FIX; Q2
(0, 4) ; D1
(0, 2); DBASE2
(0, 0.1); CL2

$OMEGA
0, FIX ; CLM2
0, FIX; VCM2
0, FIX; VPM2
```

```
0, FIX; QM2
0, FIX ; DBASEM2 INDIV
20; DBASEM2 GROUP
0, FIX; KA2
0.3; VC2
0, FIX; VP2
0, FIX; Q2
0, FIX ; D1
0.3 ; DBASE2
0, FIX; CL2

$SIGMA
0.8 ;  D2
1 ;  25OHD2 INDIVIDUALS
1; 25OHD2 ARMS

$ESTIMATION MAXEVAL=9999 PRINT=10 METHOD = 1 INT SIGDIGITS = 2 MSFO=./7170.msf
$COV MATRIX = S PRINT = E UNCONDITIONAL

 #TERM:
0MINIMIZATION SUCCESSFUL
 NO. OF FUNCTION EVALUATIONS USED:      409
 NO. OF SIG. DIGITS IN FINAL EST.:  2.1

 ETABAR IS THE ARITHMETIC MEAN OF THE ETA-ESTIMATES,
 AND THE P-VALUE IS GIVEN FOR THE NULL HYPOTHESIS THAT THE TRUE MEAN IS 0.

 ETABAR:         0.0000E+00  0.0000E+00  0.0000E+00  0.0000E+00  0.0000E+00 -8.8119E-02  0.0000E+00 -1.3193E-02  0.0000E+00  0.0000E+00
                 0.0000E+00  3.6063E-02  0.0000E+00
 SE:             0.0000E+00  0.0000E+00  0.0000E+00  0.0000E+00  0.0000E+00  1.5161E-01  0.0000E+00  3.8281E-02  0.0000E+00  0.0000E+00
                 0.0000E+00  6.5540E-02  0.0000E+00
 N:                     33          33          33          33          33          33          33          33          33          33
                        33          33          33

 P VAL.:         1.0000E+00  1.0000E+00  1.0000E+00  1.0000E+00  1.0000E+00  5.6108E-01  1.0000E+00  7.3036E-01  1.0000E+00  1.0000E+00
                 1.0000E+00  5.8215E-01  1.0000E+00

 ETAshrink(%):   1.0000E+02  1.0000E+02  1.0000E+02  1.0000E+02  1.0000E+02  4.6633E+01  1.0000E+02  5.7855E+01  1.0000E+02  1.0000E+02
                 1.0000E+02  4.5992E+01  1.0000E+02
 EBVshrink(%):   0.0000E+00  0.0000E+00  0.0000E+00  0.0000E+00  0.0000E+00  4.7209E+01  0.0000E+00  5.8632E+01  0.0000E+00  0.0000E+00
                 0.0000E+00  4.6376E+01  0.0000E+00
 EPSshrink(%):   5.1774E+00  4.2504E+00  6.5085E+00

 #TERE:
 Elapsed estimation time in seconds:   257.40
 Elapsed covariance time in seconds:    45.76
1

 ********************************************************************************************************************
 ********************                                                                        ********************
 ********************               FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION       ********************
 #OBJT:***************                     MINIMUM VALUE OF OBJECTIVE FUNCTION                ********************
 ********************                                                                        ********************
 ********************************************************************************************************************



 #OBJV:*****************************************      962.103       **************************************************
1
 ********************************************************************************************************************
 ********************                                                                        ********************
 ********************               FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION       ********************
 ********************                           FINAL PARAMETER ESTIMATE                     ********************
 ********************                                                                        ********************
 ********************************************************************************************************************


 THETA - VECTOR OF FIXED EFFECTS PARAMETERS   *********

         TH  1     TH  2     TH  3     TH  4     TH  5     TH  6     TH  7     TH  8     TH  9     TH 10     TH 11     TH 12

         2.64E-02  1.50E+01  7.29E+01  6.31E-02  2.79E+00  3.23E-01  1.71E+01  2.32E+03  1.86E-01  5.19E+00  2.58E+00  2.53E-01


 OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS  ********

            ETA1      ETA2      ETA3      ETA4      ETA5      ETA6      ETA7      ETA8      ETA9      ET10      ET11      ET12
              ET13

 ETA1
+        0.00E+00

 ETA2
+        0.00E+00  0.00E+00

 ETA3
+        0.00E+00  0.00E+00  0.00E+00

 ETA4
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00

 ETA5
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00

 ETA6
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  2.75E+00

 ETA7
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00

 ETA8
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  2.81E-01

 ETA9
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00
```

```
 ET10
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00

 ET11
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00

 ET12
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  5.01E-01

 ET13
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00
          0.00E+00


 SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS  ****

1

            EPS1      EPS2      EPS3

 EPS1
+        7.41E-01

 EPS2
+        0.00E+00  1.56E-01

 EPS3
+        0.00E+00  0.00E+00  1.01E+00

1


 OMEGA - CORR MATRIX FOR RANDOM EFFECTS - ETAS  *******

            ETA1      ETA2      ETA3      ETA4      ETA5      ETA6      ETA7      ETA8      ETA9      ET10      ET11      ET12
             ET13

 ETA1
+        0.00E+00

 ETA2
+        0.00E+00  0.00E+00

 ETA3
+        0.00E+00  0.00E+00  0.00E+00

 ETA4
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00

 ETA5
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00

 ETA6
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  1.66E+00

 ETA7
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00

 ETA8
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  5.30E-01

 ETA9
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00

 ET10
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00

 ET11
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00

 ET12
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  7.08E-01

 ET13
+        0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00  0.00E+00
          0.00E+00


 SIGMA - CORR MATRIX FOR RANDOM EFFECTS - EPSILONS  ***

            EPS1      EPS2      EPS3

 EPS1
+        8.61E-01

 EPS2
+        0.00E+00  3.95E-01

 EPS3
+        0.00E+00  0.00E+00  1.00E+00

1
 ********************************************************************************************************************************
 ********************                                                                                ********************
 ********************               FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION               ********************
 ********************                           STANDARD ERROR OF ESTIMATE                           ********************
 ********************                                                                                ********************
 ********************************************************************************************************************************


 THETA - VECTOR OF FIXED EFFECTS PARAMETERS   *********

         TH  1     TH  2     TH  3     TH  4     TH  5     TH  6     TH  7     TH  8     TH  9     TH 10     TH 11     TH 12
```

```
         1.54E-02 .........  5.81E+01  2.59E-02  2.34E-01 .........  1.37E+01 ......... .........  4.12E+00  5.84E-01  6.82E-02


 OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS  ********

            ETA1      ETA2      ETA3      ETA4      ETA5      ETA6      ETA7      ETA8      ETA9      ET10      ET11      ET12
             ET13

 ETA1
+       .........

 ETA2
+       ......... .........

 ETA3
+       ......... ......... .........

 ETA4
+       ......... ......... ......... .........

 ETA5
+       ......... ......... ......... ......... .........

 ETA6
+       ......... ......... ......... ......... .........  2.26E+00

 ETA7
+       ......... ......... ......... ......... ......... ......... .........

 ETA8
+       ......... ......... ......... ......... ......... ......... .........  3.81E-01

 ETA9
+       ......... ......... ......... ......... ......... ......... ......... ......... .........

 ET10
+       ......... ......... ......... ......... ......... ......... ......... ......... ......... .........

 ET11
+       ......... ......... ......... ......... ......... ......... ......... ......... ......... ......... .........

 ET12
+       ......... ......... ......... ......... ......... ......... ......... ......... ......... ......... .........  3.62E-01

 ET13
+       ......... ......... ......... ......... ......... ......... ......... ......... ......... ......... ......... .........
          .........


 SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS  ****

1

            EPS1      EPS2      EPS3

 EPS1
+        2.85E-01

 EPS2
+       .........  6.11E-02

 EPS3
+       ......... .........  1.48E-01

1


 OMEGA - CORR MATRIX FOR RANDOM EFFECTS - ETAS  *******

            ETA1      ETA2      ETA3      ETA4      ETA5      ETA6      ETA7      ETA8      ETA9      ET10      ET11      ET12
             ET13

 ETA1
+       .........

 ETA2
+       ......... .........

 ETA3
+       ......... ......... .........

 ETA4
+       ......... ......... ......... .........

 ETA5
+       ......... ......... ......... ......... .........

 ETA6
+       ......... ......... ......... ......... .........  6.81E-01

 ETA7
+       ......... ......... ......... ......... ......... ......... .........

 ETA8
+       ......... ......... ......... ......... ......... ......... .........  3.59E-01

 ETA9
+       ......... ......... ......... ......... ......... ......... ......... ......... .........

 ET10
+       ......... ......... ......... ......... ......... ......... ......... ......... ......... .........

 ET11
+       ......... ......... ......... ......... ......... ......... ......... ......... ......... ......... .........
```

```
 ET12
+       ......... ......... ......... ......... ......... ......... ......... ......... ......... ......... .........  2.55E-01

 ET13
+       ......... ......... ......... ......... ......... ......... ......... ......... ......... ......... ......... .........
          .........


 SIGMA - CORR MATRIX FOR RANDOM EFFECTS - EPSILONS  ***


            EPS1      EPS2      EPS3

 EPS1
+        1.65E-01

 EPS2
+       .........  7.74E-02

 EPS3
+       ......... .........  7.36E-02

1
 ************************************************************************************************************************
 ********************                                                                            ********************
 ********************              FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION            ********************
 ********************                         COVARIANCE MATRIX OF ESTIMATE                      ********************
 ********************                                                                            ********************
 ************************************************************************************************************************


    TH  1 | TH  1    TH  3 | TH  1    TH  3 | TH  3    TH  4 | TH  1    TH  4 | TH  3    TH  4 | TH  4    TH  5 | TH  1
       2.38E-04        -8.49E-01         3.37E+03        -1.82E-04         1.01E+00         6.73E-04         1.03E-03

    TH  5 | TH  3    TH  5 | TH  4    TH  5 | TH  5    TH  7 | TH  1    TH  7 | TH  3    TH  7 | TH  4    TH  7 | TH  5
      -4.54E+00        -1.83E-03         5.48E-02        -5.85E-03        -1.58E+02        -2.73E-01         7.08E-01

    TH  7 | TH  7    TH 10 | TH  1    TH 10 | TH  3    TH 10 | TH  4    TH 10 | TH  5    TH 10 | TH  7    TH 10 | TH 10
       1.89E+02         2.75E-03         4.51E+01         8.10E-02        -2.06E-01        -5.49E+01         1.70E+01

    TH 11 | TH  1    TH 11 | TH  3    TH 11 | TH  4    TH 11 | TH  5    TH 11 | TH  7    TH 11 | TH 10    TH 11 | TH 11
       2.83E-03        -5.11E+00         1.18E-03         2.45E-03         5.05E-01        -4.92E-02         3.42E-01

    TH 12 | TH  1    TH 12 | TH  3    TH 12 | TH  4    TH 12 | TH  5    TH 12 | TH  7    TH 12 | TH 10    TH 12 | TH 11
       1.09E-04         6.73E-01         1.40E-03        -3.42E-03        -8.72E-01         2.61E-01         6.35E-03

    TH 12 | TH 12   OM0606 | TH  1   OM0606 | TH  3   OM0606 | TH  4   OM0606 | TH  5   OM0606 | TH  7   OM0606 | TH 10
       4.65E-03         7.49E-03        -4.16E+01        -1.84E-02         1.48E-01         7.12E+00        -2.12E+00

   OM0606 | TH 11   OM0606 | TH 12   OM0606 | OM0606  OM0808 | TH  1   OM0808 | TH  3   OM0808 | TH  4   OM0808 | TH  5
      -1.27E-01        -3.83E-02         5.10E+00        -1.52E-04        -3.71E+00        -6.44E-03         1.70E-02

   OM0808 | TH  7   OM0808 | TH 10   OM0808 | TH 11   OM0808 | TH 12   OM0808 | OM0606  OM0808 | OM0808  OM1212 | TH  1
       4.45E+00        -1.19E+00         5.64E-03        -2.04E-02         1.71E-01         1.45E-01        -2.53E-04

   OM1212 | TH  3   OM1212 | TH  4   OM1212 | TH  5   OM1212 | TH  7   OM1212 | TH 10   OM1212 | TH 11   OM1212 | TH 12
       1.44E+00         2.52E-03        -2.81E-03        -2.50E+00         7.07E-01        -1.04E-01         8.60E-03

   OM1212 | OM0606  OM1212 | OM0808  OM1212 | OM1212  SG0101 | TH  1   SG0101 | TH  3   SG0101 | TH  4   SG0101 | TH  5
      -2.14E-02        -5.35E-02         1.31E-01         3.23E-04        -3.40E+00        -4.39E-03         1.16E-02

   SG0101 | TH  7   SG0101 | TH 10   SG0101 | TH 11   SG0101 | TH 12   SG0101 | OM0606  SG0101 | OM0808  SG0101 | OM1212
       3.16E+00        -9.19E-01         6.76E-02        -1.40E-02         9.45E-02         7.33E-02        -6.51E-02

   SG0101 | SG0101  SG0202 | TH  1   SG0202 | TH  3   SG0202 | TH  4   SG0202 | TH  5   SG0202 | TH  7   SG0202 | TH 10
       8.10E-02        -2.62E-04         1.29E+00         6.55E-04        -7.10E-03        -2.99E-01         8.74E-02

   SG0202 | TH 11   SG0202 | TH 12   SG0202 | OM0606  SG0202 | OM0808  SG0202 | OM1212  SG0202 | SG0101  SG0202 | SG0202
       9.43E-04         1.43E-03        -3.19E-02        -7.11E-03         4.37E-03        -4.82E-03         3.74E-03

   SG0303 | TH  1   SG0303 | TH  3   SG0303 | TH  4   SG0303 | TH  5   SG0303 | TH  7   SG0303 | TH 10   SG0303 | TH 11
       3.36E-04        -2.27E+00        -1.68E-03         5.77E-03         8.49E-01        -2.53E-01        -9.38E-03

   SG0303 | TH 12   SG0303 | OM0606  SG0303 | OM0808  SG0303 | OM1212  SG0303 | SG0101  SG0303 | SG0202  SG0303 | SG0303
      -4.41E-03        -1.06E-02         2.01E-02        -8.06E-03         1.26E-02        -2.22E-03         2.18E-02
1
 ************************************************************************************************************************
 ********************                                                                            ********************
 ********************              FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION            ********************
 ********************                         CORRELATION MATRIX OF ESTIMATE                     ********************
 ********************                                                                            ********************
 ************************************************************************************************************************


    TH  1 | TH  1    TH  3 | TH  1    TH  3 | TH  3    TH  4 | TH  1    TH  4 | TH  3    TH  4 | TH  4    TH  5 | TH  1
       1.54E-02        -9.47E-01         5.81E+01        -4.54E-01         6.71E-01         2.59E-02         2.87E-01

    TH  5 | TH  3    TH  5 | TH  4    TH  5 | TH  5    TH  7 | TH  1    TH  7 | TH  3    TH  7 | TH  4    TH  7 | TH  5
      -3.34E-01        -3.02E-01         2.34E-01        -2.76E-02        -1.98E-01        -7.66E-01         2.20E-01

    TH  7 | TH  7    TH 10 | TH  1    TH 10 | TH  3    TH 10 | TH  4    TH 10 | TH  5    TH 10 | TH  7    TH 10 | TH 10
       1.37E+01         4.32E-02         1.88E-01         7.57E-01        -2.14E-01        -9.70E-01         4.12E+00

    TH 11 | TH  1    TH 11 | TH  3    TH 11 | TH  4    TH 11 | TH  5    TH 11 | TH  7    TH 11 | TH 10    TH 11 | TH 11
       3.14E-01        -1.51E-01         7.79E-02         1.79E-02         6.29E-02        -2.05E-02         5.84E-01

    TH 12 | TH  1    TH 12 | TH  3    TH 12 | TH  4    TH 12 | TH  5    TH 12 | TH  7    TH 12 | TH 10    TH 12 | TH 11
       1.04E-01         1.70E-01         7.91E-01        -2.15E-01        -9.31E-01         9.30E-01         1.59E-01

    TH 12 | TH 12   OM0606 | TH  1   OM0606 | TH  3   OM0606 | TH  4   OM0606 | TH  5   OM0606 | TH  7   OM0606 | TH 10
       6.82E-02         2.15E-01        -3.17E-01        -3.14E-01         2.80E-01         2.30E-01        -2.28E-01

   OM0606 | TH 11   OM0606 | TH 12   OM0606 | OM0606  OM0808 | TH  1   OM0808 | TH  3   OM0808 | TH  4   OM0808 | TH  5
      -9.61E-02        -2.49E-01         2.26E+00        -2.59E-02        -1.68E-01        -6.52E-01         1.91E-01

   OM0808 | TH  7   OM0808 | TH 10   OM0808 | TH 11   OM0808 | TH 12   OM0808 | OM0606  OM0808 | OM0808  OM1212 | TH  1
       8.52E-01        -7.61E-01         2.53E-02        -7.87E-01         1.99E-01         3.81E-01        -4.54E-02

   OM1212 | TH  3   OM1212 | TH  4   OM1212 | TH  5   OM1212 | TH  7   OM1212 | TH 10   OM1212 | TH 11   OM1212 | TH 12
```

```
       6.86E-02         2.69E-01        -3.32E-02        -5.03E-01         4.74E-01        -4.91E-01         3.49E-01

   OM1212 | OM0606  OM1212 | OM0808  OM1212 | OM1212  SG0101 | TH  1   SG0101 | TH  3   SG0101 | TH  4   SG0101 | TH  5
      -2.62E-02        -3.88E-01         3.62E-01         7.35E-02        -2.05E-01        -5.94E-01         1.75E-01

   SG0101 | TH  7   SG0101 | TH 10   SG0101 | TH 11   SG0101 | TH 12   SG0101 | OM0606  SG0101 | OM0808  SG0101 | OM1212
       8.09E-01        -7.84E-01         4.06E-01        -7.20E-01         1.47E-01         6.77E-01        -6.33E-01

   SG0101 | SG0101  SG0202 | TH  1   SG0202 | TH  3   SG0202 | TH  4   SG0202 | TH  5   SG0202 | TH  7   SG0202 | TH 10
       2.85E-01        -2.78E-01         3.64E-01         4.13E-01        -4.96E-01        -3.56E-01         3.47E-01

   SG0202 | TH 11   SG0202 | TH 12   SG0202 | OM0606  SG0202 | OM0808  SG0202 | OM1212  SG0202 | SG0101  SG0202 | SG0202
       2.64E-02         3.44E-01        -2.31E-01        -3.06E-01         1.97E-01        -2.77E-01         6.11E-02

   SG0303 | TH  1   SG0303 | TH  3   SG0303 | TH  4   SG0303 | TH  5   SG0303 | TH  7   SG0303 | TH 10   SG0303 | TH 11
       1.48E-01        -2.64E-01        -4.40E-01         1.67E-01         4.19E-01        -4.15E-01        -1.09E-01

   SG0303 | TH 12   SG0303 | OM0606  SG0303 | OM0808  SG0303 | OM1212  SG0303 | SG0101  SG0303 | SG0202  SG0303 | SG0303
      -4.38E-01        -3.17E-02         3.57E-01        -1.51E-01         3.01E-01        -2.46E-01         1.48E-01
1
 ************************************************************************************************************************
 ********************                                                                            ********************
 ********************              FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION            ********************
 ********************                    INVERSE COVARIANCE MATRIX OF ESTIMATE                   ********************
 ********************                                                                            ********************
 ************************************************************************************************************************


    TH  1 | TH  1    TH  3 | TH  1    TH  3 | TH  3    TH  4 | TH  1    TH  4 | TH  3    TH  4 | TH  4    TH  5 | TH  1
       3.28E+05         7.71E+01         2.02E-02         2.60E+04         1.73E-01         2.41E+04        -3.73E+02

    TH  5 | TH  3    TH  5 | TH  4    TH  5 | TH  5    TH  7 | TH  1    TH  7 | TH  3    TH  7 | TH  4    TH  7 | TH  5
      -6.66E-02        -6.86E+01         2.68E+01         1.27E-01        -2.75E-03         9.93E+00        -3.58E-02

    TH  7 | TH  7    TH 10 | TH  1    TH 10 | TH  3    TH 10 | TH  4    TH 10 | TH  5    TH 10 | TH  7    TH 10 | TH 10
       2.28E-01        -1.45E+00        -6.73E-04         9.23E-01        -1.75E-03         4.56E-01         1.52E+00

    TH 11 | TH  1    TH 11 | TH  3    TH 11 | TH  4    TH 11 | TH  5    TH 11 | TH  7    TH 11 | TH 10    TH 11 | TH 11
      -1.18E+03        -2.32E-01        -2.12E+02         3.91E-01        -3.42E-01        -2.33E-01         1.36E+01

    TH 12 | TH  1    TH 12 | TH  3    TH 12 | TH  4    TH 12 | TH  5    TH 12 | TH  7    TH 12 | TH 10    TH 12 | TH 11
      -2.58E+04        -4.97E+00        -6.21E+03         3.95E+01         5.64E+00        -1.51E+01         2.65E+01

    TH 12 | TH 12   OM0606 | TH  1   OM0606 | TH  3   OM0606 | TH  4   OM0606 | TH  5   OM0606 | TH  7   OM0606 | TH 10
       5.88E+03         3.61E+01         1.53E-02        -1.08E+01        -4.25E-01        -1.26E-02        -2.14E-03

   OM0606 | TH 11   OM0606 | TH 12   OM0606 | OM0606  OM0808 | TH  1   OM0808 | TH  3   OM0808 | TH  4   OM0808 | TH  5
       1.14E-01         6.87E-01         2.68E-01        -2.33E+00        -6.17E-04        -1.87E-01         6.61E-02

   OM0808 | TH  7   OM0808 | TH 10   OM0808 | TH 11   OM0808 | TH 12   OM0808 | OM0606  OM0808 | OM0808  OM1212 | TH  1
      -1.86E+00        -3.84E+00         1.49E+00         1.29E+01         2.48E-03         3.52E+01        -1.94E+01

   OM1212 | TH  3   OM1212 | TH  4   OM1212 | TH  5   OM1212 | TH  7   OM1212 | TH 10   OM1212 | TH 11   OM1212 | TH 12
       1.18E-02        -2.15E+01        -2.51E+00         2.56E-01        -2.06E-02         2.87E+00         3.89E+01

   OM1212 | OM0606  OM1212 | OM0808  OM1212 | OM1212  SG0101 | TH  1   SG0101 | TH  3   SG0101 | TH  4   SG0101 | TH  5
       3.05E-02        -1.62E+00         1.60E+01         1.05E-03        -4.28E-07         7.77E-04         0.00E+00

   SG0101 | TH  7   SG0101 | TH 10   SG0101 | TH 11   SG0101 | TH 12   SG0101 | OM0606  SG0101 | OM0808  SG0101 | OM1212
      -7.02E-02         5.66E-01        -1.35E+01         1.47E+02         0.00E+00        -2.99E+00         7.15E+00

   SG0101 | SG0101  SG0202 | TH  1   SG0202 | TH  3   SG0202 | TH  4   SG0202 | TH  5   SG0202 | TH  7   SG0202 | TH 10
       6.66E+01         1.27E+03         1.17E-01         4.30E+02         4.04E+01         4.59E-01         5.94E-02

   SG0202 | TH 11   SG0202 | TH 12   SG0202 | OM0606  SG0202 | OM0808  SG0202 | OM1212  SG0202 | SG0101  SG0202 | SG0202
      -1.34E+01        -1.51E+02         0.00E+00         7.71E-01        -1.25E+01         0.00E+00         4.30E+02

   SG0303 | TH  1   SG0303 | TH  3   SG0303 | TH  4   SG0303 | TH  5   SG0303 | TH  7   SG0303 | TH 10   SG0303 | TH 11
      -5.24E+02        -3.84E-02        -1.93E+02        -1.11E+00        -2.20E-01        -3.84E-02         5.59E+00

   SG0303 | TH 12   SG0303 | OM0606  SG0303 | OM0808  SG0303 | OM1212  SG0303 | SG0101  SG0303 | SG0202  SG0303 | SG0303
       9.86E+01         1.10E+00         1.16E-01         1.40E+00         0.00E+00         0.00E+00         6.68E+01
1


 ************************************************************************************************************************
 ********************                                                                            ********************
 ********************              FIRST ORDER CONDITIONAL ESTIMATION WITH INTERACTION            ********************
 ********************                    EIGENVALUES OF COR MATRIX OF ESTIMATE                   ********************
 ********************                                                                            ********************
 ************************************************************************************************************************


            1         2         3         4         5         6         7         8         9        10        11        12
           13        14

        6.52E-03  1.56E-02  3.50E-02  6.60E-02  1.44E-01  2.84E-01  3.43E-01  5.23E-01  5.48E-01  9.34E-01  1.06E+00  1.69E+00
         2.40E+00  5.95E+00

 #CPUT: Total CPU Time in Seconds,     1646.427
Stop Time:
Thu Jul  9 18:45:38 UTC 2015
```

Appendix B

D3-25OHD3 Model Appendix

Figure B.1: Observed parent (D3) concentration data after D3 supplementation. The size of the points indicates the size of the of the treatment arm

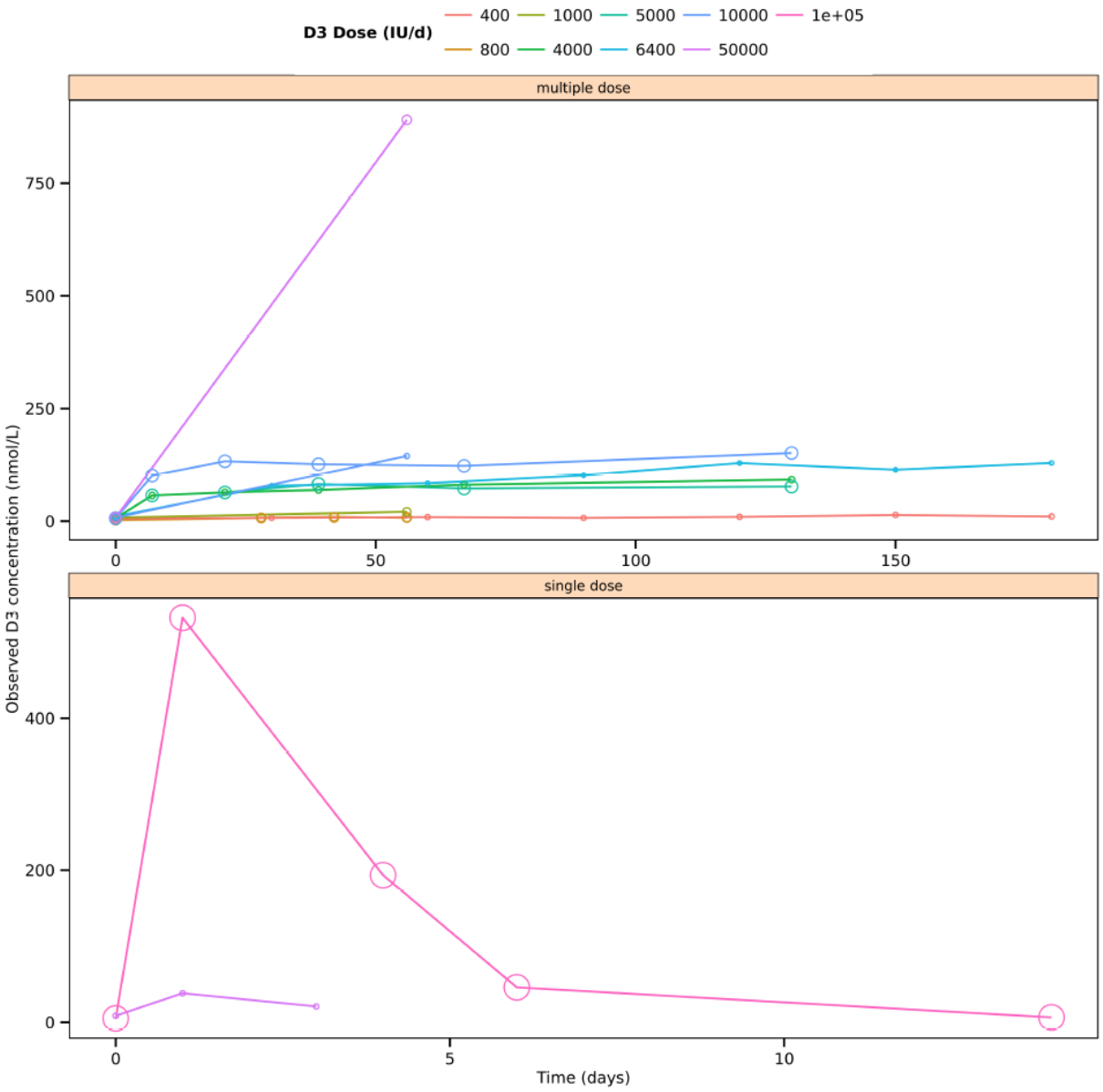

Figure B.2: Observed metabolite (25OHD3) concentration data after 25OHD3 supplementation. The size of the points indicates the size of the of the treatment arm

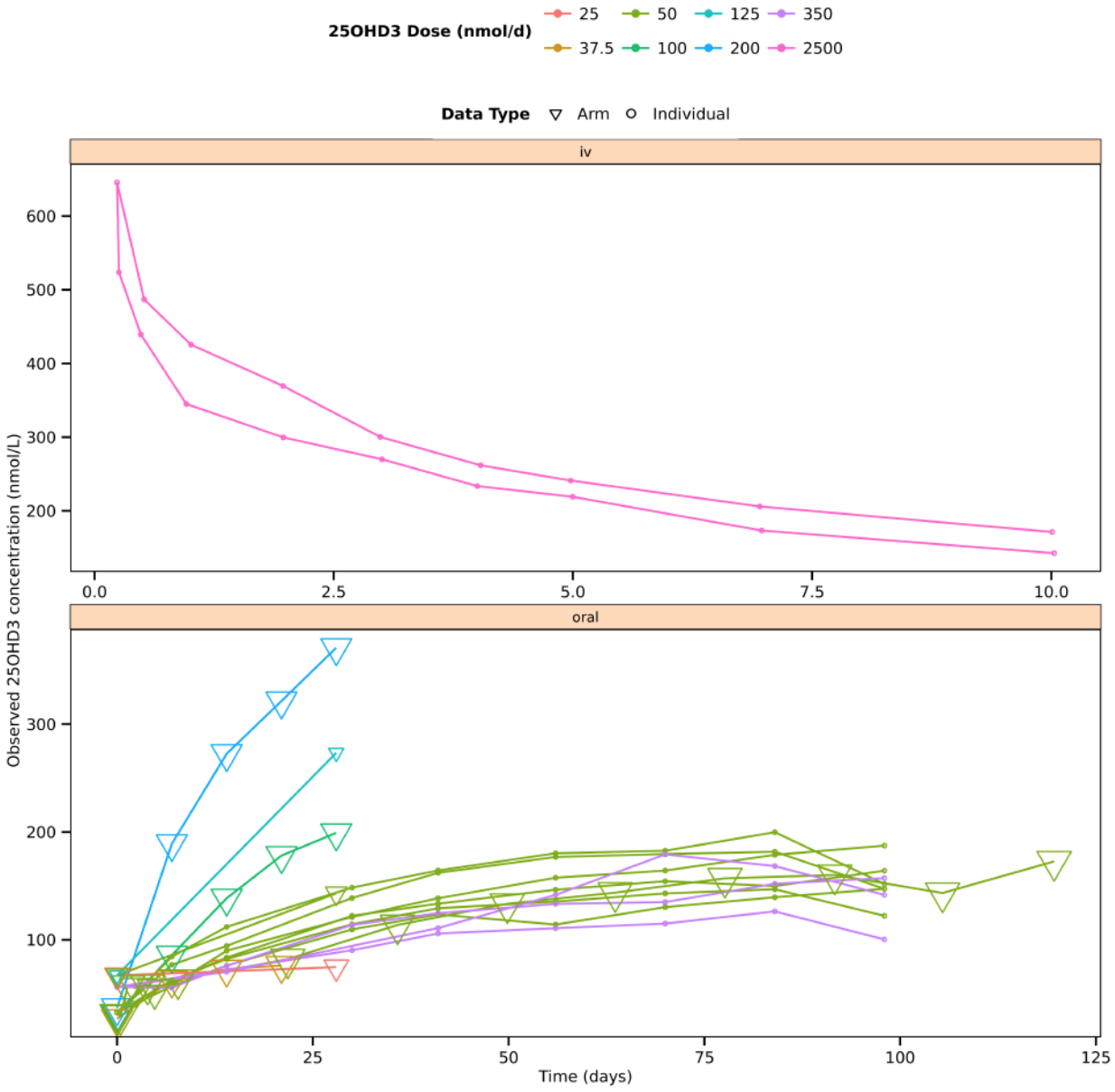

Figure B.3: Observed metabolite (25OHD3) concentration data after D3 supplementation. The size of the points indicates the size of the of the treatment arm. Panel labels include assay types.

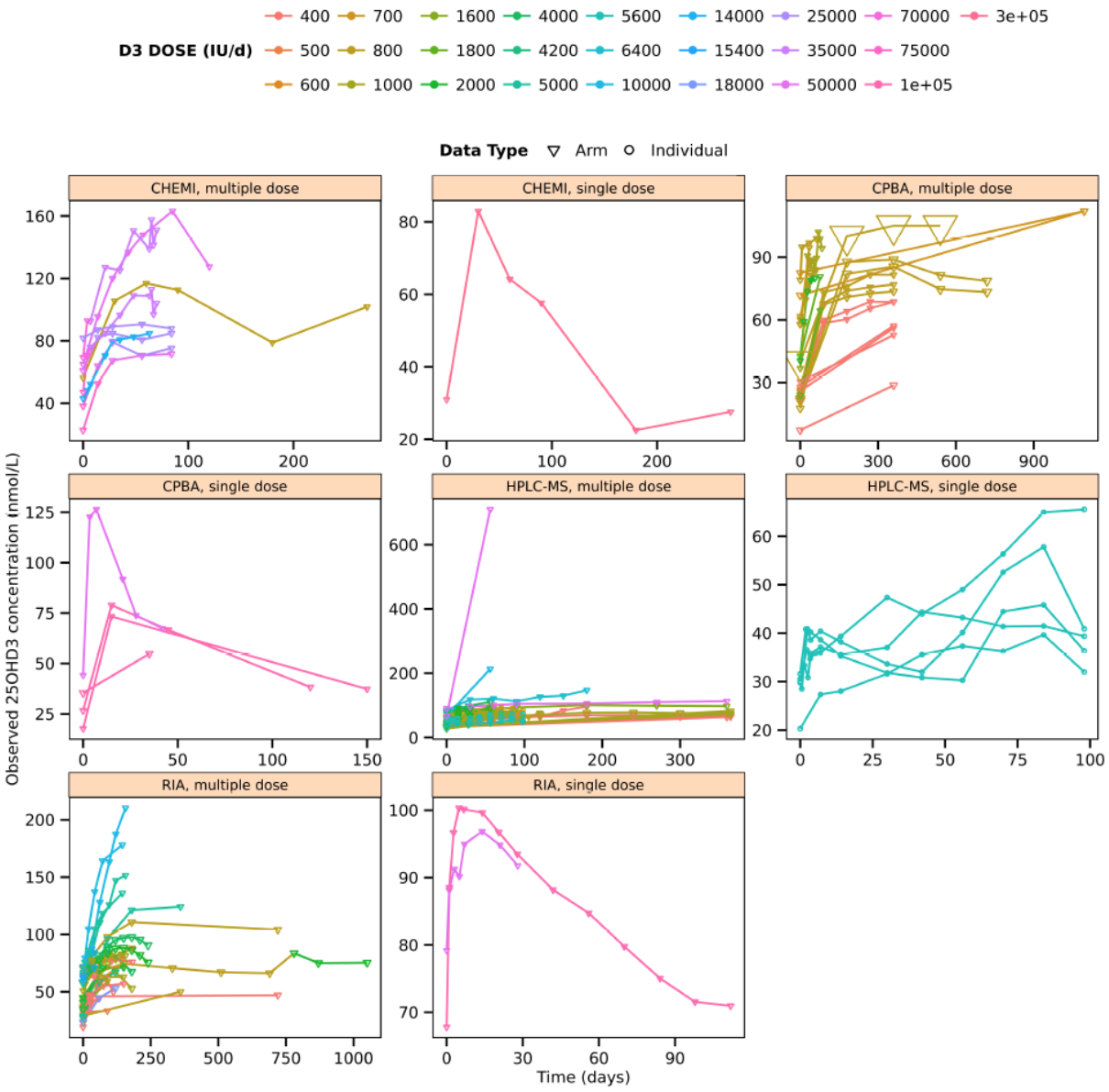

TABLE OF STUDIES INCLUDED IN META-ANALYSIS

Author	Year	Population*	LOC*	%White	Design*	Duration	Sampling Times	nTOT*	nARM Units*	nIND Units*	TRT*	DV*	Original Dose	Original Dose Units	Reg*	BMI (kg/m^2)	Weight (kg)	Assay*
Haddad et al [1]	1976	healthy adults (21-40y)	St. Louis, MO, USA	NA	NR; OL	10 days	0-10 days; every 24 h	2	0	2	25D3	25D3	1	mg/d	single; IV	NA	NA	CPBA
Stamp et al [2]	1977	patients requiring treatment with vitamin D including deficiency; X-linked hypophosphatemic rickets or osteomalacia; osteoporosis; HOPTH; healthy adult volunteers	St. Louis, MO, USA	NA	parallel	4 weeks	0; 1; 2; 3; 4 weeks	27	3 (n =9 each)	0	25D3	25D3	80; 40; 15	ug/d	mult; PO	NA	NA	CPBA
Whyte et al [3]	1979	young adults and lab personnel	St. Louis, MO, USA; London, England	NA	R;C	7 weeks	0; 0.5; 1; 3; 4; 6 weeks	5	1	0	D3	25D3	1.25	mg/d	single; PO	NA	NA	CPBA
Tjellesen et al [4]	1986	healthy adults (22-49 y)	Aalborg, Denmark	NA	R; DB	8 weeks	0; 4; 8 weeks	10	1	0	D3	25D3	4000	IU/d	mult; PO	NA	NA	HPLC-MS
Weisman et al [5]	1986	elderly adults (72-94y)	Tel Aviv, Israel	NA	R; C	5 months	0; 1; 2; 5 months	57	2 (n = 13; n = 44)	0	D3	25D3	1.00E+05	IU/d	single; PO	NA	NA	CPBA
Hartwell et al [6]	1987	premenopausal women (22-49 y)	Copenhagen, Denmark	NA	R	8 weeks	0; 4; 8 weeks	18	9	0	D3	25D3	4000	IU/d	mult; PO	NA	NA	HPLC-MS
Lips et al [7]	1988	healthy adults (mean 82 y)	Arnhem/ Amsterdam, The Netherlands	NA	R; C	1 year	0; 3; 6; 9; 12 months	142	4	0	D3	25D3	800	IU/d	mult; PO	NA	NA	CPBA
Himmelstein et al [8]	1990	healthy elderly adults (mean 81.1 y)	Paramus, NJ, USA	~100%	R; PC; DB	6 weeks	2 weeks pre-BL; 0;2; 4; 6; 7 weeks	15	1	0	D3	25D3	50	ug/d	mult; PO	NA	NA	CPBA
Honkanen et al [9]	1990	independent and institutionalized elderly women (mean 75 y)	Kuopio, Finland	NA	R; C	11 weeks	0; 11 weeks	55	2 (n = 25; n = 30)	0	D3	25D3	1800	IU/d	mult; PO	NA	70.7; 62.1	CPBA
Chapuy et al [10]	1992	healthy women (69-106y)	Lyon, France	NA	R; PC	18 months	0; 6; 12; 18 months	1634	1	0	D3	25D3	800	IU/d	mult; PO	NA	56	CPBA
Khaw et al [11]	1994	healthy adults (63-76y)	Cambridge, UK	NA	R; PC; DB	5 weeks	0; 5 weeks	95	1	0	D3	25D3	1.00E+05	IU/d	single; PO	NA	70.6	CPBA
Vanderklis et al [12]	1996	pre- and PM white women in	The Nether-	NA, 100%,	R; PC	4 weeks pre-	0; 5 weeks; 0; 4 weeks;	85	3 (n = 38; n =	0	D3	25D3	15; 20; 20	ug/d	mult; PO	NA	NA	CPBA

		Netherlands (mean 30y; 61 y) ; elderly black & white women in Curacao (mean 75 y)	lands	100%		menopausal; 5 weeks PM; 9 weeks black & white	0; 1; 5; 9 weeks		6; n = 41)									
Graafmans et al [13]	1997	elderly women (> 70 y)	Rotter-dam/Am-sterdam, The Nether-lands	NA	R; PC	2 years	0; 2 years	46	3 (n = 13; n = 22; n = 11)	0	D3	25D3	400	IU/d	mult; PO	28.2; 29.2; 27.3	NA	CPBA
Dawson-Hughes et al [14]	1997	healthy men and women (> 65 y)	Boston, MA, USA	NA	R; PC	18 months	0; 18 months	187	2 (n = 86 ; n = 101)	0	D3	25D3	700	IU/d	mult; PO	NA	82.4; 67.6	CPBA
Chel et al [15]	1998	Elderly women with secondary hyper-parathyroidism	War-mond, The Nether-lands	NA	R; C	12 weeks	0; 2; 4; 8; 12 weeks	15	1	0	D3	25D3	400	IU/d	mult; PO	NA	NA	RIA
Trang et al [16]	1998	healthy adults (mean 38 y)	Toronto, Canada	NA	R; DB; C	2 weeks	0; 2 weeks	55	1	0	D3	25D3	4000	IU/d	mult; PO	NA	NA	RIA
Barger-Lux et al [17]	1998	healthy men (mean 28 y)	Omaha, NE, USA	NA	OL	D3: 8 weeks; 25D3: 4 weeks	D3: 0; 8 weeks; 25D3: 0; 4 weeks	54	6 (n = 13; n = 10; n =14; n = 7; n = 6; n = 4)	0	D3; 25D3	D3; 25D3	D3: 25; 250 1250; 25D3: 10; 20; 50	ug/d	mult; PO	25.7	81.9	HPLC-MS
Hunter et al [18]	2000	twins (47-70y)	London, England	NA	R; PC; DB	2 years	0; 12; 18; 24 months	79	1	0	D3	25D3	800	IU/d	mult; PO	24.1	62.4	RIA
Harris et al [19]	2002	healthy men (18-79y)	Boston, MA, USA	NA	R; C	2 months	0; 4; 6; 8 weeks	27	1	0	D3	D3; 25D3	20	ug/d	mult; PO	29	NA	HPLC-MS; CPBA
Chapuy et al [20]	2002	elderly women (64-99y)	NA	NA	R; PC; DB	2 years	0; 6; 12; 18; 24 months	393	2 (n = 199; n = 194)	0	D3	25D3	800	IU/d	mult; PO	NA	58.7; 59	CPBA
Tangpricha et al [21]	2003	healthy adults (19-68y)	Boston, MA, USA	NA	R; C; DB	12 weeks	0-12 weeks; once every week	14	1	0	D3	25D3	1000	IU/d	mult; PO	NA	NA	CPBA
Grados et al [22]	2003	elderly women (> 65 y)	Amiens/ Paris/ Poitiers/ Tours/ Rouen/ Biarritz/ Evreux/ Nantes/ Reims/ and St-Julien	NA	R; DB; PC	1 year	0; 3; 6; 9; 12 months	95	1	0	D3	25D3	800	IU/d	mult; PO	26.8	NA	CPBA

			des Landes, France															
Heaney et al [23], [24]	2003; 2008	healthy men (mean 38.7 y)	Omaha, NE, USA	NA	R; C	5 months	1000 IU/d group: 0-5 months; every month; others: 1; 3; 6; 10; 20 weeks	51	4 (n =17; n = 17; n = 17; n = 11)	0	D3	D3; 25D3	1000; 4000; 5000; 10000	IU/d	mult; PO	27.4; 24.3;NA	71.6; 71.2; NA	HPLC-MS; RIA
Armas et al [25]	2004	healthy men (20-61y)	Omaha, NE, USA	NA	R; C	1 month	0; 1; 3; 5-7; 14; 28 days	10	1	0	D3	D3; 25D3	50000	IU/d	single; PO	27.14	89.36	RIA
Larsen et al [26]	2004	healthy Danish adults (65-103 y)	Randers, Denmark	NA	F;C; CR; PG	2 years	0; 1; 24 months	67	1	0	D3	25D3	400	IU/d	mult; PO	NA	69.2	RIA
Harwood et al [27]	2004	elderly women with history of hip fracture	Notting-ham, England	NA	R; PC	1 year	0; 3; 6; 12 months	76	39	0	D3	25D3	800	IU/d	mult; PO	NA	NA	RIA
Brazier et al [28]	2005	healthy women (74.6 y)	France	NA	R; PC; DB	1 year	0; 3; 6; 9; 12 months	95	1	0	D3	25D3	800	IU/d	mult; PO	27	65.2	CPBA
Natri et al [29]	2006	healthy women (12-45y)	Helsinki, Finland	NA	R; C; SB	3 weeks	0; 3 weeks	31	3 (n = 10; n = 10; n = 11)	0	D3	25D3	10	ug/d	mult; PO	NA	NA	RIA
Wagner et al [30]	2006	lactating women at 1-month postpartum (mean ~30y)	Charle-ston, SC, USA	66.7%; 88.9%	R; PC; DB	6 months	0-6 months; once per month	19	2 (n = 10; n = 9)	0	D3	D3; 25D3	400; 6400	IU/d	mult; PO	NA	NA	HPLC-MS
Talwar et al [31]	2007	healthy; black PM women	Long Island, NY, USA	0%	R; PC	3 years	0; 3; 6; 12; 18; 24; 27; 30; 36 months	104	1		D3	25D3	800 for 2 years; 2000 for 1 year	IU/d	mult; PO	29	78	RIA
Ilahi et al [32]	2008	healthy adults (27-91y)	Omaha, NE, USA	mostly Cau-casian	R; C; OL	4 months	0; 1; 3; 5; 7; 14; 21; 28; 42; 56; 70; 84; 96; 112 days	30	1	0	D3	D3; 25D3	1.00E+05	IU/d	single; PO	NA	NA	RIA; HPLC-MS
Premaor et al [33]	2008	adults (>= 65 y) with SHPT*	Porto Alegre, Brazil	NA	R; C; DB	9 months	0; 1; 2; 3; 6; 9 months	28	2 (n = 14 each)	0	D3	25D3	300000; 800	IU/d	single; mult; PO	23.4; 24.4	62.8; 64.9	CHEMI
Holick et al [34]	2008	healthy adults (18-84 y)	Boston, MA, USA	30%	R; PC; DB	11 weeks	0-11 weeks; once a week	20	1	0	D3	25D3	1000	IU/d	mult; PO	30	NA	HPLC-MS
Chel et al [35]	2008	elderly adults (mean 84 y)	Amster-dam, The Nether-lands	NA	R; PC	4 months	0; 2; 4 months	166	3 (n = 55; n = 54; n = 57)	0	D3	25D3	600; 4200; 18000	IU/d; IU/week; IU/month	mult; PO	NA	NA	RIA
Viljakainen et al [36]	2009	healthy white men (21-49 y)	Helsinki, Finland	100%	R; PC; DB	6 months	0; 5; 10; 15; 20; 25 weeks	36	2 (n = 16 each)	0	D3	25D3	400; 800	IU/d	mult; PO	NA	80; 78.4	RIA
Smith et al [37]	2009	healthy men and women in Antarctica	Mc-Murdo, Ant-	100%	R; DB	5 months	0; 2.5; 5 months	62	3 (n = 18; n = 19; n =	0	D3	25D3	400; 1000; 2000	IU/d	mult; PO	29; 31; 28	91; 95; 89	RIA

			arctica						18)									
Glendenning et al [38]	2009	hip fracture patients (mean 84 y)	Perth, Australia	NA	R; DB	3 months	0; 3 months	47	1	0	D3	25D3	1000	IU/d	mult; PO	NA	NA	HPLC-MS
Mocanu et al [39], [40]	2009 ; 2013	healthy adults (58-89y)	Iasi, Romania	NA	single arm	12 months	0; 3; 6; 9; 12 months	37	1	0	D3	25D3	5000	IU/d	mult; PO	NA	74.8	RIA
Biancuzzo et al [41]	2010	healthy adults (18-84y)	Boston, MA, USA	11.1%; 30%	R; PC; DB	11 weeks	0-11 weeks; once a week	38	2 (n = 18; n = 20)	0	D3	25D3	1000	IU/d	mult; PO	29.9 ; 29.1	NA	HPLC-MS
Binkley et al [42]	2011	healthy adults (> 65 y)	Madison, WI, USA	NA	R; DB; PC	1 year	0; 1; 2; 3; 6; 9; 12 months	36	2 (n = 16 each)	0	D3	25D3	1600; 50000	IU/d; IU/month	mult; PO	28.1; 26.1	NA	HPLC-MS
Heaney et al [43]	2011	healthy adults (mean ~ 50 y)	Omaha, NE, USA	100%	R; SB	12 weeks	0; 2; 4; 6; 8; 12; 17	17	17		D3	25D3	50000	IU/week	mult; PO	25.5	NA	CHEMI
Bischoff-Ferarri et al [44]	2012	PM women (mean 61.5 y)	Zurich, Switzer-land	100%	R	4 months	0; 0.06; 0.195; 0.36; 0.56; 0.69; 1.11; 3.12; 5.12; 7.12; 9.09; 11.09; 13.09; 15.06; 17.09 weeks	20	2 (n = 10 each)	0	D3	25D3	800	IU/d	mult; PO	25.49; 23.24	NA	HPLC-MS
Lehmann et al [45]	2013	healthy adults (19-67 y)	Halle, Germany	NA	R; PC; DB	8 weeks	0; 4; 8 weeks	42	1	0	D3	25D3	50	ug/d	mult; PO	24	NA	HPLC-MS
Macdonald et al [46]	2013	PM women from Scotland (60-70y)	northeast Scotland	100%	R; PC; DB	1 year	0; 2; 4; 6; 8; 10; 12 months	174	2 (n = 84; n = 90)	0	D3	25D3	400; 1000	IU/d	mult; PO	25.3; 25.2	68.1; 69.4	HPLC-MS
Bonjour et al [47]	2013	institutionalized women (mean 85.5 y)	France	NA	R; PC; DB	56 days	0; 28; 56 days	31	1	0	D3	25D3	10	ug/d	mult; PO	26.6	66.6	RIA
Cavalier et al [48]	2013	white healthy adults (> 50 y)	Belgium	100%	R; PC; DB	12 weeks (8 weeks supplementation; 4 week follow-up)	0; 2; 4; 8; 12	140	3 (n = 40; n = 40; n = 40; n = 20)	0	D3	25D3	75000 then 50000; 75000 then 50000 then 25000; 50000 then 25000 ; 25000	IU/d	single; mult; PO	26.5; 26.2; 25.6; 25.8	NA	CHEMI
Roth et al [49]	2013	pregnant (27-<31 weeks gestation) and non-pregnant women (18 - < 35 years)	Dhaka, Bangla-desh	NA	R; PC	pregnant: until delivery; non-pregnant: 10 weeks	pregnant: 0; 4; 7; 35; 63; 67; non-pregnant: 0; 4; 21; 49; 65; 70	44	3 (n = 16; n = 14; n = 14)	0	D3	25D3	70000 then 35000; 70000 then 35000; 14000	IU/week	mult; PO	NA	NA	CHEMI

Wagner et al [50]	2013	pregnant women 12-16 weeks gestation	Columbia /North Charle-ston, SC, USA	9.2%; 13%	R; DB	8 months	0; 1; 2; 3; 4; 5; 6 months	257	2 (n = 130; n = 127)	0	D3	25D3	2000; 4000	IU/d	mult; PO	38	NA	RIA
Lagunova et al [51]	2013	healthy adults (23-61 y)	Oslo, Norway	NA	R	1 month	0; 15; 30 days	22	4 (n = 5; n = 6; n = 4; n = 7)	0	D3	25D3	2000	IU/d	mult; PO	22.4; 22.4; 22.9; 22.9	NA	HPLC-MS
Nimitphong et al [52]	2013	healthy adults (15-70y)	Thailand	NA	R; UB	3 months	0; 1; 3; months	20	1	0	D3	25D3	400	IU/d	mult; PO	22.4	56.9	HPLC-MS
Drincic et al [53]	2013	healthy adults (19-68 y)	Omaha, NE, USA	NA	R; SB	21 weeks	0; 1;3;6; 10 weeks	62	3 (n = 22; n = 20; n = 20)	0	D3	25D3	1000; 5000; 10000	IU/d	mult; PO	36.7; 36.1; 37.9	105.8; 109.4; 106.5	RIA
Wood et al [54]	2013	Caucasian PM women	northeast Scotland	100%	R; PC; DB	1 year	0; 12 months	193	6 (n =37; n = 35; n = 44; n = 45; n = 16; n = 16)	0	D3	25D3	400; 1000; 400; 1000; 400; 1000	IU/d	mult; PO	21.5; 21.5; 27; 27; 30; 30	59.1; 59.1; 70.7; 70.7; 85.7; 85.7	HPLC-MS
Agarwal et al [55]	2013	healthy PM Indian women	New Delhi, India	NA	R; C; OL	3 months	0; 3 months	40	2 (n =25; n = 15)	0	D3	25D3	500; 1000	IU/d	mult; PO	28.87; 28.34	NA	RIA
Shapses et al [56]	2013	PM women (50-70y)	New Bruns-wick, NJ, USA	81% of total that com-pleted the study	R; PC; DB	6 weeks	0; 6 weeks	82	4 (n = 19; n = 20; n = 22; n = 21)	0	D3	25D3	385; 385; 10; 10	ug/week	mult; PO	30.1; 30.4; 30.9; 29.2	79.1; 80.1; 84.3; 73.3	RIA
Ng et al [57]	2013	African American adults (mean ~50 y)	Boston, MA	0%	R; PC; DB	6 months	0; 3; 6; months	247	3 (n=81; n = 83; n = 83)	0	D3	25D3	1000; 2000; 4000	IU/d	mult; PO	30.5; 31.9; 31.4	NA	RIA
Jetter et al [58]	2014	women (mean 60 y)	Zurich, Switzer-land	100%	R; C; DB	15 weeks	0-15 weeks; every 1- 2 weeks	23	0	23	D3; 25D3	25D3	20; 140	ug/d; ug/week	mult; PO	NA	NA	HPLC-MS

*Design: R = randomized, NR = non-randomized, PC = placebo-controlled, DB = double blind, UB = un-blinded, SB = single-blinded, C = control, OL = open label, F = factorial, CR = cluster randomized, PG = pragmatic study; Population: PM = postmenopausal; SHPT = secondary hyper-parathyroidism; HOPTH = hypoparathyroidism; Column Names: LOC = location of study; nTOT = total number of subjects from study included in the analysis; nARM Units = number of treatment arms from study included in the analysis; nIND Units = number of individual profiles from study included in the analysis; TRT = treatment(s) administered during the study; DV = study endpoints used in the analysis; Reg = Regimen: IV = intravenous, PO = oral, mult = multiple dose; Assay: RIA = radioimmunoassay, HPLC-MS = high pressure liquid chromatography tandem mass spectrometry, CPBA = competitive protein binding assay, CHEMI = chemiluminescence

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Figure B.4: Dose-normalized concentration-time profiles following oral D3 administration: (a) metabolite (25OHD3) concentrations collected over a 12-month period and D3 dose range of 400-50000 IU/day; (b) parent (D3) concentrations collected over a 6-month period and a D3 dose range of 400-10000 IU/day.

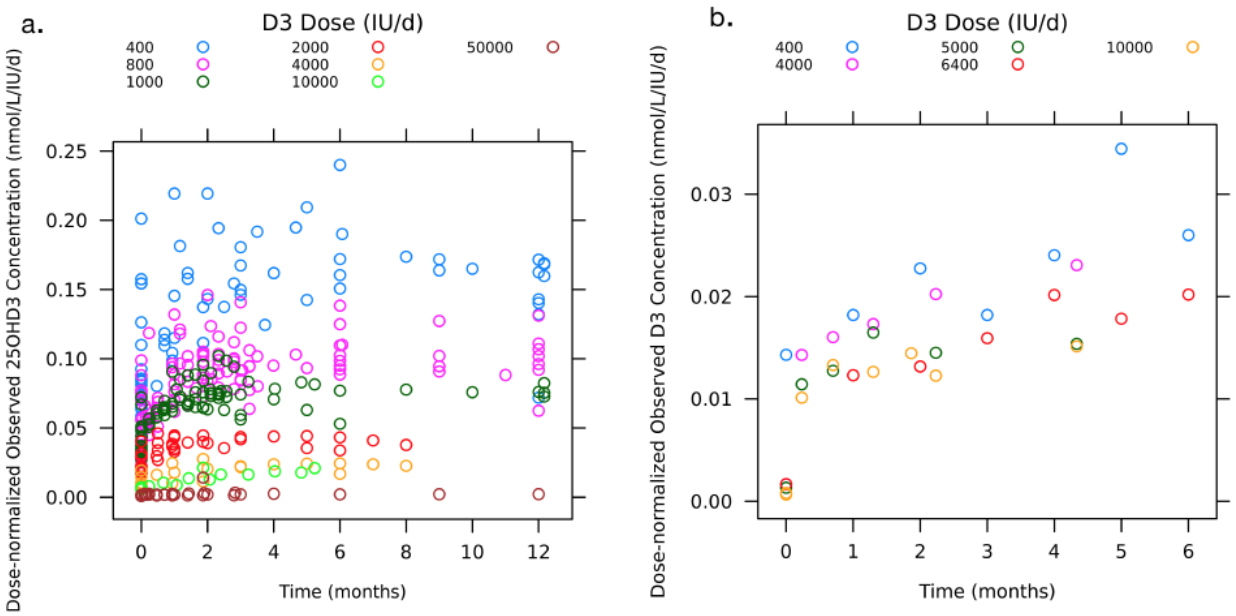


Figure B.5: Sample selection of visual posterior predictive checks for 25OHD3 (D3-25OHD3 dataset) on the unit-level. Solid red line is the simulated median; the blue shaded region indicates the 25th and 75th percentiles around the simulated median when uncertainty is included only around the fixed effects estimates; dashed blue lines indicate the 25th and 75th percentiles of the simulated median with uncertainty included around both the fixed effects and residual error estimates; black dots indicate observed data; RIA: Assay = 1; CPBA: Assay = 2; HPLC-MS: Assay = 3; CHEMI: Assay = 4

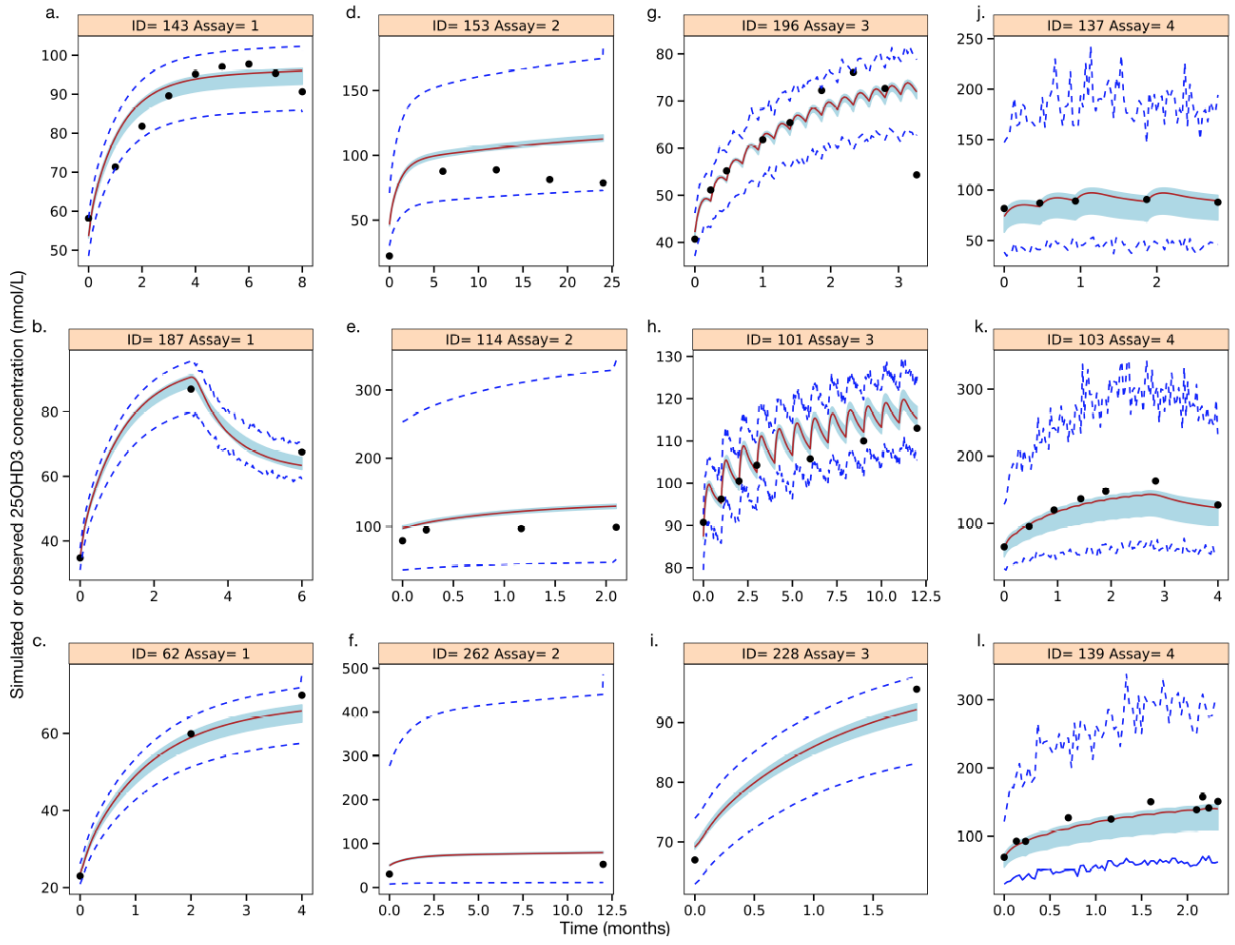


Figure B.6: Visual posterior predictive checks for D3 on the unit-level. Solid red line is the simulated median; the blue shaded regions indicate the 25th and 75th percentiles around the simulated median when uncertainty is included only around the fixed effects estimates; dashed blue lines indicate the 25th and 75th percentiles of the simulated median with uncertainty included around both the fixed effects and residual error estimates; black dots indicate observed data

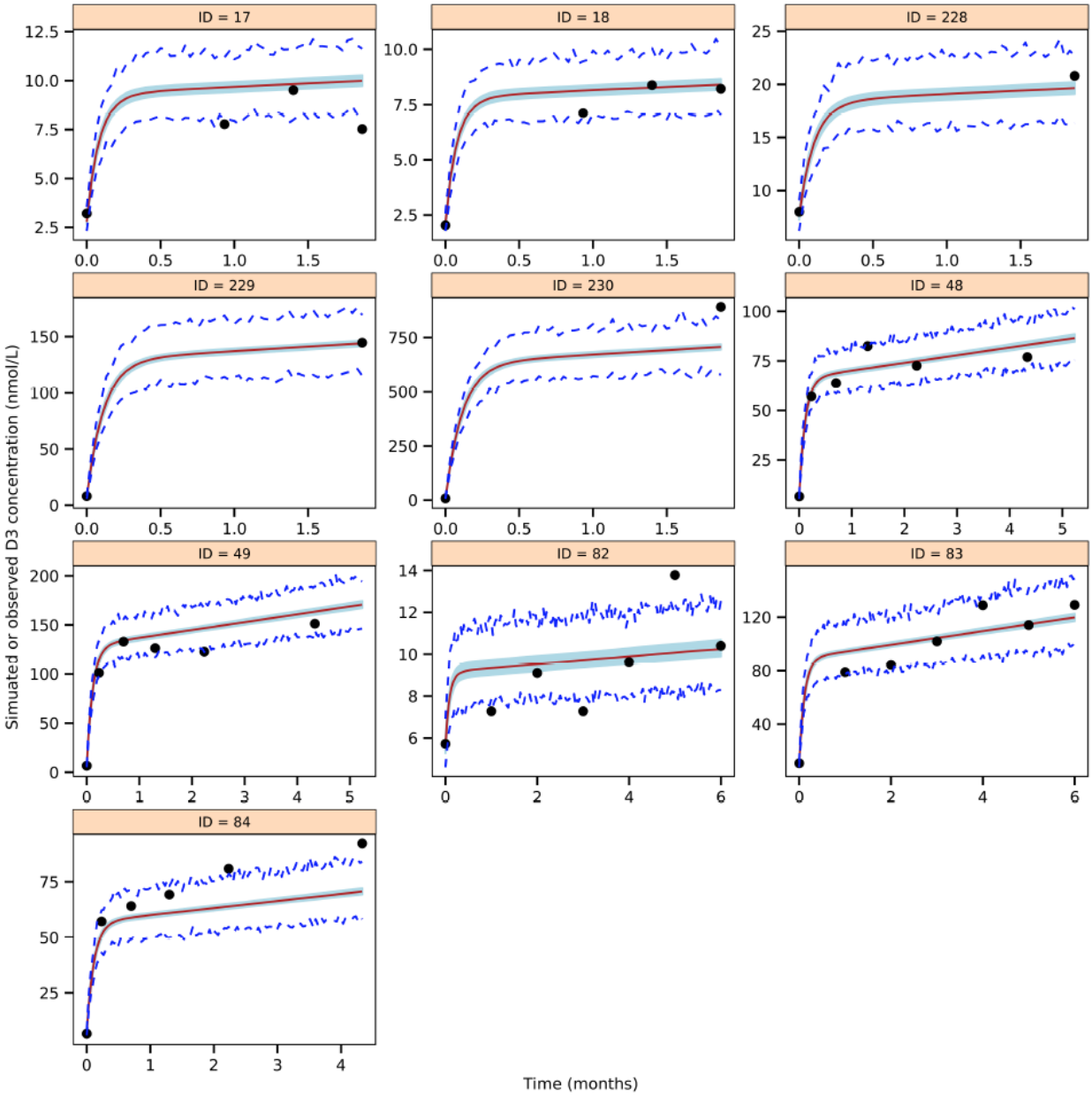


Figure B.7: Visual posterior predictive checks for 25OHD3 (25OHD3-25OHD3 dataset) on the unit level. Solid red line is the simulated median; the blue shaded regions indicate the 25th and 75th percentiles around the simulated median when uncertainty is included only around the fixed effects estimates; dashed blue lines indicate the 25th and 75th percentiles of the simulated median with uncertainty included around both the fixed effects and residual error estimates; black dots indicate observed data

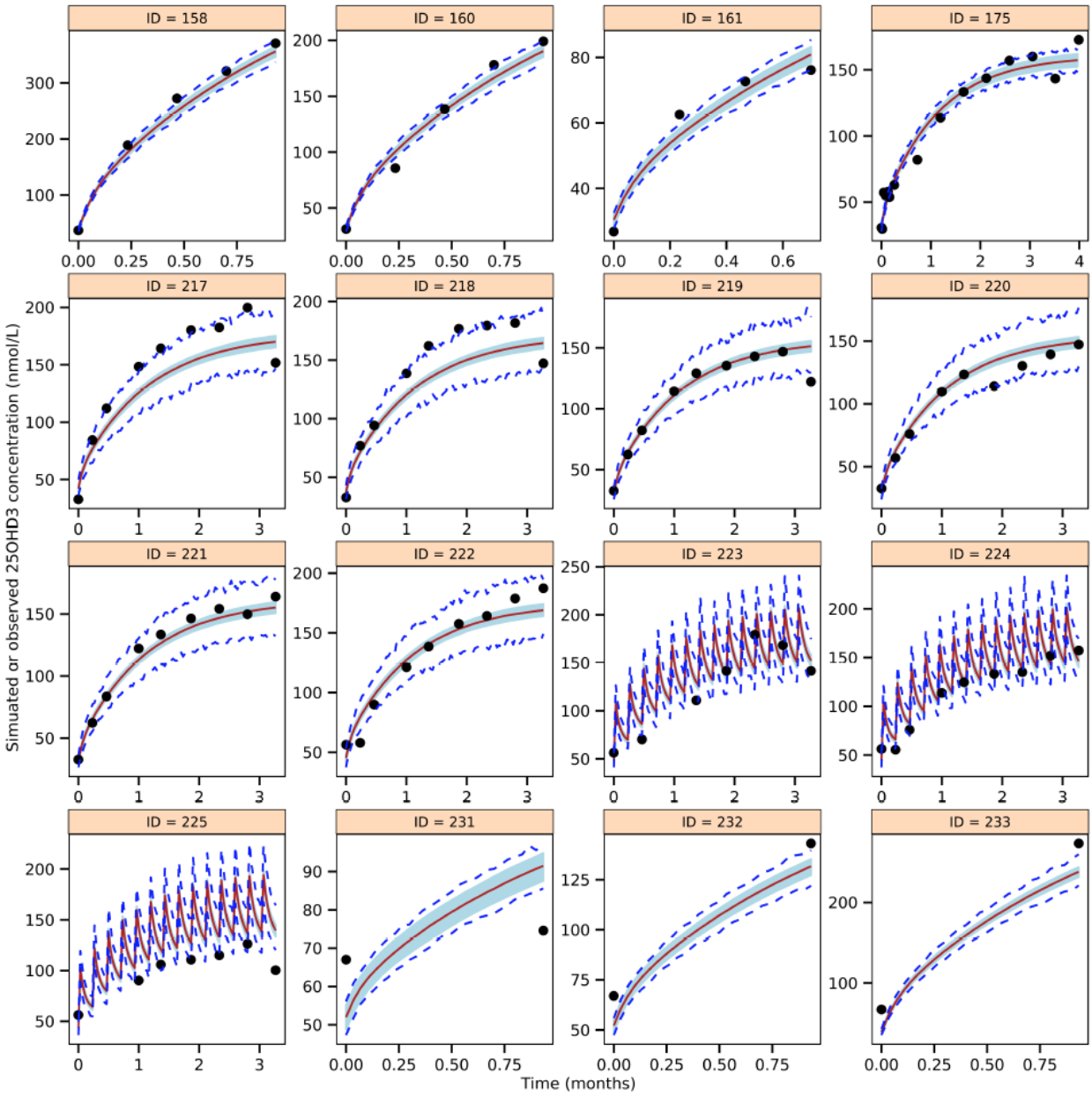


Figure B.8: 25OHD3 prediction-corrected visual predictive checks (pcVPC) for a) treatment arms measured by RIA b) treatment arms measured by CPBA c) treatment arms measured by HPLC-MS (reference) d) treatment arms measured by CHEMI. Solid red line is simulated median; red band is the simulated 80% confidence interval around the simulated median; black dots are the observed medians at a given time bin; black horizontal bars indicate time bin ranges; green triangles are the observed data; size of the triangles indicate observed sample size

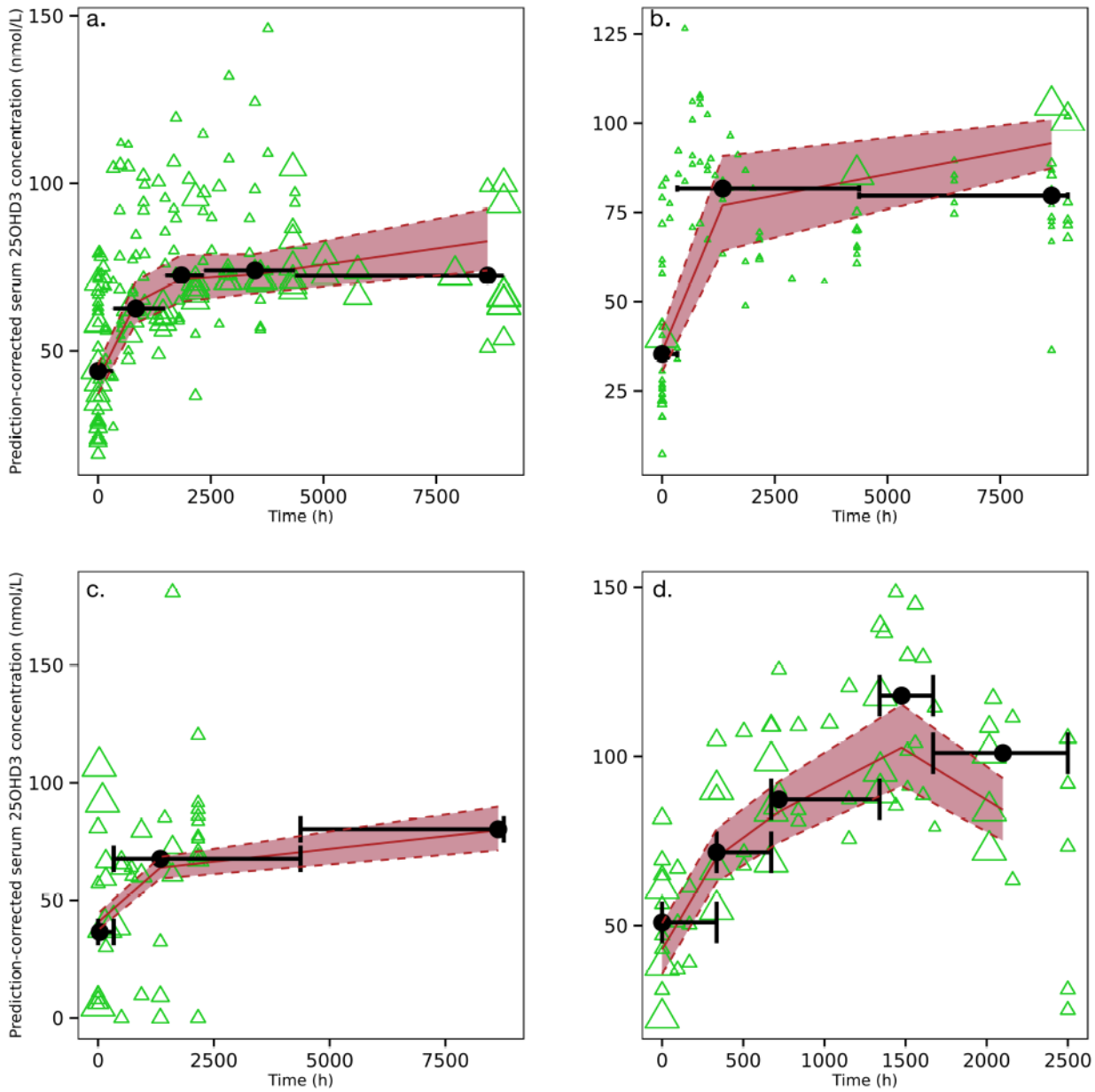


Figure B.9: Prediction-corrected visual predictive checks for a) the parent only model (D3-D3 dataset) b) metabolite only model (25D3-25D3 dataset). The solid red line is simulated median; red band is the simulated 80% confidence interval around the simulated median; black dots are the observed medians at a given time bin; black horizontal bars indicate time bin ranges; green triangles are the observed data; size of the triangles indicate observed sample size

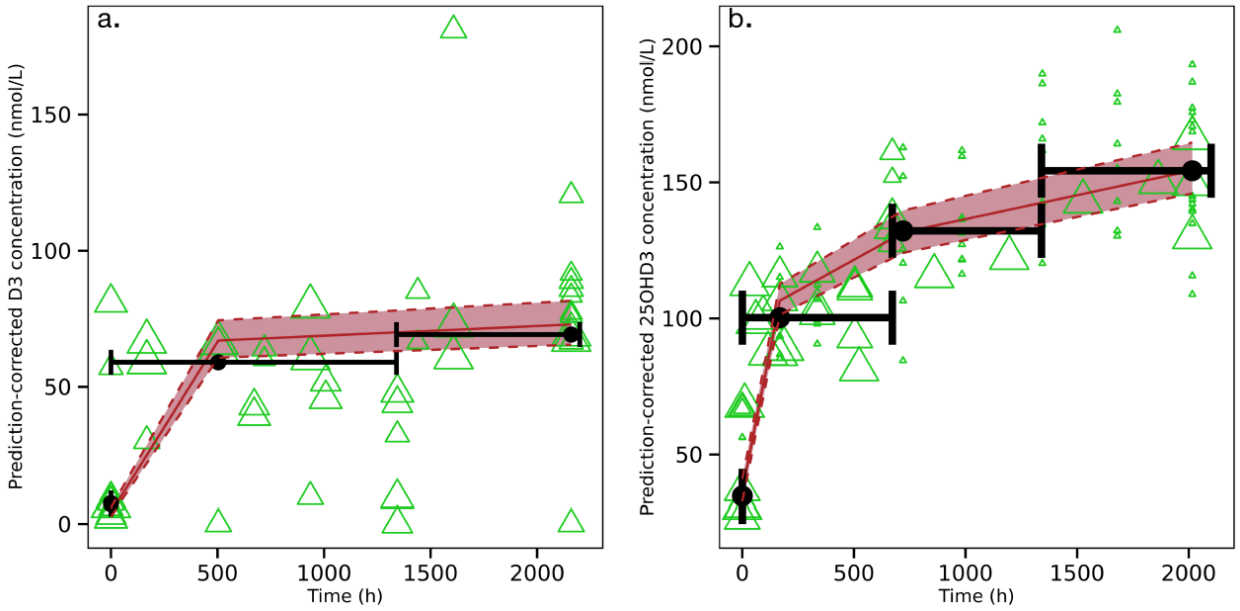
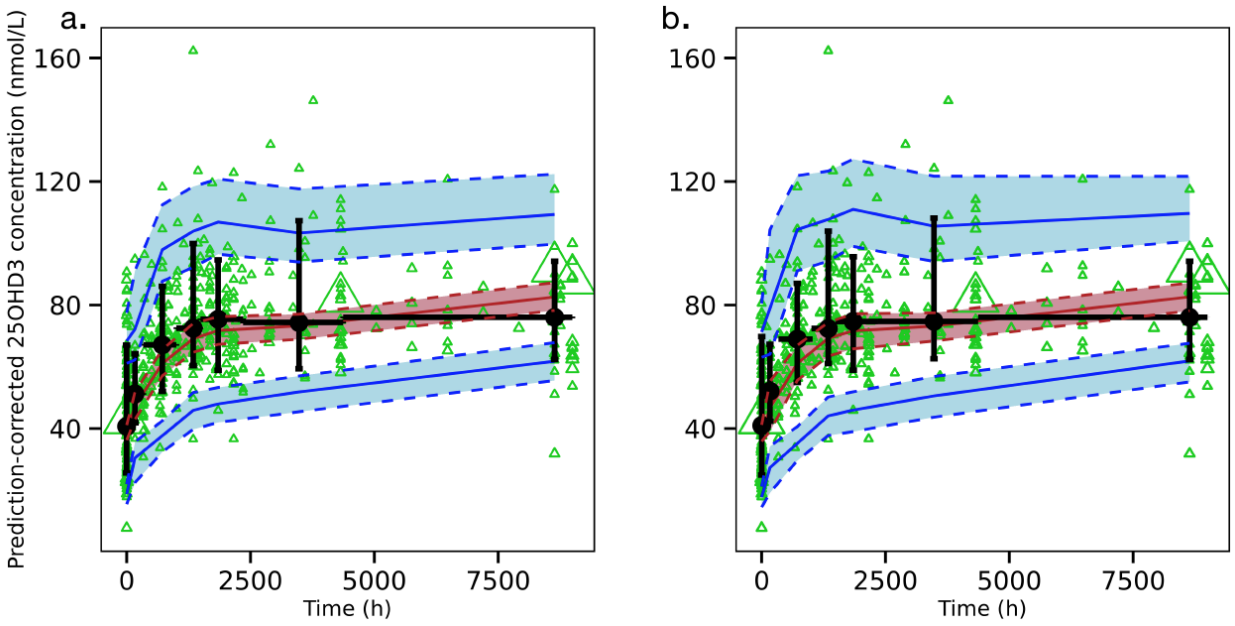


Figure B.10: Prediction-corrected visual predictive checks for a) both arm- and individual-level 25OHD3 multiple-dose data (D3-25OHD3 dataset) b) arm-level multiple-dose data only. Solid red line is simulated median; solid blue lines are the simulated 5th and 95th percentiles; red band is the simulated 80% confidence interval around the simulated median; blue bands are the simulated 80% confidence intervals around the simulated 5th and 95th percentiles of the median; black dots are the observed medians at a given time bin; black vertical bars are the observed 5th & 95th percentiles of the observed data; black horizontal bars indicate time bin ranges; green triangles are the observed data; size of the triangles indicate observed sample size



Appendix C

D2-25OHD2 Model Appendix

Figure C.1: Observed a) parent (D2) and b) metabolite (25OHD2) concentration data after D2 supplementation. The size of the points indicates the size of the of the treatment arm

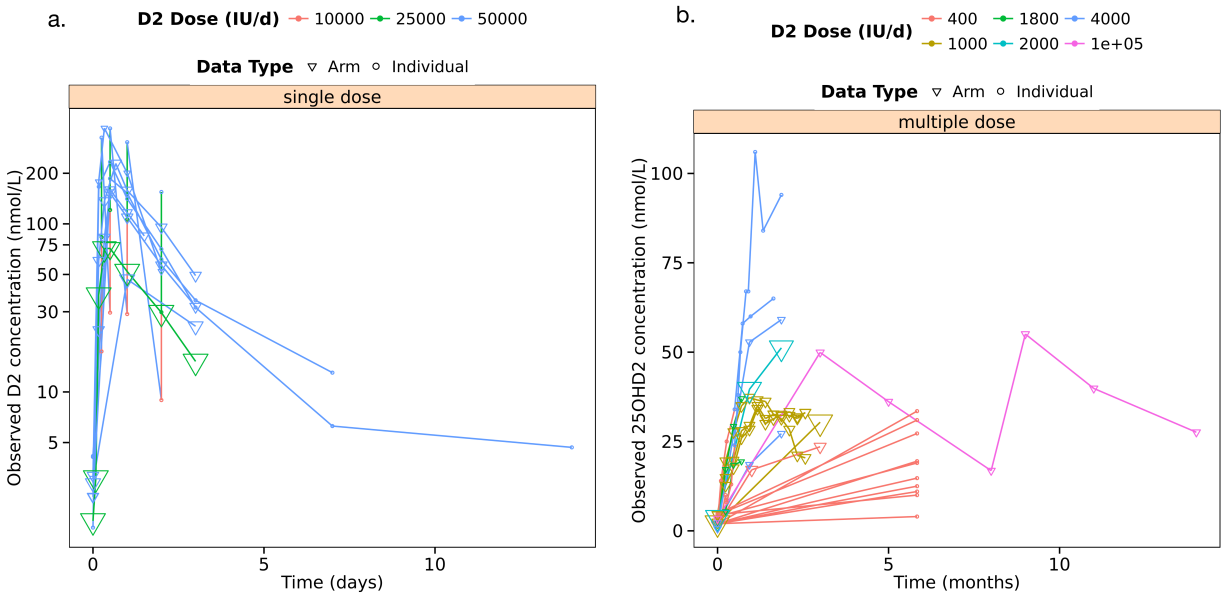


Figure C.2: Dose-normalized observed a) parent (D2) and b) metabolite (25OHD2) concentration data after D2 supplementation. The size of the points indicates the size of the of the treatment arm

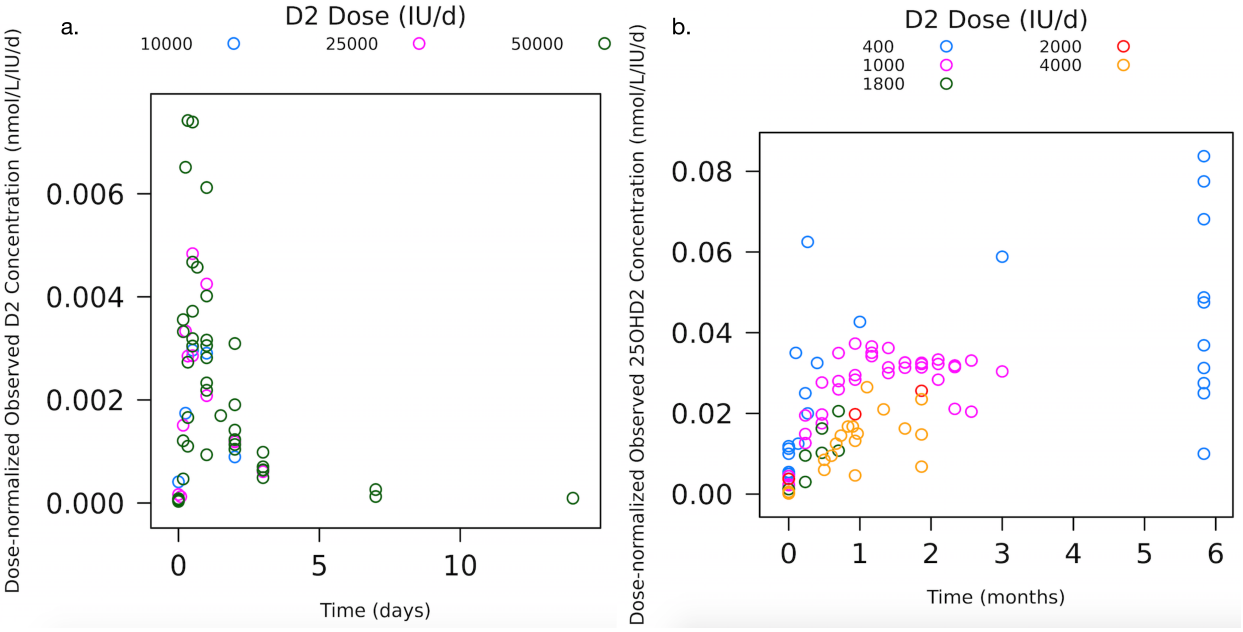


TABLE OF STUDIES INCLUDED IN D2-25OHD2 META-ANALYSIS

Author	Year	Population	Design*	DUR*	Sampling Times	nTOT*	nARM Units*	nIND Units*	TRT*	DV*	Original Dose	Original Dose Units	Reg*	BMI (kg/m^2)	Weight (kg)	Assay*
Glendenning [1]	2009	fracture patients; Vit D insufficient (< 50 nmol/L)	R; DB	3 months	0; 3 months	48	1	0	D2	25OHD2	1000	IU/d	mult; PO	NA	NA	HPLC
Lehmann [2]	2013	healthy volunteers	R; DB	2 months	0; 4; 8 weeks	46	1	0	D2	25OHD2	50	ug/d	mult; PO	23.7	NA	LC-MS
Biancuzzo [3]	2010	healthy volunteers; excluded those taking a Vit D supplement > 400 IU/d	R; PC; DB	11 weeks	weekly 1-11 weeks	34	2	0	D2	25OHD2	1000 (both arms)	IU/d	mult; PO	27; 30.4	NA	LC-MS
Holick [4]	2008	healthy adults 18-84 y; allowed a multi-vitamin of 400 IU/d	R; DB	11 weeks	weekly 1-11 weeks	16	1	0	D2	25OHD2	1000	IU/d	mult; PO	31	NA	HPLC
Tjellesen [5]	1986	healthy volunteers; PM women 22-49 years; members of lab	DB	2 months	0; 4; 8 weeks	9	1	0	D2	25OHD2	4000	IU/d	mult; PO	NA	NA	UV detection after HPLC
Harris [6]	1999	old and young men with D2 intake < 200 IU/d	R; C	3 weeks	weekly 0-3 weeks	11	2	0	D2	25OHD2	1800 (both arms)	IU/d	mult; PO	32.8; 26.1	NA	HPLC
Nimitphong [7]	2013	healthy volunteers (mean 36.7 y)	R; C	3 months	0; 1; 3 months	19	1	0	D2	25OHD2	400	IU/d	mult; PO	23.2	59.5	LC-MS/MS
Davies [8]	1985	elderly institutionalized subjects; one quarter deficient (25OHD < 12.5 nmol/L)	R	1 year	0; 6 months	20	2	0	D2	25OHD2	100000	IU/d	single dose; twice a year; PO	NA	NA	HPLC
Hartwell [9]	1987	premenopausal women (22-49 y)	R	2 months	0; 4; 8 weeks	9	1	0	D2	25OHD2	4000	IU/d	mult; PO	NA	NA	HPLC
Mawer [10]	1998	men and women with	archived samples	2 months	0; 4; 7; 8; 12;	2	0	2	D2	25OHD2	400 then	IU/d	mult for 8	NA	NA	HPLC

		osteomalacia; low D; or HPT			15; 18; 20; 25; 27; 33; 40; 56 days; 0; 3; 8; 15; 22; 29; 49 days						4000 (both arms)		days; then mult for 1.5 months; PO			
Markestad [11]	1984	pregnant women	P	6 months	0; 6 months	10	0	10	D2	25OHD2	400 (all arms)	IU/d	mult; PO	NA	NA	HPLC
Lo [12]	1985	healthy volunteers	SA	single dose	0; 4h; 12h; 1 day; 2 days; 3 days; 7 days; 14 days; 0; 8h; 12h; 1 day; 2 days; 3 days; 7 days; 0; 6h; 12h; 1 day; 2 days; 0; 4h; 8h; 12h; 1 day; 2 days; 3 days	10	1	3	D2	D2	50000; 50000; 10000; 25000; 50000; 50000	IU/d	single dose	NA	NA	HPLC-MS
Armas [13]	2004	healthy male volunteers	R; C	single dose	0; 1; 3 days	10	1	0	D2	D2	50000	IU/d	single dose	NA	NA	RIA
Clemens [14]	1986	healthy; elderly volunteers	SA; C	single dose	0; 4h; 8h; 16h; 1 day; 2 days; 3 days	7	1	0	D2	D2	50000	IU/d	single dose	NA	NA	RIA
Tangpricha [15]	2003	healthy volunteers	DB; R	single dose	0; 2h; 4h; 8h; 12h; 1 day; 2 days; 3 days	18	1	0	D2	D2	25000	IU/d	single dose	NA	NA	CPBA
Haddad [16]	1993	healthy male volunteers	SA	single dose	0; 4 h; 8h; 1 day; 2	3	1	0	D2	D2	50000	IU/d	single dose	NA	NA	HPLC-MS

					days											
Matsuoka [17]	1992	healthy volunteers	R	single dose	0; 12h; 1 day; 1.5 days	6	1	0	D2	D2	50000	IU/d	single dose	NA	NA	HPLC-MS

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TABLE OF STUDIES INCLUDED IN EXTERNAL VALIDATION FOR D3-25D3 MODEL

Author	Year	Population*	LOC*	%White	Design	Duration	Sampling Times	nTOT*	nARM Units*	TRT*	DV*	Orig-inal Dose	Original Dose Units	Reg*	BMI (kg/m^2)	Weight (kg)	Assay*
Al-khalidi [1]	2015	healthy adults	Toronto Ontario	27; 22	R; DB; C	10 weeks	0; 10 weeks	96	2	D3	25OHD3	200; 28000	IU/week	PO; MD	25.6; 25.8	NA	CHEMI
Aloia [2]	2015	healthy volunteers	New York	75	R; PC	10 weeks	0; 10 weeks	57	3	D3	25OHD3	800; 2000; 4000	IU/d	PO; MD	NA	NA	RIA
Arora [3]	2014	high blood pressure and Vit D deficient patients	Boston MA; Hartford CT; Minne-apolis MN	46	R; DB; PC	6 months	0; 2; 4; 6 months	534	2	D3	25OHD3	400; 4000	IU/d	PO; MD	28.1; 28.1	NA	CHEMI
Asemi [4]	2015	pregnant women at risk for pre-eclampsia	Kashan Iran	0	R; DB; PC	9 weeks	0; 9 weeks	23	1	D3	25OHD3	400	IU/d	PO; MD	27	71	immune-assays
Bhaga-twala [5]	2015	obese African Americans	Augusta GA	0	R; DB; PC	16 weeks	0; 8; 16 weeks	53	3	D3	25OHD3	18000; 60000; 120000	IU/mont h	PO; MD	34.56; 37.08; 34.42	93.39; 99.16; 92.77	immuno-assays
Cangussu [6]	2015	Brazilian PM women	Sao Paulo Brazil	NA	R; DB; PC	9 months	0; 9 months	80	1	D3	25OHD3	1000	IU/d	PO; MD	29.2	NA	HPLC-MS
Cashman [7]	2014	elderly adults	Cork Ireland	NA	R; DB; PC	15 weeks	0; 8; 15 weeks	64	1	D3	25OHD3	20	ug/d	PO; MD	26.7	72.5	HPLC-MS
Caval-cante [8]	2015	PM women with type 2 diabetes	Brazil	NA	R; PC	3 months	0; 3 months	38	19	D3	25OHD3	943	IU/d	PO; MD	27.6	NA	CHEMI

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Figure C.3: Prediction-corrected visual predictive check for 25OHD3 data not included in the optimization process. The solid red line is simulated median; red band is the simulated 80% confidence interval around the simulated median; black dots are the observed medians at a given time bin; black horizontal bars indicate time bin ranges; green triangles are the observed data; size of the triangles indicate observed sample size

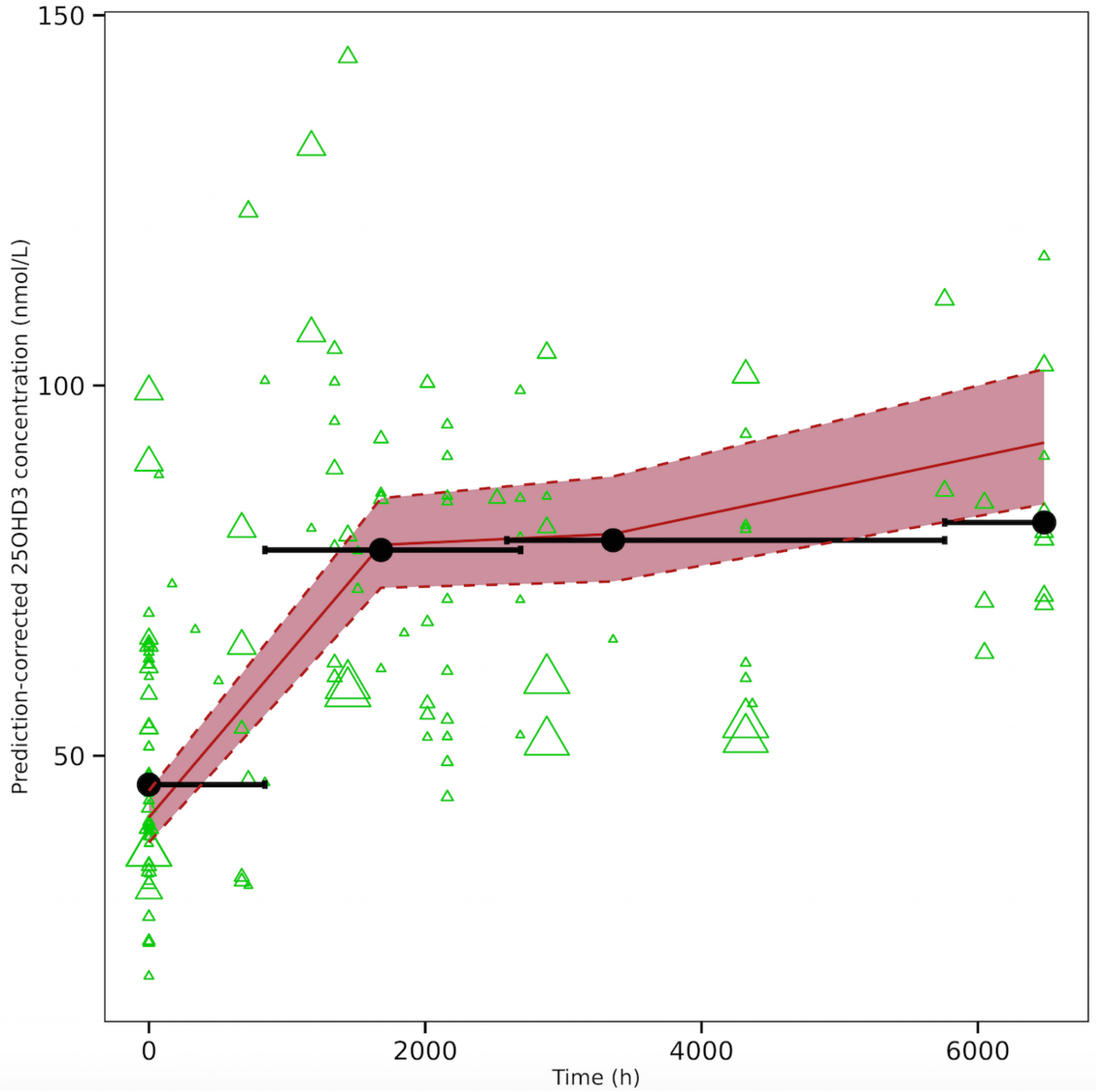


Figure C.4: Results from the global sensitivity analysis on parameters related to 25OHD3 assay types. The solid lines are trendlines through 2000 replicates of simulated 25OHD3 data, resulting from 3 months of different doses of Vitamin D3

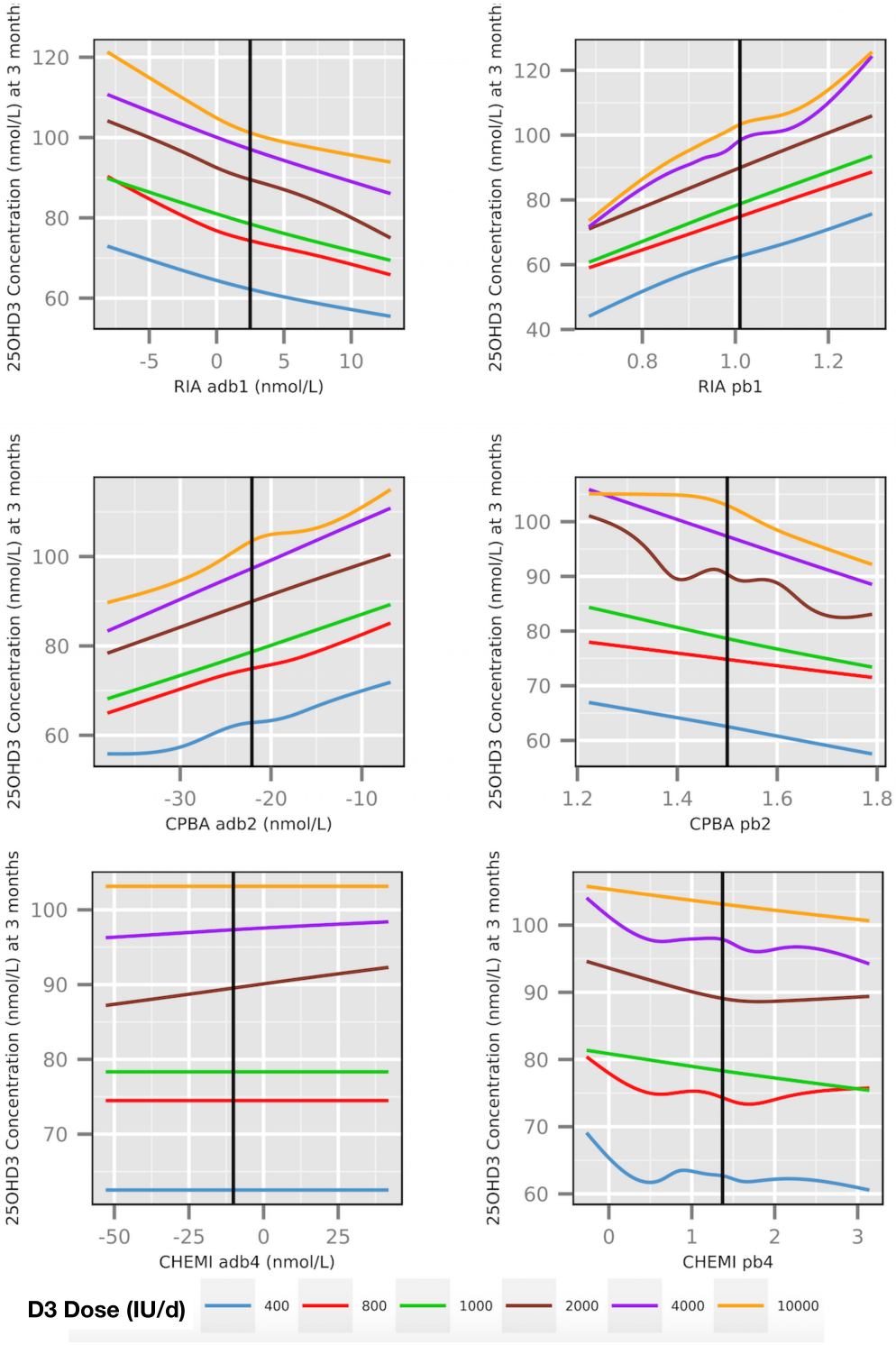


Figure C.5: Results from the global sensitivity analysis on parameters related to D3 and 25OHD3 kinetics by 25OHD3 assay type. The lines are trendlines through 2000 replicates of simulated 25OHD3 data, resulting from 3 months of different doses of Vitamin D3

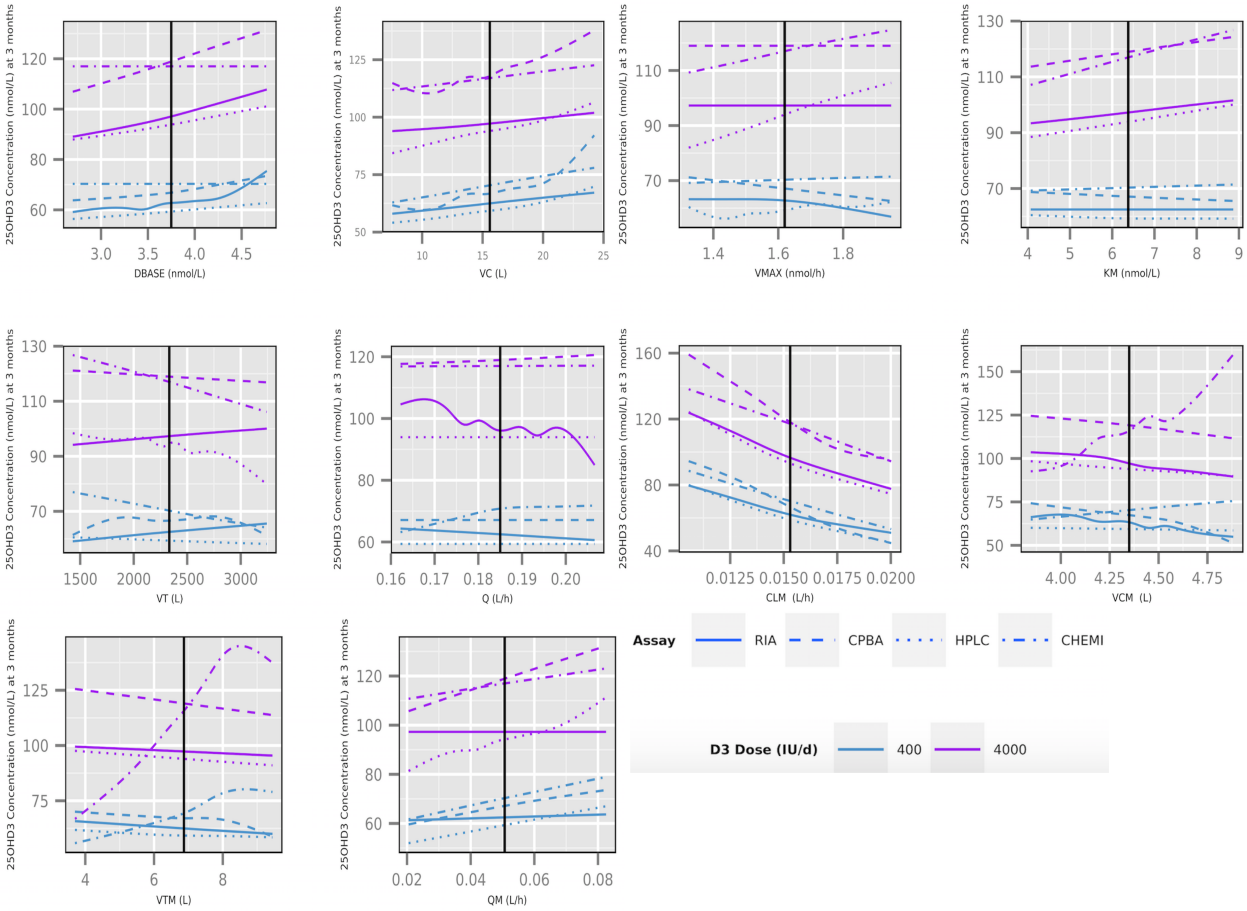


Figure C.6: Results from the global sensitivity analysis on parameters related to D2 and 25OHD2 kinetics. The solid lines are trendlines through 2000 replicates of simulated 25OHD2 data, resulting from 3 months of different doses of Vitamin D2

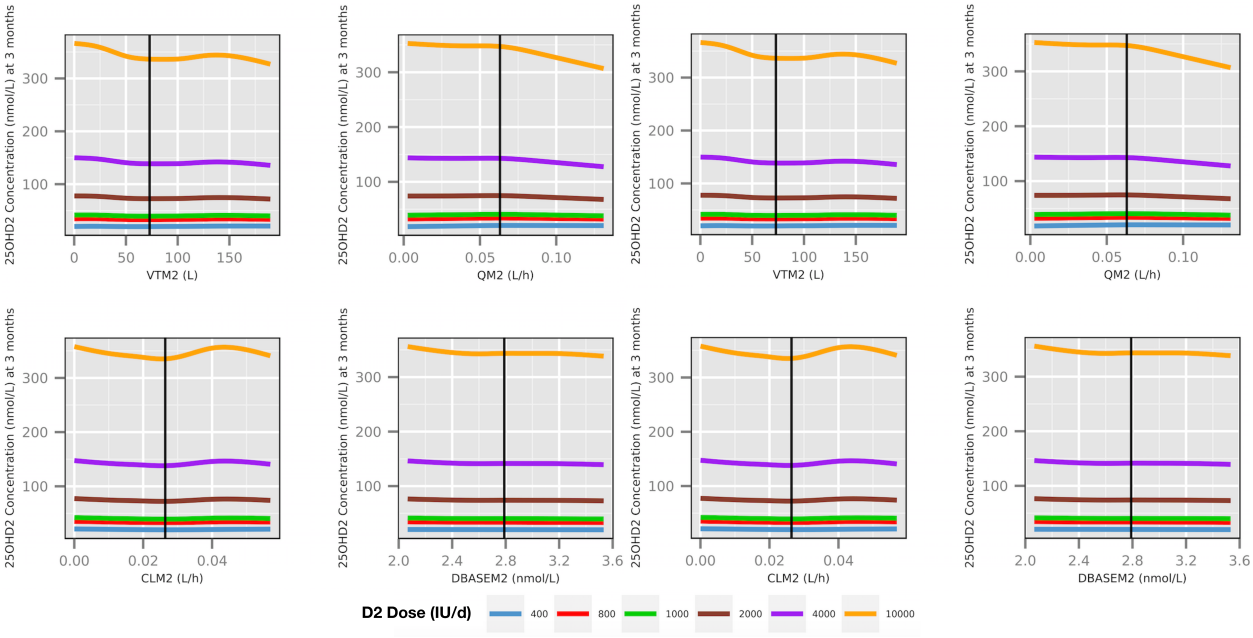


Figure C.7: Results from the local sensitivity analysis on parameters related to D2 and 25OHD2 kinetics. The solid lines are trendlines through 2000 replicates of simulated 25OHD2 data, resulting from 3 months of different doses of Vitamin D2

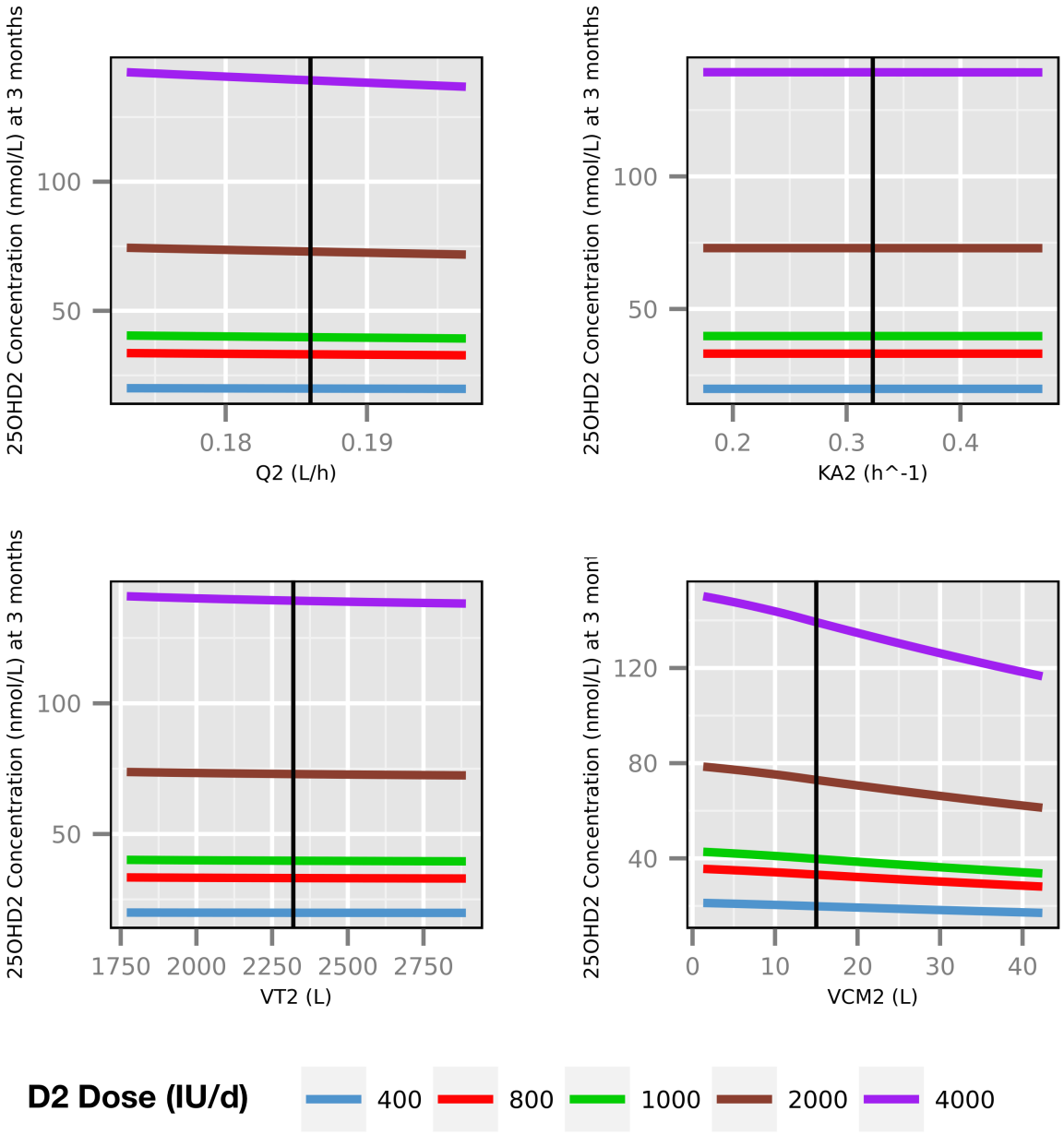
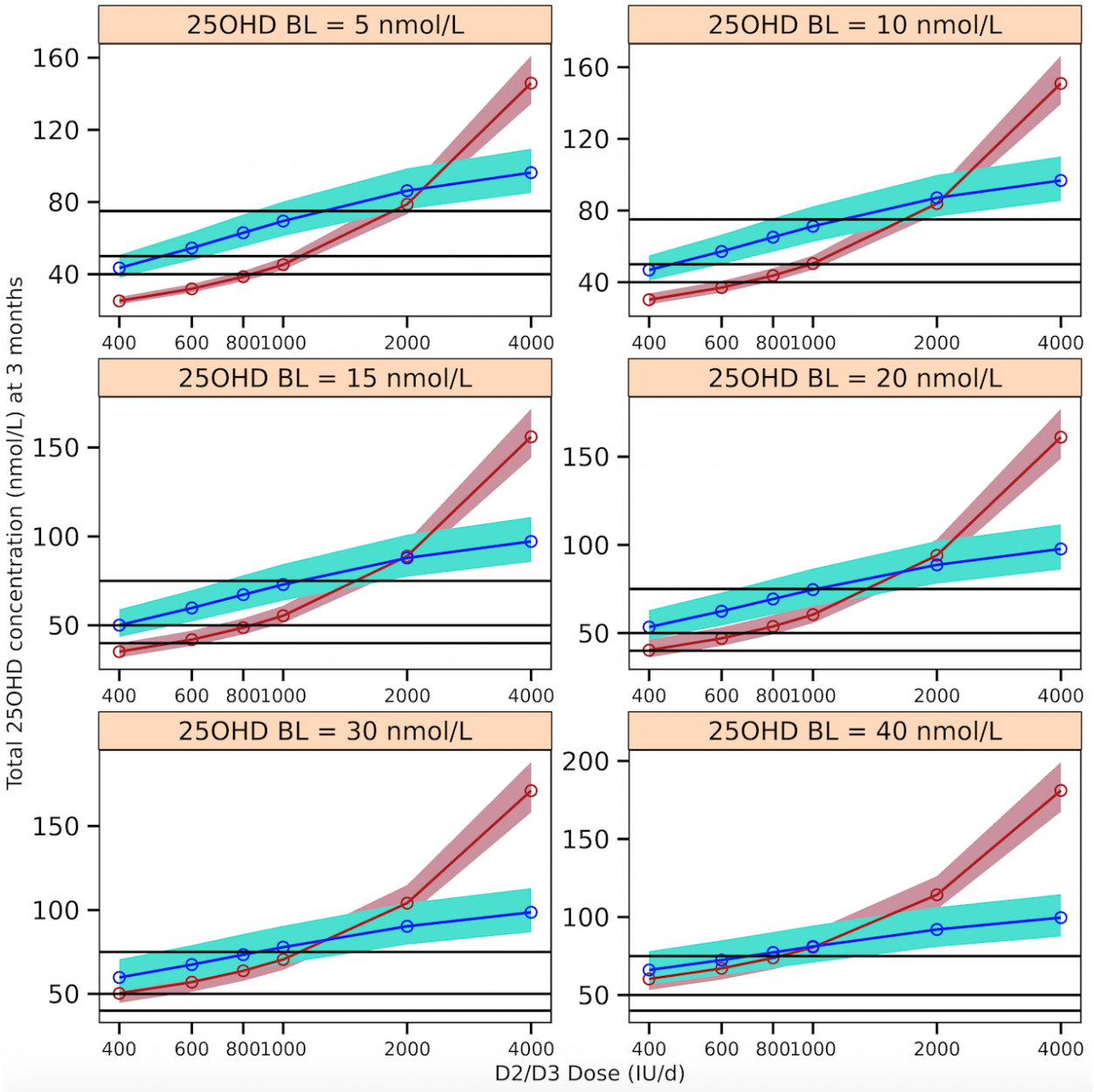


Figure C.8: Simulated total 25OHD concentration after 3 months of daily D2 or D3 doses. The solid red/blue line is the median 25OHD concentration following D2/D3 administration. The red/blue bands are simulated 90% confidence intervals around the median. The black horizontal lines are target 25OHD thresholds.



Appendix D

Vitamin D-MSPM Integration Appendix

Figure D.1: Observed serum calcitriol data used to optimize parameters related to the conversion of 25OHD3 to calcitriol. Peach horizontal strip indicates Vitamin D3 and calcium dose, respectively.

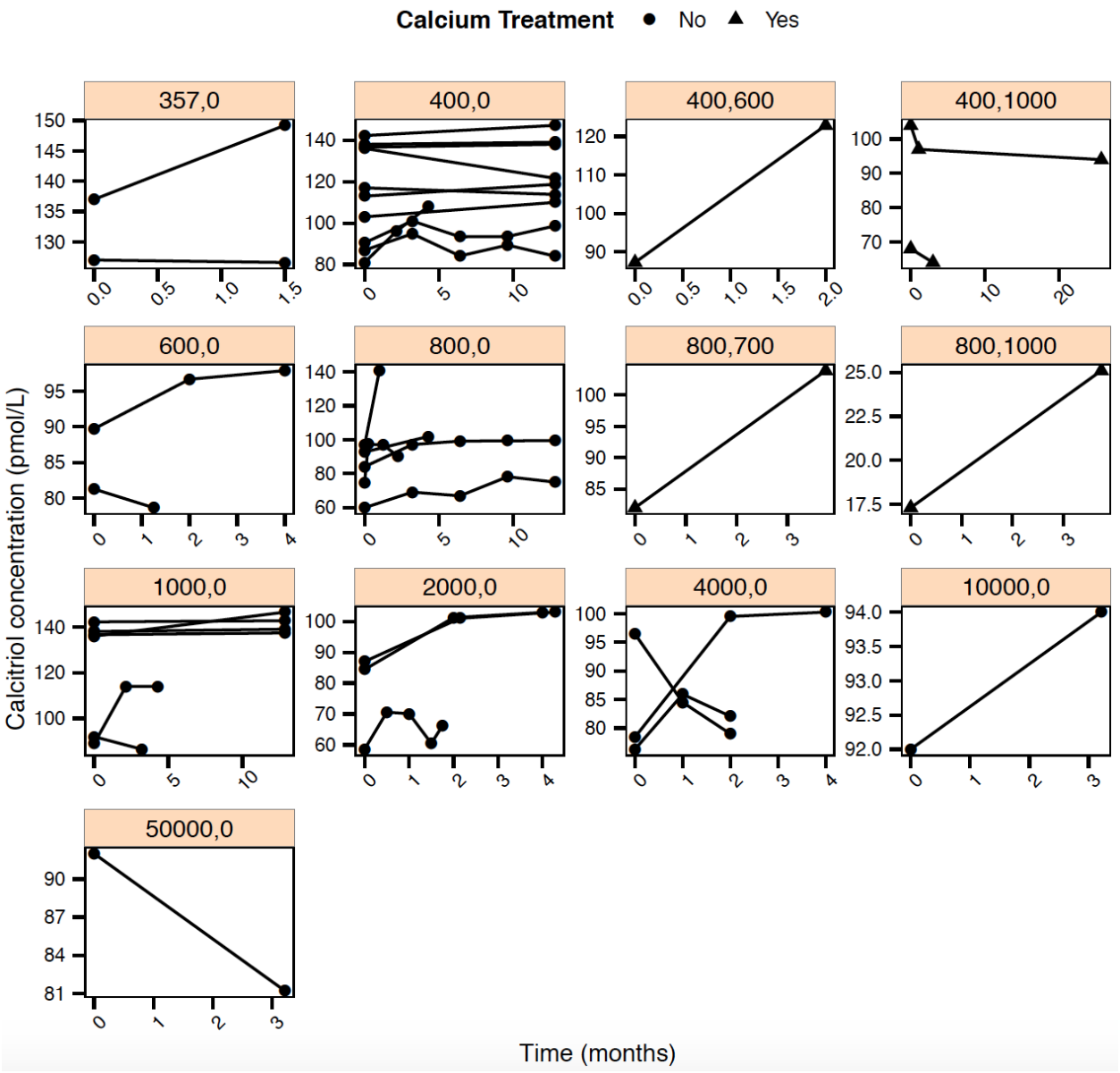


Figure D.2: Observed BMDLS data used to re-estimate parameter related to osteoclast bone resorption. Peach horizontal strip indicates Vitamin D3 and calcium dose, respectively.

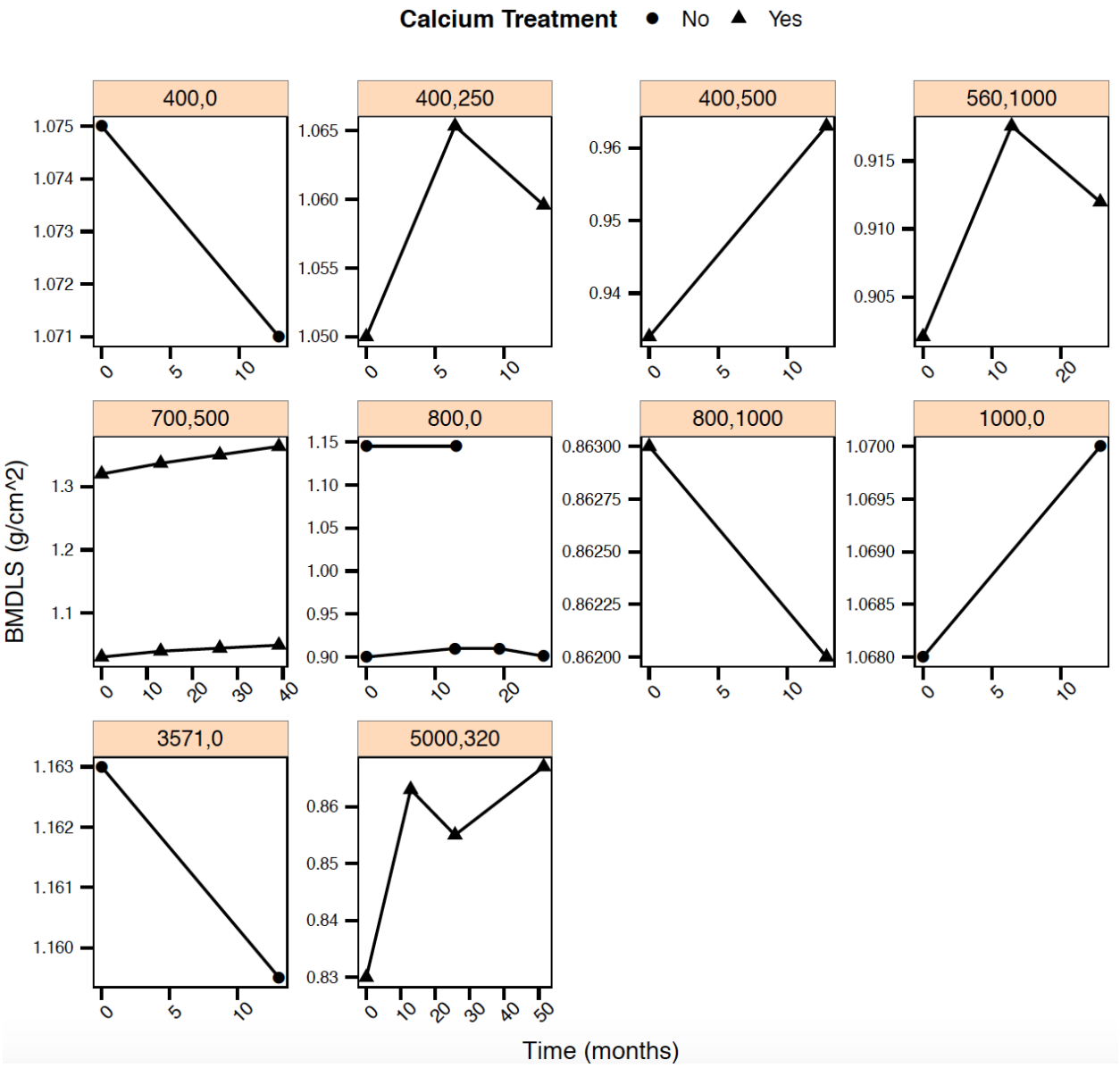


Figure D.3: Observed serum PTH data used to validate integrated Vitamin D and MSPM. Peach horizontal strip indicates Vitamin D3 and calcium dose, respectively.

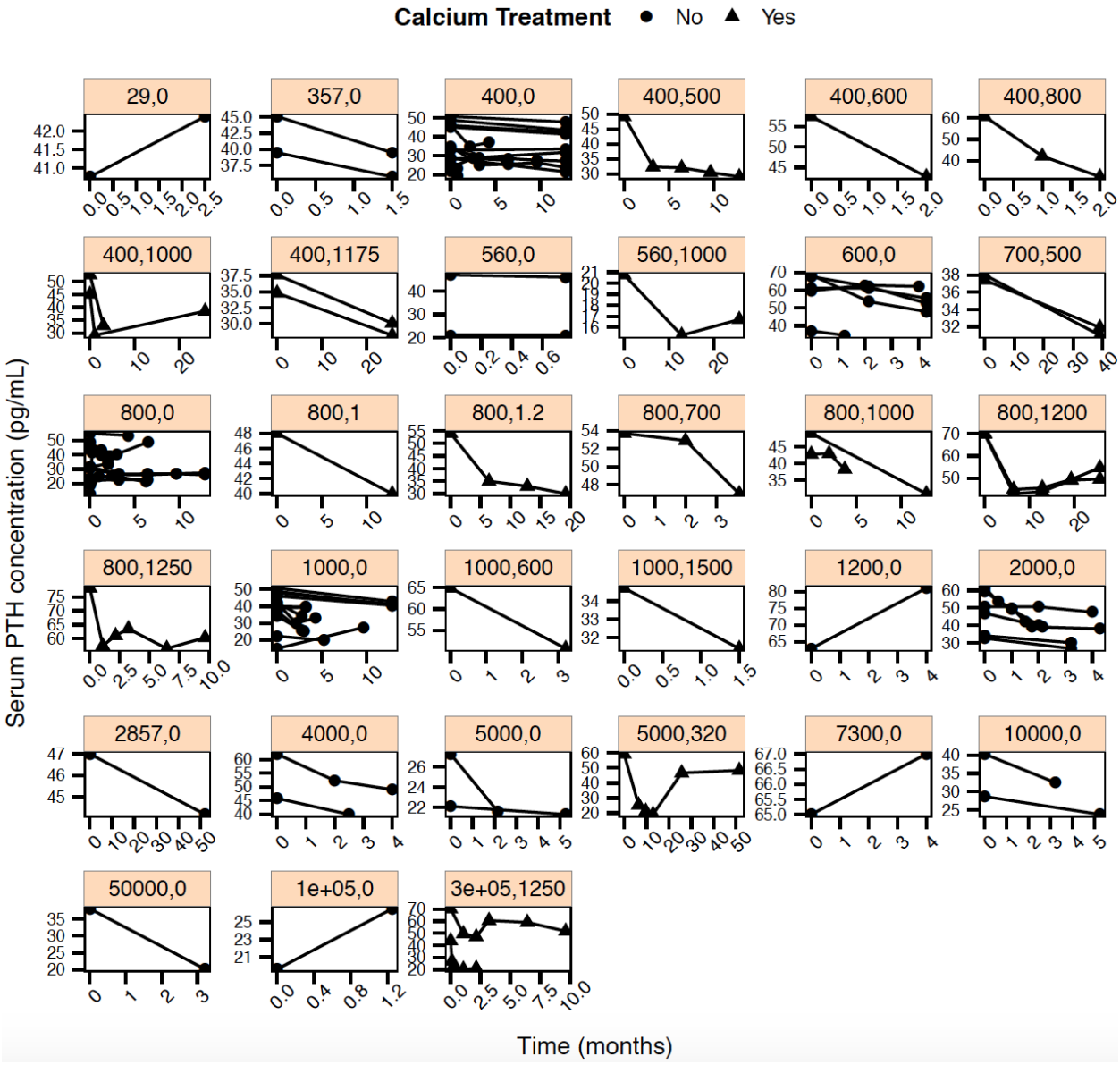


Figure D.4: Observed serum calcium data used to validate integrated Vitamin D and MSPM. Peach horizontal strip indicates Vitamin D3 and calcium dose, respectively.

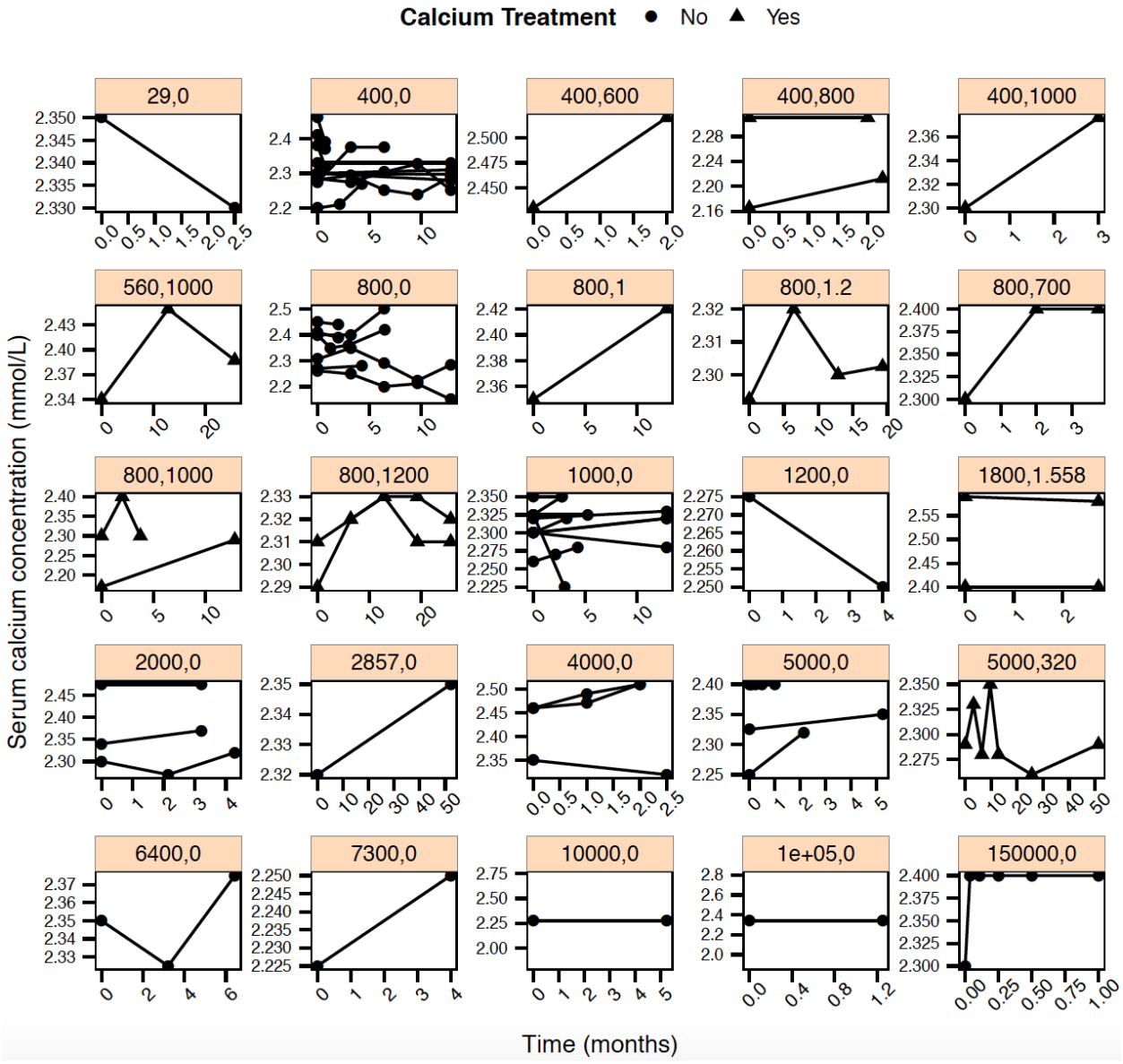


Figure D.5: Observed serum BSAP data used to validate integrated Vitamin D and MSPM. Peach horizontal strip indicates Vitamin D3 and calcium dose, respectively.

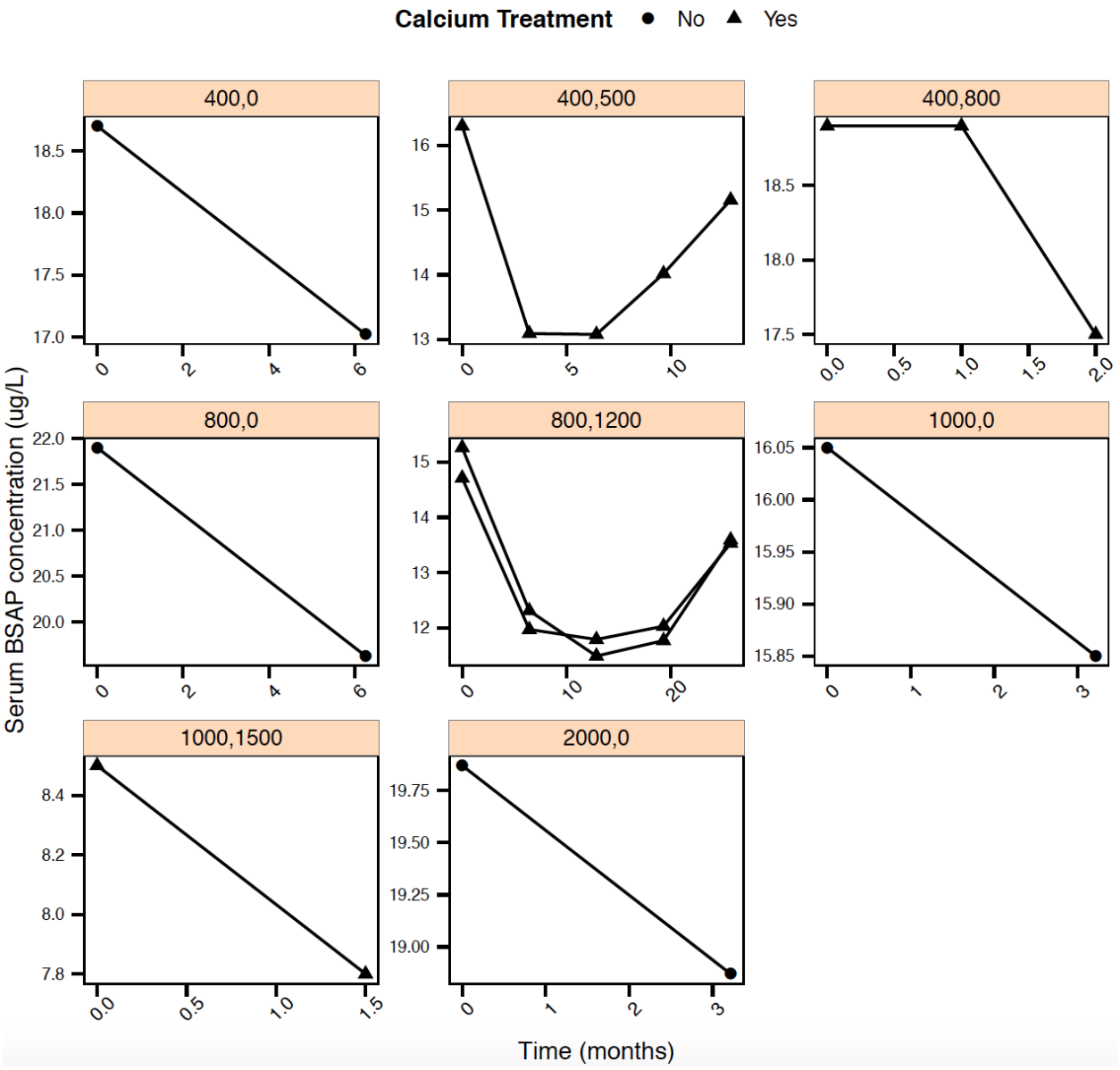


Figure D.6: Observed serum CTX data used to validate integrated Vitamin D and MSPM. Peach horizontal strip indicates Vitamin D3 and calcium dose, respectively.

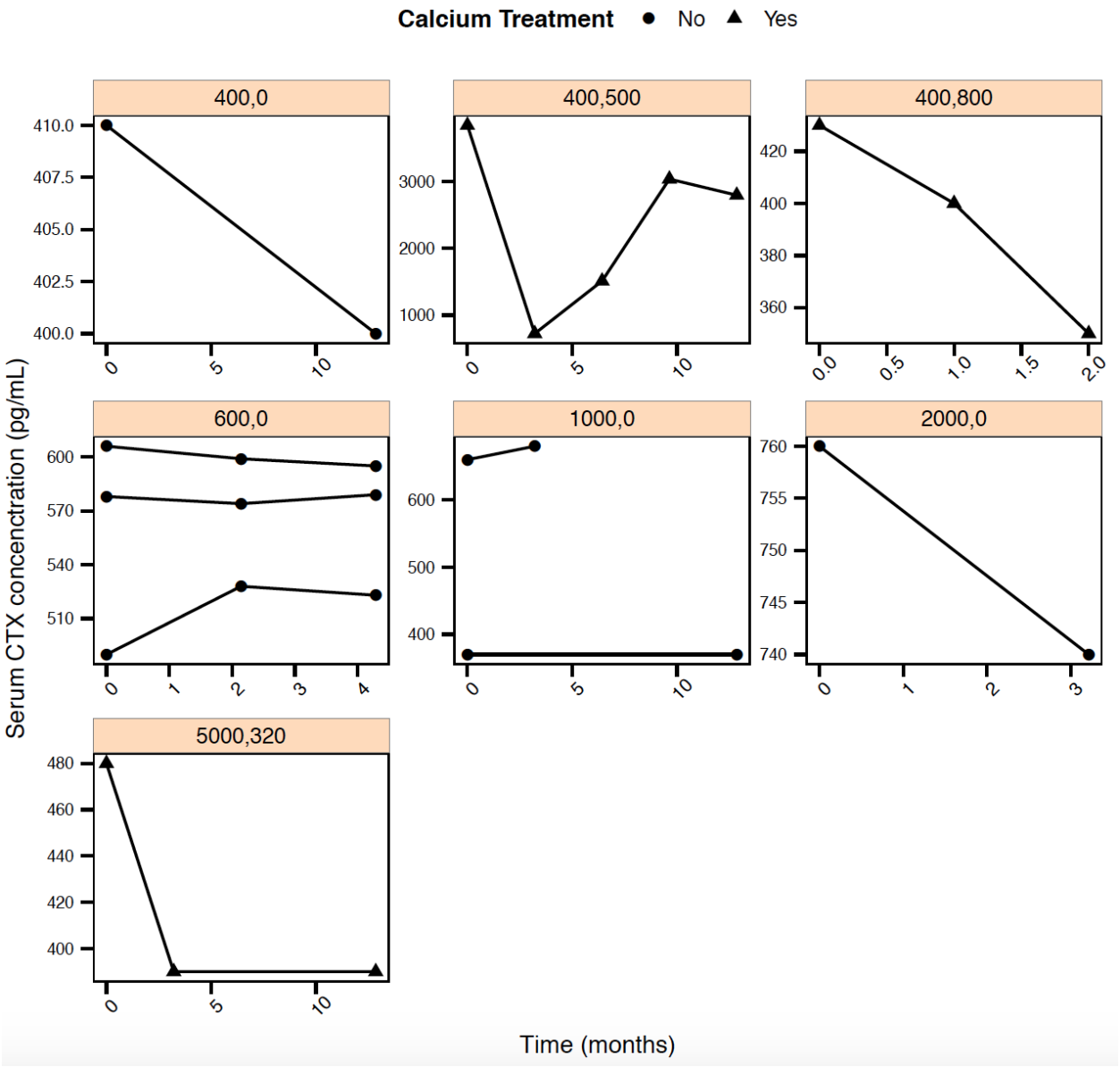


Figure D.7: Observed serum P1NP data used to validate integrated Vitamin D and MSPM. Peach horizontal strip indicates Vitamin D3 and calcium dose, respectively.

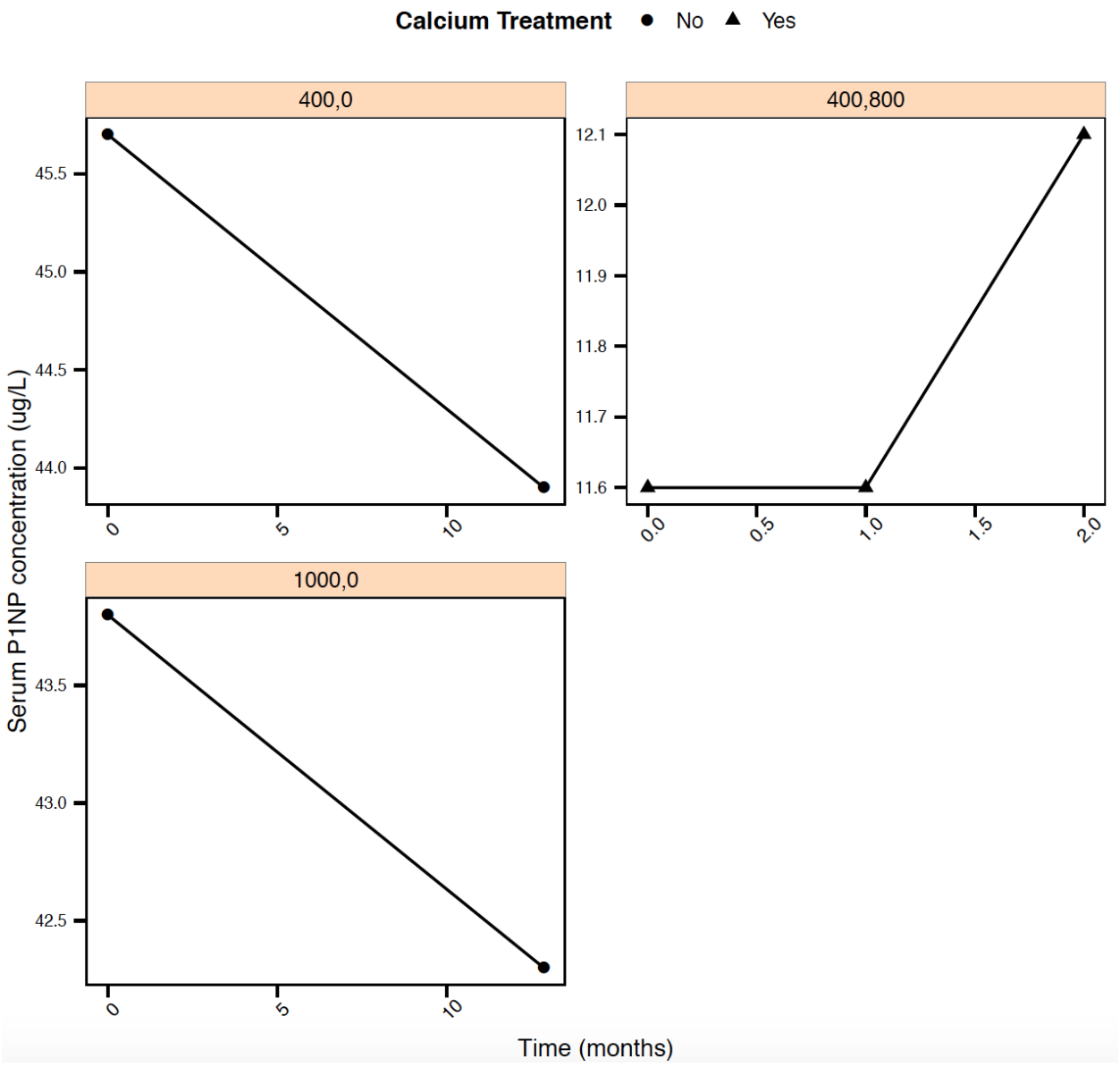


TABLE OF STUDIES USED IN MSPM META-ANALYSIS

Author	Year	Population*	Design*	Duration	Sampling Times	nTOT*	nARM Units*	TRT*	DV*	Original Dose	Regimen	Mean BMI (kg/m^2)	Mean Weight (kg)
Chapuy [1]	2002	healthy adults	R; DB; PC	24 months	0; 6; 12; 18; 24 mo	393	2	D3 + calcium	serum calcium; serum PTH; serum BSAP	800 IU/d D3 + 1200 mg/d calcium (both arms)	MD; PO	NA	58.7; 84.9
Chapuy [2]	1992	healthy ambulatory women	R; PC	18 months	0; 6; 12; 18 mo	1634	1	D3 + calcium	serum calcium; serum PTH	800 IU/d D3 + 1200 mg/d calcium	MD; PO	NA	56
Grados [3]	2003	elderly female patients	R; DB; PC	12 months	0; 3; 6; 9; 12 mo; 0; 12 mo	95	1	D3 + calcium	serum calcium; serum PTH; serum BSAP; serum CTX; BMD LS; BMD FN	400 IU/d D3 + 500 mg/d calcium	MD; PO	26.8	NA
Hunter [4]	2000	PM identical twins	R; DB; PC	24 months	0; 3; 6; mo; 0; 12; 18; 24 mo	79	1	D3	serum calcium; serum PTH; BMD LS	800 IU/d D3	MD; PO	24.1	62.4
Lips [5]	1988	aged people's home or nursing home	R; C	12 months	0; 3; 6; 9; 12 mo	112	4	D3	serum calcium; serum PTH; serum calcitriol	400 IU/d D3 (2 arms); 800 IU/d D3 (both arms)	MD; PO	NA	NA
Premaor [6]	2008	elderly people with SHPT living in low-income housing	R; DB; C	9 months; SD	0; 1; 2; 3; 6; 9 mo	28	2	D3 + calcium	serum PTH	800 IU/d D3 + 1250 mg/d calcium; 300000 IU/d D3 + 1250 mg/d calcium	MD; SD; PO	23.4; 24.4	28.6; 30.8
Romag-noli [7]	2008	elderly people in nursing homes	R; P	SD	0; 3; 7; 30; 60 d	8	1	D3 + calcium	serum PTH	300000 IU/d D3 + 1250 mg/d calcium	SD; PO	NA	NA
Roth [8]	2013	pregnant women from Dhaka; Bangladesh	R; DB; PC	2 months	0; 2 mo	80	1	D3	serum calcium; serum PTH	5000 IU/d D3	MD; PO	21.1	52.2
Vilja-kainen [9]	2009	white men from Finland	R; DB; PC	6 months	0; 10; 25 weeks	109	2	D3	serum PTH	400 IU/d D3; 800 IU/d D3	MD; PO	NA	70.4; 80.0
Van der Klis [10]	1996	PM black and white women from Curacao	R; PC	9 weeks; 5 weeks; 4 weeks	0; 1; 5; 9 weeks; 0; 5 weeks; 0; 4 weeks	85	3	D3	serum calcitriol; serum PTH	800 IU/d D3; 600 IU/d D3; 800 IU/d D3	MD; PO	NA	NA
Bhaga-twala [11]	2015	overweight African Americans with low vitamin D status	R; DB; PC	16 weeks	0; 8; 16 weeks	53	3	D3	serum calcitriol; serum PTH	600 IU/d D3; 2000 IU/d D3; 4000 IU/d D3	MD; PO	34.56; 37.08; 34.42	93.39; 99.16; 92.77
Hansen	2015	PM women with	R; DB; PC	1 year	0; 1 year	150	2	D3	BMD LS	800 IU/d D3;	MD; PO	31.2; 30.7	82; 80

[12]		Vitamin D deficiency								3571 IU/d D3			
Hartwell [13]	1987	healthy adults	R	8 weeks	0; 4; 8 weeks	9	1	D3	serum calcitriol; serum calcium	4000 IU/d D3	MD; PO	NA	NA
Brazier [14]	2005[15]	community-dwelling ambulatory women with vitamin D insufficiency	R; DB; PC	1 year	0; 12 mo	95	1	D3 + calcium	serum calcium; serum PTH	800 IU/d D3 + 1000 mg/d calcium	MD; PO	27	65.2
Graaf-mans [16]	1997	elderly women living in communities	R; PC	1 year	0; 12 mo	46	3	D3	serum calcitriol; serum PTH	400 IU/d D3 (all 3 arms)	MD; PO	28.2; 29.2; 27.3	NA
Dawson-Hughes [17]	1997	elderly women	R; DB; PC	3 years	0; 3 years; 0; 1; 2; 3; years	187	2	D3 + calcium	serum PTH; BMD LS	700 IU/d D3 + 500 mg/d calcium (both arms)	MD; PO	NA	82.4; 67.6
Honk-anen [18]	1990	elderly women living at home	R; C	11 weeks	0; 11 weeks	55	2	D3 + calcium	serum calcium	1800 IU/d D3 + 1558 mg/d calcium (both arms)	MD; PO	NA	70.7; 62.1
Har-wood [19]	2004	elderly women after hip fractures	R; C	12 months	0; 12 mo	39	1	D3 + calcium	serum calcium; serum PTH; BMD LS	800 IU/d D3 + 1000 mg/d calcium	MD; PO	NA	NA
Drincic [15]	2013	obsese adults	R; SB	21 weeks	0; 21 weeks	62	3	D3	serum calcium; serum PTH	1000 IU/d D3; 5000 IU/d D3; 10000 IU/d D3	MD; PO	36.7; 36.1; 37.9	105.8; 109.4; 106.5
Mac-Donald [20]	2013	PM women	R; DB; PC	1 year	0; 12 mo	174	2	D3	serum calcium; serum calcitriol; serum PTH; serum CTX; serum P1NP; BMD LS	400 IU/d D3; 1000 IU/d D3	MD; PO	25.3; 25.2	68.1; 69.4
Bonjour [21]	2013	institutionalized women	R; DB; C	8 weeks	0; 56 days; 0; 28; 56 days	59	2	D3 + calcium	serum calcium; serum PTH; serum BSAP; serum P1NP; serum CTX	400 IU/d D3 + 800 mg/d calcium (both arms)	MD; PO	26.2	63.2
Khaw [22]	1994	healthy adults	R; DB; C	SD	0; 5 weeks	95	1	D3	serum calcium; serum PTH	100000 IU/d D3	SD; PO	NA	70.6
Leh-mann [23]	2013	healthy adults	R; DB; PC	8 weeks	0; 4; 8 weeks	42	1	D3	serum PTH	2000 IU/d D3	MD; PO	24	NA
Glen-denning [24]	2009	hip fracture patients	R; DB	3 months	0; 3 mo	36	1	D3 + calcium	serum PTH	1000 IU/d D3 + 600 mg/d calcium	MD; PO	NA	NA

Larsen [25]	2004	community dwelling residents	F; CR; P	2 years	0; 1; 24 mo	67	1	D3 + calcium	serum PTH; serum calcitriol	400 IU/d D3 + 1000 mg/d calcium	MD; PO	NA	NA
Shapses [26]	2013	PM women	R; DB; PC	5 weeks	0; 6 weeks	39	2	D3	serum calcitriol; serum PTH	357 IU/d D3 (both arms)	MD; PO	30.1; 30.4	NA
Mocanu [27]	2009	nursing home residents	SA	1 year	0; 3; 6; 9; 12; 24; 48 mo	45	1	D3 + calcium	serum calcium serum PTH; serum CTX; BMD LS	5000 IU/d D3 + 320 mg/d calcium	MD; PO	NA	74.8
Chel [28]	2008	nursing home residents	R; PC	4 months	0; 2; 4; mo	166	3	D3	serum PTH; serum CTX	600 IU/d D3 (all 3 arms)	MD; PO	NA	NA
Himmel-stein [29]	1990	subjects from long-term care facility	R; DB; PC	6 weeks	0; 2; 4; 6; 7 weeks	15	1	D3	serum calcitriol; serum PTH	2000 IU/d D3	MD; PO	NA	NA
Wood [30]	2013	caucasian postmenopausal women	R; DB; PC	1 year	0; 12 mo	192	6	D3	serum calcitriol; serum PTH	4000 IU/d D3 (3 arms); 1000 IU/d D3 (3 arms)	MD; PO	NA	NA
Tjellesen [31]	1986	premenopausal women	R; DB	8 weeks	0; 4; 8 weeks	10	1	D3	serum calcitriol; serum calcium	4000 IU/d D3	MD; PO	NA	NA
Chel [32]	1998	elderly nursing home patients	R; C	12 weeks	0; 12 weeks	15	1	D3 + calcium	serum calcium; serum PTH; serum calcitriol	400 IU/d D3 + 1000 mg/d calcium	MD; PO	NA	NA
Smith [33]	2009	healthy volunteers in Antarctica	PR; R; DB; C	5 months	0; 2; 4 mo	55	3	D3	serum calcitriol; serum PTH; serum calcium; serum BSAP	400 IU/d D3; 1000 IU/d D3; 2000 IU/d D3	MD; PO	29; 31; 28	NA
Talwar [34]	2007	healthy black PM women	R; PC	3 years	0; 3; 24; 27 mo	104	1	D3 + calcium	serum calcium; serum calcitriol; serum PTH	800 IU/d D3 for 2 years then 2000 IU/d for 1 year + 1300 mg/d calcium	MD; PO	29	78
Harris [35]	2002	healthy men	R; C	8 weeks	0; 8 weeks	35	2	D3	serum calcium; serum PTH	800 IU/d D3 (both arms)	MD; PO	25; 29	NA
Natri [36]	2005	healthy women	R; SB; PC	3 weeks	0; 3 weeks	32	3	D3	serum calcium; serum PTH	400 IU/ D3 (all 3 arms)	MD; PO	22.3; 23.6; 22.1	62.3; 61.7; 61.2
Tang-pricha [37]	2003	healthy adults	R; DB; C	12 weeks	0; 12 weeks	14	1	D3	serum calcium; serum PTH	1000 IU/d D3	MD; PO	NA	NA
Wagner [38]	2006	lactating women	R; DB; PC	6 months	0; 3; 6 mo	19	2	D3	serum calcium	400 IU/d D3; 6400 IU/d D3	MD; PO	NA	NA
Nimit-phong [39]	2013	healthy adults	R; C	3 months	0; 24 mo	20	1	D3 + calcium	serum PTH	400 IU/d D3 + 1175 mg/d calcium	MD; PO	22.4	56.9

Bischoff-Ferrari [40]	2012	PM women	R; DB; C	4 months	0; 4 mo	10	1	D3	serum calcium; serum PTH; serum calcitriol	800 IU/d D3	MD; PO	25.49	NA
Barger-Lux [41]	1998	healthy men	R; C	3 months	0; 3 mo	38	3	D3	serum calcitriol; serum PTH	1000 IU/d D3; 10000 IU/d D3; 50000 IU/d D3	MD; PO	NA	NA
Bian-cuzzo [42]	2008	healthy adults	R; DB; PC	11 weeks	0; 11 weeks	37	2	D3	serum PTH; serum calcitriol	1000 IU/d D3 (both arms)	MD; PO	29.9; 29.1	NA
Al-khalidi [43]	2015	healthy adults	R; DB; C	10 weeks	0; 10 weeks	96	2	D3	serum calcium; serum PTH	29 IU/d D3; 4000 IU/d D3	MD; PO	25.6; 25.8	NA
Asemi [44]	2015	pregnant women at risk for pre-eclampsia	R; DB; C	9 weeks	0; 9 weeks	23	1	D3 + calcium	serum calcium	400 IU/d D3 + 800 mg/d calcium	MD; PO	27	NA
Can-gussu [45]	2015	Brazilian PM women	R; DB; PC	9 months	0; 9 mo	80	1	D3	serum PTH	1000 IU/d D3	MD; PO	29.2	NA
Cash-man [46]	2015	elderly adults	R; DB; PC	15 weeks	0; 15 weeks; 0; 8; 15 weeks	128	2	D3 + calcium	serum calcium; serum calcitriol; serum PTH	800 IU/d D3 + 700 mg/d calcium; 800 IU/d D3 + 1000 mg/d calcium	MD; PO	NA	NA
Didrik-sen [47]	2015	diabetes patients	R; PC	4 years	0; 4 years	18	1	D3	serum calcium; serum PTH	2857 IU/d D3	MD; PO	30.7	NA
Gasier [48]	2015	submariners with limited sunlight	R; PC	3 months	0; 3 mo	37	2	D3	serum calcium; serum PTH; serum CTX; serum BSAP	1000 IU/d D3; 2000 IU/d D3	MD; PO	27.4; 30.0	85.2; 95.5
Mehrota [49]	2014	pre-diabetic; D deficient	R; PC	4 months	0; 16 weeks	16	2	D3	serum PTH; serum calcium	1200 IU/d D3; 7300 IU/d D3	MD; PO	NA	NA
Nader [50]	2014	obese adolescents	R; DB; PC	3 months	0; 3 mo	20	1	D3	serum PTH; serum calcium	2000 IU/d D3	MD; PO	35.3	NA
Schild [51]	2015	PM women	SA	9 months	0; 3; 6; 9 mo	24	1	D3	serum BSAP	400 IU/d D3 for 3 mo then 1000 IU/d for 3 mo then 2000 IU/d for 3 mo	MD; PO	NA	NA
Schleck [52]	2015	vitamin D deficient adults	R; DB	SD; 12 weeks	0; 12 weeks	150	3	D3	serum calcium	10000 IU/d D3 SD then 1667 IU/d D3 for 12 weeks; 20000 IU/d D3 SD then 3333 IU/d D3	MD; SD; PO	22.8; 23.9; 23.1	NA

										for 12 weeks; 50000 IU/d D3 SD then 833 IU/d D3 for 12 weeks			
Wijnen [53]	2015	nursing home patients with 25OHD levels < 50 nmol/L	R; OL	12 weeks	0; 5; 12; 26 weeks	14	1	D3	serum PTH; serum calcium	800 IU/d D3	MD; PO	NA	NA
Meekins [54]	2014	healthy women	R	28 days	0; 1; 3; 7; 14; 28 days	39	2	D3	serum calcium	5000 IU/d D3; 150000 IU/d D3	MD; PO	23.4; 22.8	64.1; 63.4
Pfeifer [55]	2001	elderly women	R; DB; C	8 weeks	0; 8 weeks	148	2	D3 + calcium	serum calcium; serum calcitriol; serum PTH	400 IU/d D3 + 600 mg/d calcium (both arms)	MD; PO	NA	65.2
Prest-wood [56]	1996	elderly women	SA	6 weeks	0; 6 weeks	12	1	D3 + calcium	serum PTH; serum BSAP	1000 IU/d D3 + 1500 mg/d calcium	MD; PO	NA	NA
Ramly [57]	2014	urban premenopausal women in tropical country	R; DB; PC	1 year	0; 6; 12 mo	93	1	D3	serum PTH; serum calcium	7143 IU/d D3 for 6 mo then 1667 IU/d D3 for 6 mo	MD; PO	27.23	NA
Dawson-Hughes [58]	1991	PM women	R; DB; PC	1 year	0; 6; 12 mo	249	1	D3 + calcium	BMD LS	400 IU/d D3 + 250 mg/d calcium	MD; PO	NA	68.5
Baeks-gaard [59]	1998	healthy women	R; DB; PC	2 years	0; 12; 24 mo	80	1	D3 + calcium	serum calcium; serum PTH; BMD LS	560 IU/d D3 + 1000 mg/d calcium	MD; PO	NA	NA
Aloia [60]	2005	black PM women	R; DB; PC	3 years	0; 6; 12; 18; 24; 30; 36 mo; 0; 24; 36 mo	208	2	D3 + calcium	serum calcium; BMD LS	800 IIU/d D3 + 1350 mg/d calcium for 2 years then 2000 IU/d D3 + 1350 mg/d calcium for 1 year (both arms)	MD; PO	29	78

*Design: R = randomized, PC = placebo-controlled, DB = double blind, SB = single-blinded, C = control, OL = open label, F = factorial, CR = cluster randomized, P = pragmatic ; PR= prospective; SA = single arm;
Population: PM = postmenopausal; Column Names: nTOT = total number of subjects from study included in the analysis; nARM Units =number of treatment arms from study included in the analysis; TRT = treatment(s) administered during the study; DV = study endpoints used in the analysis; Regimen = PO = oral, MD= multiple dose, SD = single dose

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Figure D.8: Observed relationship between calcitriol baseline and response following Vitamin D3 supplementation

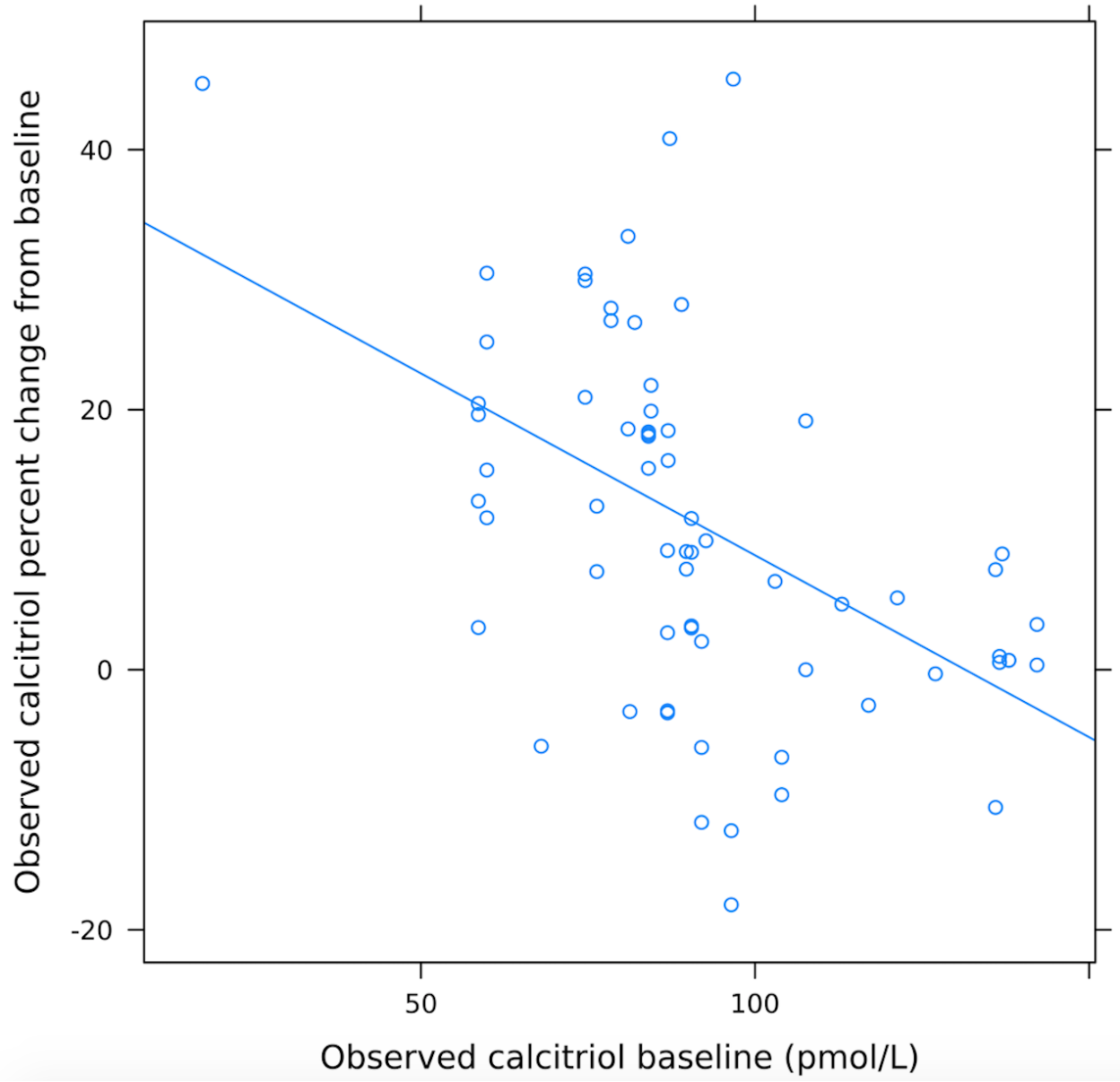

Figure D.9: Final model predictions into observed data not included in the optimization process for serum PTH following Vitamin D3 with or without calcium supplementation by ID. Peach horizontal strip indicates Vitamin D3 dose, calcium dose, and arm ID, respectively. Blue band is prediction region where θ_1 = point estimate +/- 50%*point estimate

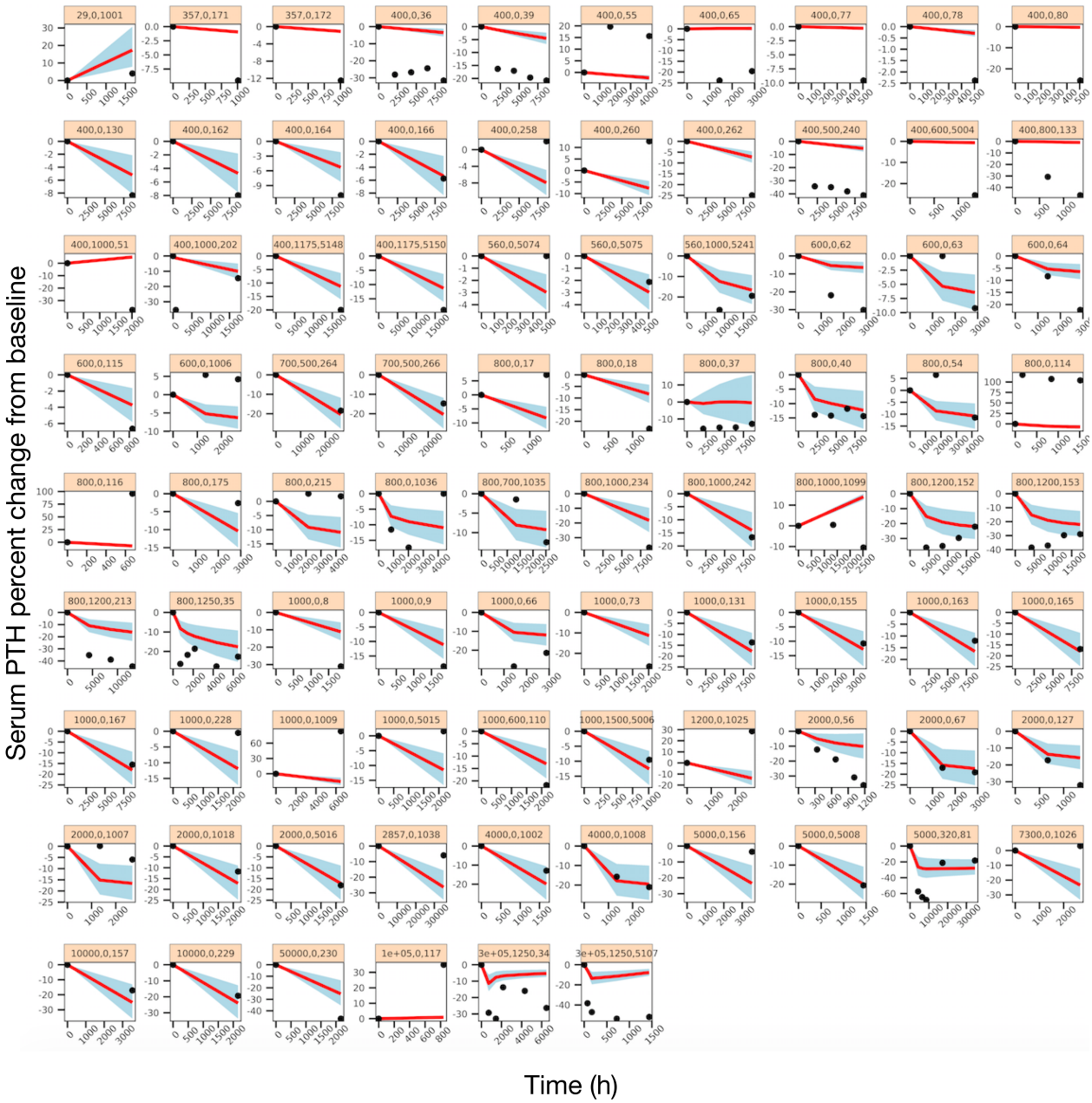

Figure D.10: Final model predictions into observed data not included in the optimization process for serum calcium following Vitamin D3 with or without calcium supplementation by ID. Peach horizontal strip indicates Vitamin D3 dose, calcium dose, and arm ID, respectively. Blue band is prediction region where θ_1 = point estimate +/- 50%*point estimate

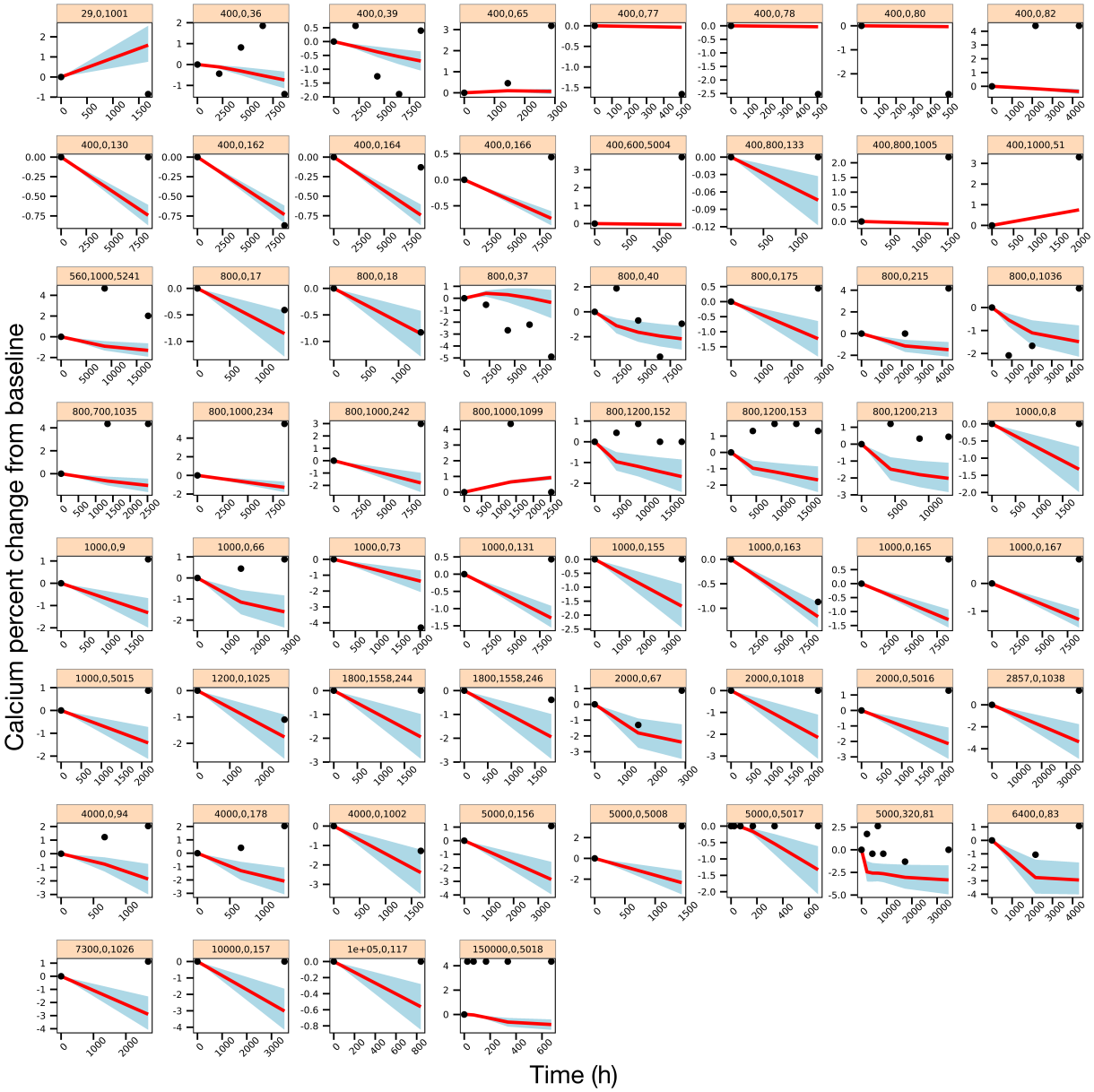

Figure D.11: Final model predictions into observed data not included in the optimization process for serum CTX following Vitamin D3 with or without calcium supplementation by ID. Peach horizontal strip indicates Vitamin D3 dose, calcium dose, and arm ID, respectively. Blue band is prediction region where θ_1 = point estimate +/- 50%*point estimate

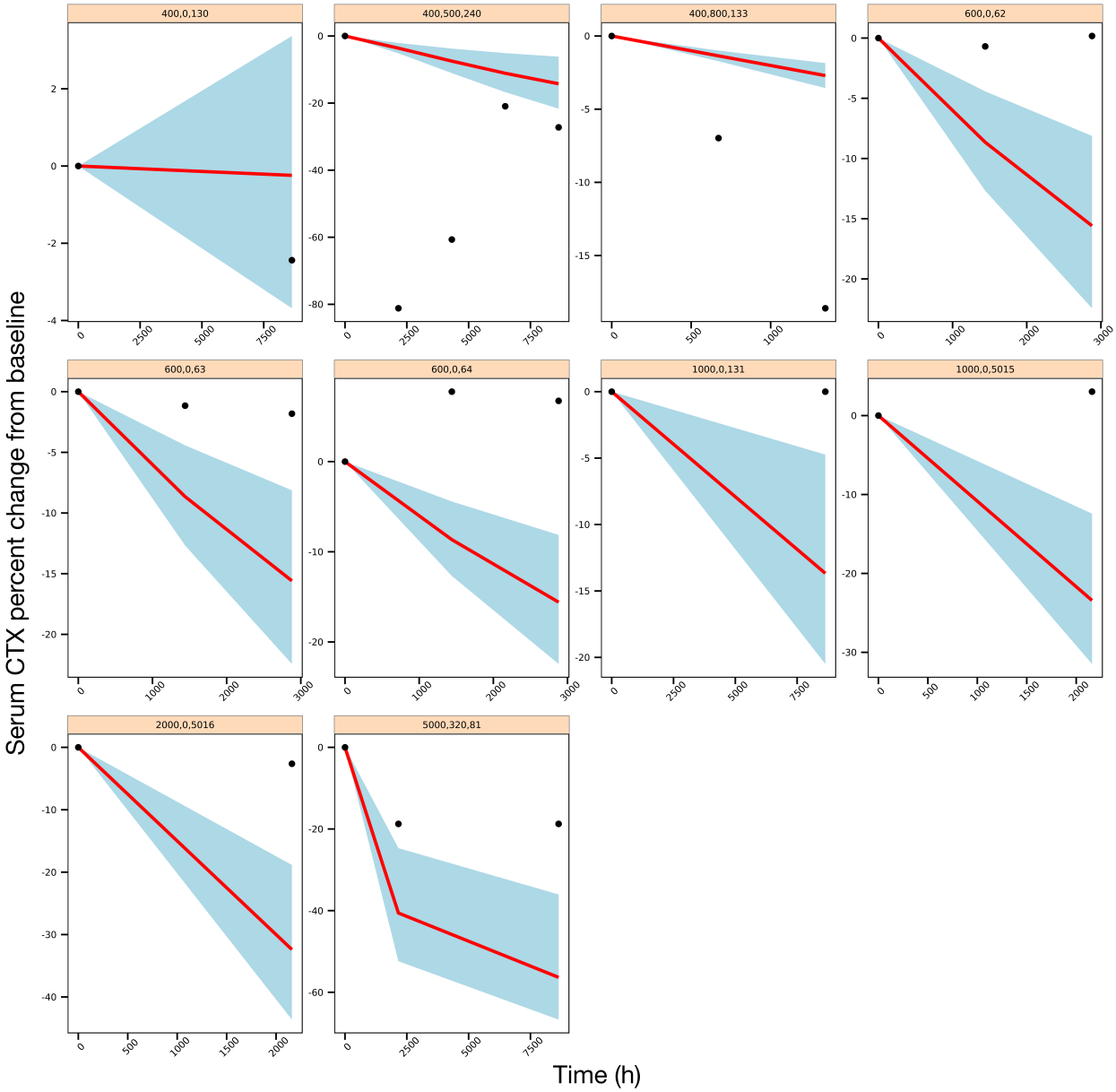

Figure D.12: Final model predictions into observed data not included in the optimization process for serum BSAP following Vitamin D3 with or without calcium supplementation by ID. Peach horizontal strip indicates Vitamin D3 dose, calcium dose, and arm ID, respectively. Blue band is prediction region where θ_1 = point estimate +/- 50%*point estimate

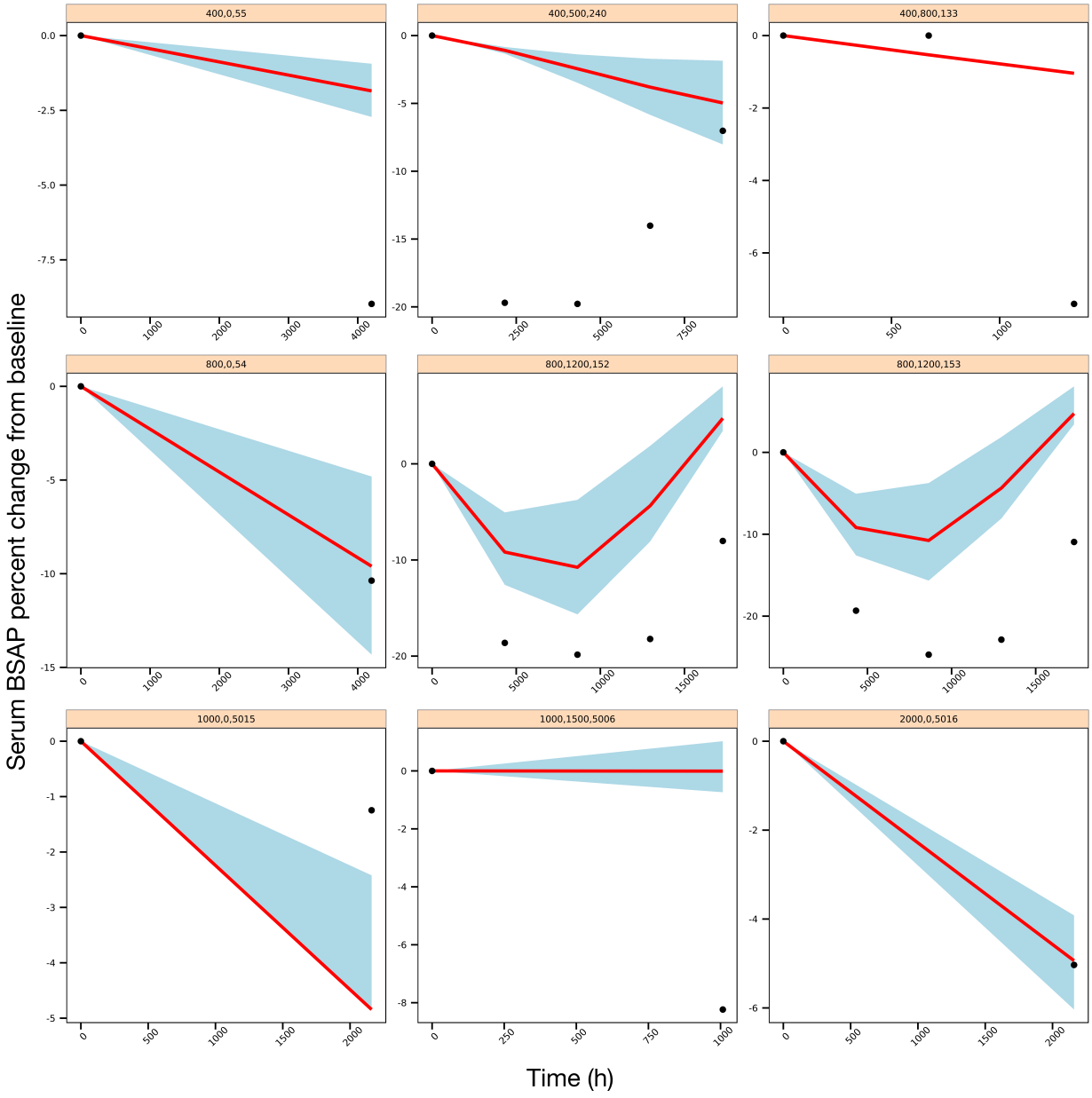

Figure D.13: Simulated serum calcitriol, serum PTH, and BMDLS time profiles, following 1 year of different Vitamin D3 and calcium regimens

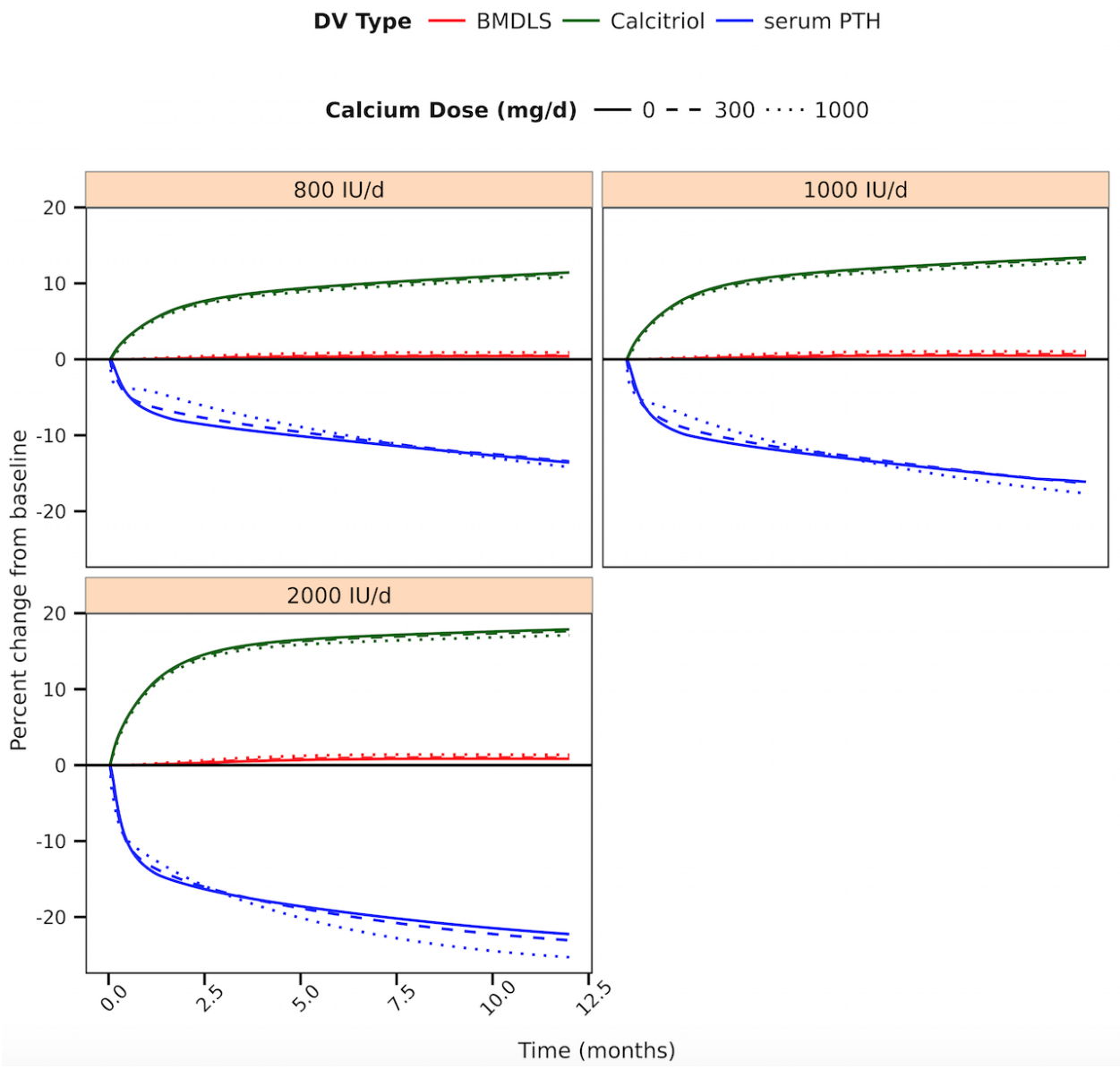

Table D.1: Vitamin D3 doses and 25OHD3 levels (assay = RIA) necessary for reaching BMDLS percent increase targets at 1 year (calcium dose = 1000 mg/d); 25OHD3 BL = 30 nmol/L; PTH BL = 50-60 pg/mL

Target BMDLS % Increase	D3 Dose (IU/d)	D3CA Dose (IU/d)	25OHD3 after D3 (nmol/L)	25OHD3 after D3CA (nmol/L)	PTH after D3 (pg/mL)	PTH after D3CA (pg/mL)
0.5	300	300	50	50	46	42
1.0	400	400	65	65.2	38	35
1.5	1000	700	84	77	32	30
2.0	3100	2000	100	90	28	26
2.5	>5000	5000	>106	105	<27	23

Table D.2: Vitamin D3 doses and 25OHD3 levels (assay = CPBA) necessary for reaching BMDLS percent increase targets at 1 year (calcium dose = 1000 mg/d); 25OHD3 BL = 30 nmol/L; PTH BL = 50-60 pg/mL

Target BMDLS% Increase	D3 Dose (IU/d)	D3CA Dose (IU/d)	25OHD3 after D3 (nmol/L)	25OHD3 after D3CA (nmol/L)	PTH after D3 (pg/mL)	PTH after D3CA (pg/mL)
0.5	300	300	54	54	46	42
1.0	400	400	71	71	38	35
1.5	1000	700	99	88	32	30
2.0	3100	2000	123	110	28	26
2.5	>5000	5000	>130	131	<27	23

Table D.3: Vitamin D3 doses and 25OHD3 levels (assay = CHEMI) necessary for reaching BMDLS percent increase targets at 1 year (calcium dose = 1000 mg/d); 25OHD3 BL = 30 nmol/L; PTH BL = 50-60 pg/mL

Target BMDLS % Increase	D3 Dose (IU/d)	D3CA Dose (IU/d)	25OHD3 after D3 (nmol/L)	25OHD3 after D3CA (nmol/L)	PTH after D3 (pg/mL)	PTH after D3CA (pg/mL)
0.5	300	300	60	59	46	42
1.0	400	400	75	75	38	35
1.5	1000	700	100	90	32	30
2.0	3100	2000	122	109	28	26
2.5	>5000	5000	>131	129	<27	23

Table D.4: Vitamin D3 doses and 25OHD3 levels (assay = RIA) necessary for reaching serum PTH targets at 1 year (calcium dose = 1000 mg/d); 25OHD3 BL = 30 nmol/L; PTH BL = 50-60 pg/mL

Target PTH (pg/mL)	D3 Dose (IU/d)	D3CA Dose (IU/d)	25OHD3 after D3 (nmol/L)	25OHD3 after D3CA (nmol/L)	BMDLS % after D3	BMDLS % after D3CA
>= 50	200	200	<46	<46	0	0
40	300-400	300	60	54	0.7	0.6
30	1500	700	87	73	1.7	1.6
25	>5000	2000	>106	91	>2.1	2

Table D.5: Vitamin D3 and calcium doses and 25OHD3 levels (assay = CPBA) necessary for reaching serum PTH targets at 1 year (calcium dose = 1000 mg/d); 25OHD3 BL = 30 nmol/L; PTH BL = 50-60 pg/mL

Target PTH (pg/mL)	D3 Dose (IU/d)	D3CA Dose (IU/d)	25OHD3 after D3 (nmol/L)	25OHD3 after D3CA (nmol/L)	BMDLS % after D3	BMDLS % after D3CA
>= 50	200	200	<43	<43	0	0
40	300-400	300	63	54	0.7	0.6
30	1500	700	104	83	1.7	1.6
25	>5000	2000	>132	110	>2.1	2

Table D.6: Vitamin D3 and calcium doses and 25OHD3 levels (assay = CHEMI) necessary for reaching serum PTH targets at 1 year (calcium dose = 1000 mg/d); 25OHD3 BL = 30 nmol/L; PTH BL = 50-60 pg/mL

Target PTH (pg/mL)	D3 Dose (IU/d)	D3CA Dose (IU/d)	25OHD3 after D3 (nmol/L)	25OHD3 after D3CA (nmol/L)	BMDLS % after D3	BMDLS % after D3CA
>= 50	200	200	<49	<50	0	0
40	300-400	300	68	59	0.7	0.6
30	1500	700	105	86	1.7	1.6
25	>5000	2000	>130	110	>2.1	2