

# Physiologically Based Pharmacokinetic Modeling of Vitamin D<sub>3</sub> and Metabolites in Vitamin D–Insufficient Patients<sup>S</sup>

Colton W. Sawyer, Stacey M. Tuey, Raymond E. West III, Thomas D. Nolin, and  
Melanie S. Joy

*Department of Mathematics, Southern New Hampshire University, Manchester, New Hampshire (C.W.S.); Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado, Aurora, Colorado (S.M.T., M.S.J.); and University of Pittsburgh, School of Pharmacy, Department of Pharmacy and Therapeutics, Pittsburgh Pennsylvania (R.E.W., T.D.N.)*

Received August 12, 2021; accepted June 13, 2022

## ABSTRACT

A physiologically based pharmacokinetic (PBPK) model of vitamin D<sub>3</sub> and metabolites [25(OH)D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub>, and 24,25(OH)<sub>2</sub>D<sub>3</sub>] is presented. In this study, patients with 25(OH)D<sub>3</sub> plasma concentrations below 30 ng/ml were studied after a single dose of 5000 I.U. (125 μg) cholecalciferol, provided with 5000 I.U. daily cholecalciferol supplementation until vitamin D replete [25(OH)D<sub>3</sub> plasma concentrations above 30 ng/ml], and had serial plasma samples were collected at each phase for 14 days. Total concentrations of vitamin D<sub>3</sub> and metabolites were measured by ultra-high performance liquid chromatography tandem mass spectrometry. A nine-compartment PBPK model was built using MATLAB to represent the triphasic study nature (insufficient, replenishing, and sufficient). The stimulatory and inhibitory effect of 1,25(OH)<sub>2</sub>D<sub>3</sub> were incorporated by fold-changes in the primary metabolic enzymes CYP27B1 and CYP24A1, respectively. Incorporation of

dynamic adipose partition coefficients for vitamin D<sub>3</sub> and 25(OH)D<sub>3</sub> and variable enzymatic reactions aided in model fitting. Measures of model predictions agreed well with data from metabolites, with 97%, 88%, and 98% of the data for 25(OH)D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub>, and 1,25(OH)<sub>2</sub>D<sub>3</sub>, respectively, within twofold of unity (fold error values between 0.5 and 2.0). Bootstrapping was performed and optimized parameters were reported with 95% confidence intervals. This PBPK model could be a useful tool for understanding the connections between vitamin D and its metabolites under a variety of clinical situations.

## SIGNIFICANCE STATEMENT

This study developed a physiologically based pharmacokinetic (PBPK) model of vitamin D<sub>3</sub> and metabolites for patients moving from an insufficient to a repleted state over a period of 16 weeks.

## Introduction

Vitamin D is a fat-soluble, prohormone that plays an essential role in regulating calcium and phosphorus to maintain musculoskeletal health. The main source of vitamin D is through endogenous production of cholecalciferol (VitD<sub>3</sub>) in the skin upon UV B exposure from the sun. The active form of VitD<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub> (calcitriol, 1,25D<sub>3</sub>) has been found to exhibit many pleiotropic actions beyond calcium and phosphorus homeostasis (e.g., musculoskeletal health), that are often referred to as “nonclassical” actions. Additionally, 1,25D<sub>3</sub> controls over 200 genes, including those responsible for the regulation of hormone secretion, immune function, and cell proliferation and differentiation (Holick, 2006).

VitD insufficiency is a global health issue, and it has been estimated that up to 80% of men and women in the United States, Canada, and Europe meet this classification (Ganji et al., 2012; van Schoor and Lips, 2018). VitD status is based on concentrations of 25-hydroxyvitamin D, 25(OH)D (calcifediol, 25D), given that it is the major circulating form and it is readily assayed in hospital laboratories. There is a lack of consensus on the serum concentrations of 25D considered to be adequate,

but it is commonly agreed that 25D concentrations should be above 20 ng/ml (1 ng/ml = 2.5 nmol/l) (Ganji et al., 2012, van Schoor and Lips, 2018). The Institute of Medicine (IOM) defines deficiency as 25D < 12 ng/ml and recommends a target serum concentration of 20 ng/ml (Ross, 2011). The Endocrine Society (ENDO) defines VitD deficiency as 25D < 20 ng/ml and recommends a target concentration of 30 ng/ml (Holick et al., 2011). However, other leading experts define VitD insufficiency as 25D concentrations between 20 and 30 ng/ml and deficiency as concentrations <20 ng/ml (Dawson-Hughes et al., 2005; Holick, 2007; Holick et al., 2011; Cianferotti and Marcocci, 2012). Although many people with VitD insufficiency take supplements, treatment recommendations are not consistent. The IOM recommended a daily intake of 600 I.U. VitD for children, adolescents, and adults, and 800 I.U. for adults over the age of 70 to maintain 25D concentrations of 20 ng/ml (Jernigan and Andress, 2003). ENDO recommended 1500–2000 I.U./d to prevent or treat VitD insufficiency and achieve 25D concentrations above 30 ng/ml, with a preferred range of 40–60 ng/ml (Holick et al., 2011). Whereas these are the common guidelines in the United States, recommendations in other regions of the world differ based on many population-specific factors, such as sunlight exposure, skin pigmentation, clothing, and dietary practices (Pérez-López et al., 2012; Society, 2012; Pludowski et al., 2013; Rizzoli et al., 2013; Munns et al., 2016; Haq et al., 2018).

Activation of VitD occurs through two sequential hydroxylation steps to generate the metabolically active metabolite 1,25D. Vitamin D is hydroxylated in the liver primarily by the cytochrome P450 (P450) enzyme CYP2R1 but also through pathways with CYP27A1 (Sakaki et al.,

This research was supported by the National Institutes of Health National Institute of General Medical Sciences [Grant R01-GM107122] (to M.S.J., T.D.N.).

No author has an actual or perceived conflict of interest with the contents of this article.

**Primary laboratory of origin:** M.S.J. and T.D.N.

dx.doi.org/10.1124/dmd.121.000609.

<sup>S</sup> This article has supplemental material available at [dmd.aspetjournals.org](http://dmd.aspetjournals.org).

**ABBREVIATIONS:** 25D<sub>3</sub>, 25(OH)D<sub>3</sub>; 1,25D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub>; 24,25D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub>; P450, cytochrome P450; VitD<sub>3</sub>, vitamin D<sub>3</sub>.

2005) and CYP3A4 (Battault et al., 2013; Wang et al., 2013), to form 25D, the major circulating metabolite. 25D can then be hydroxylated in the kidney by either CYP27B1 to the active metabolite, 1,25D, or by CYP24A1 to the inactive metabolite 24,25-dihydroxyvitamin D (24,25D). Phase 2 metabolism pathways have also been described for VitD but appear to be of lesser importance (Wang et al., 2014; Gao et al., 2017; Wong et al., 2018). 1,25D is tightly regulated in a feedback loop with CYP27B1 and CYP24A1; high concentrations of 1,25D will respectively suppress and stimulate expressions of CYP27B1 and CYP24A1.

Despite VitD being used clinically for decades, there are a limited number of published studies that include assessments of the parent VitD compound and metabolites using pharmacokinetic or physiologically based pharmacokinetic (PBPK) approaches (Kimura et al., 1991; Levine and Song, 1996; Bailie and Johnson, 2002; Armas et al., 2004; Ilahi et al., 2008; Roth et al., 2012; Benaboud et al., 2013; Jetter et al., 2014; Meekins et al., 2014; Ocampo-Pelland et al., 2016, 2017; Ramakrishnan et al., 2016; Fassio et al., 2020; Hsu et al., 2021). The current study sought to develop a PBPK model of VitD<sub>3</sub> and metabolites in VitD insufficient patients in the United States who were treated with moderate (5000 I.U.) daily doses of VitD<sub>3</sub> for up to 16 weeks to achieve replacement as defined by 25D  $\geq$  30 ng/ml. Through establishing a model that might align well with a triphasic clinical experience of a patient—an initial visit for establishing VitD concentrations, time to achieve repletion on a dosing regimen, and a follow up visit—this study aimed to better understand the longitudinal effects of daily supplementation of moderate doses of VitD<sub>3</sub>.

## Materials and Methods

### Design Overview

The parent study from which the data were obtained was conducted in accordance with the Declaration of Helsinki. All subjects provided informed consent and the protocols were approved by the Institutional Review Boards at the University of Colorado and the University of Pittsburgh. The parent study (NCT02360644) was registered under ClinicalTrials.gov and can be referenced for additional details.

**Study Participants.** Healthy subjects from the University of Colorado and University of Pittsburgh with VitD insufficiency defined in the study as total 25D below 30 ng/ml and not receiving VitD replacement therapy were recruited. Other eligibility criteria included age 18 to 75 years, predicted compliance with study visits, not pregnant or lactating, no changes in medications within 4 weeks, no predisposition to hypercalcemia, and hemoglobin  $\geq$  10 g/dl. Exclusion criteria were active autoimmune disease, active or recent infections requiring antimicrobial treatment, and hepatic insufficiency. Note that while the parent clinical study focused on patients with chronic kidney disease, this study used data from a control group of healthy study participants from the parent clinical study. Baseline characteristics for study participants ( $n = 11$ ) can be found in Table 1.

**Clinical Study Design.** Vitamin D insufficient subjects were admitted to the Clinical and Translational Research Centers (CTRC) at the University of Colorado or University of Pittsburgh for a 12 hour stay, followed by visits at 24, 48, 168, and 336 hours. Participants were required to be fasting at the beginning of the study. Any prescribed medications were withheld for the first two hours of the study. Subjects were given a single 5000 I.U. (125  $\mu$ g) oral dose of VitD<sub>3</sub> (Jarrow Formulas, Los Angeles, CA). Serial blood samples (7.5 ml) were collected at baseline and at 0.5, 1, 2, 4, 8, 12, 24, 48, 168, and 336 hours into heparinized vacutainers. Blood samples were centrifuged immediately following collection for 10 minutes at 3000  $\times$ G at 4°C and plasma samples were stored at  $-80$  C until analysis. After the blood draw at 336 hours, participants were given up to 16 weeks of daily supplementation of 5000 I.U. VitD<sub>3</sub> to attain replete concentrations ( $\geq$ 30 ng/ml). At the repleted study phase, participants were given a final dose of 5000 I.U. VitD<sub>3</sub> followed by collection of blood samples as previously described for up to 336 hours. The total study reflected three phases—the insufficient phase, the replenishing phase, and the repleted phase. Data are available for subjects in the insufficient and repleted phases.

**Analytical Assay.** Total concentrations of VitD<sub>3</sub> and metabolites [25D<sub>3</sub>, 1,25D<sub>3</sub>, and 24,25D<sub>3</sub>] were determined by a novel ultra-high performance liquid

TABLE 1  
Baseline characteristics of study participants ( $n = 11$ )

Baseline Characteristics	$n$ (%) or Median (IQR)
Study Site	
University of Pittsburgh	9 (82)
University of Colorado	2 (18)
Gender	
Female	6 (55)
Male	5 (45)
Race	
White	6 (55)
Black	5 (45)
Ethnicity	
Non-Hispanic	11 (100)
Age (years)	57 (11.5)
Weight (kg)	89.2 (42.6)
BMI (kg/m <sup>2</sup> )	30.5 (13.2)
eGFR (ml/min per 1.73 m <sup>2</sup> )	92 (10.5)
Serum Albumin (g/dl)	4.3 (0.2)
Serum VDBP ( $\mu$ g/ml)	294 (88.7)

Data are presented as median (IQR) or number (%). eGFR, estimated glomerular filtration rate; VDBP, vitamin D binding protein.

chromatography-tandem mass spectrometry (UHPLC-MS/MS) assay capable of detecting all four analytes simultaneously as previously described (Stubbs et al., 2014) with minor modifications. UHPLC was performed with a Waters Acquity UPLC I-class (Waters, Milford, MA, USA), which includes a sample manager and a binary solvent manager. Briefly, 500  $\mu$ l samples were precipitated with acetonitrile, extracted with methyl tert butyl ether, then derivatized with 4-phenyl-1,2,4-triazoline-3,5-dione. Separation of derivatized VitD analytes was achieved using a Waters Acquity BEH C18 column (150 mm  $\times$  2.1 mm, 1.7  $\mu$ m particles) with a gradient elution of water with 0.1% formic acid and acetonitrile. The flow rate was 500  $\mu$ l per min and the total run time was 8 minutes. Detection of analytes was achieved using positive atmospheric pressure chemical ionization and selected reaction monitoring on a TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA). Standards and quality control samples were constructed using blank human serum. Standard curve ranges were 0.10–15 ng/ml for VitD<sub>3</sub> and 24,25D<sub>3</sub>, 0.0100–0.500 ng/ml for 1,25D<sub>3</sub>, and 1.0–100 ng/ml for 25D<sub>3</sub>. Mean correlation coefficients were  $\geq$ 0.994 for all calibration curves. The within-run and between-run accuracy and precision percentage coefficient of variation were  $<$ 10.6% for all analytes.

### Physiologically Based Pharmacokinetic Model Development

A nine-compartment PBPK model (adipose tissue, brain, heart, kidney, liver, rapidly perfused tissue, slowly perfused tissue, and plasma) was developed for VitD<sub>3</sub> and its metabolites using MATLAB (version R2020a, The Mathworks Inc, Natick, MA). A schematic of the overall PBPK model for each compound is shown in Fig. 1 with the connections between each metabolite PBPK model shown in Fig. 2.

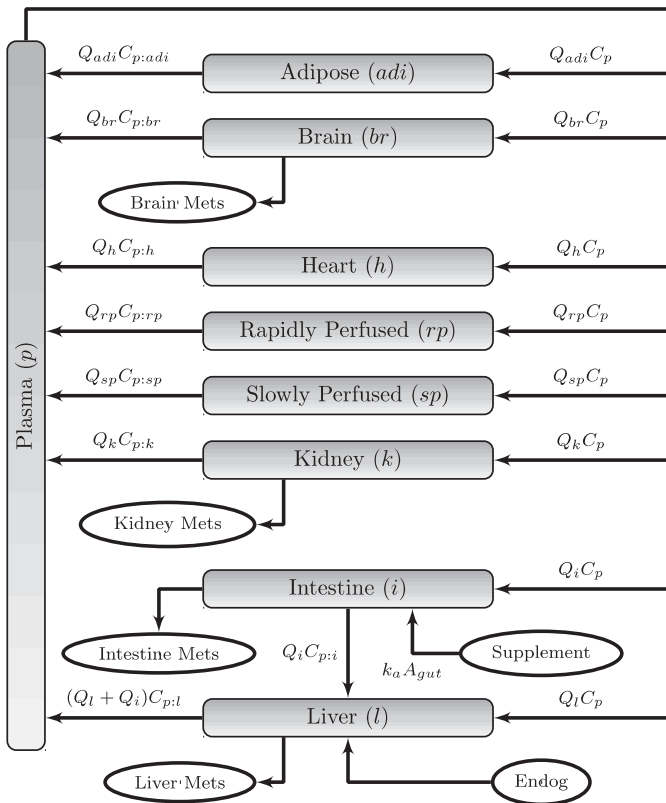
Physiologic parameters were obtained from a previous publication (Davies and Morris, 1993). The slowly perfused compartment was composed of skin and muscle. The rapidly perfused compartment served as a mass balance compartment and comprised all tissue not previously named. Fractional blood flow rate for the slowly perfused compartment was equal to the sum of the rates of its components. The fractional blood flow rate of the rapidly perfused compartment was equal to the remaining fractional blood flow rates. Following the methods of Ramakrishnan et al. (2016), this study assumed no binding to red blood cells and scaled the blood flow to tissues by multiplying the blood flow  $Q_B$  and the blood volume  $V_B$  by  $BP$ , the blood:plasma ratio, to model the rate of flow of plasma, as shown in eqs. 1 and 2:

$$V_P = \cdot BP \cdot \cdot \cdot V_B \quad (1)$$

$$Q_P = \cdot BP \cdot \cdot \cdot Q_B \quad (2)$$

The PBPK model was comprised of conventional mass balance equations, assuming well-stirred distribution into each model compartment with detailed equations for the PBPK model found in the Appendix (eqs. A1 to A14). Calculations for relevant partition coefficients and physiologic values used in this study are found in the supplemental materials (Supplemental Tables 1 and 2).

**Metabolic Routes.** The metabolic cascade for VitD<sub>3</sub> considered in this study consists of three P450 enzymes: CYP2R1, CYP27B1, and CYP24A1. Although



**Fig. 1.** General physiologically based pharmacokinetic model diagram for VitD<sub>3</sub> and metabolites. Symbols are defined as the following:  $Q$  is the plasma flow rate,  $C_{ap}$  is the arterial concentration of the compound;  $C_{vp:T} = A_T(V_T * P_T)$  is the apparent concentration of the compound in tissue  $T$  defined as the amount of compound divided by the volume of the tissue times the tissue:plasma partition coefficient;  $k_a$  is the absorption rate from the gut.

the P450 enzyme CYP2R1, a microsomal enzyme found mainly in the liver, is primarily responsible for the 25-hydroxylation of VitD<sub>3</sub> to 25D<sub>3</sub> following Michaelis-Menten kinetics (Cheng et al., 2004; Shinkyo et al., 2004), there are several other enzymatic pathways that may be involved in the 25-hydroxylation pathway, including CYP27A1 (Sakaki et al., 2005), CYP2J2, and CYP3A4 (Battault et al., 2013; Wang et al., 2013). This study chose to focus on the actions of CYP2R1, with the assumption that  $V_{max}$  may vary depending on levels of sufficiency (Abramson, 1986). The variable rate of  $V_{max}$  is given by eq. 3:

$$V_{max} = \frac{V'}{1 + \left(\frac{[D]}{D_{50}}\right)^{h_V}}, \quad (3)$$

where  $V'$  is the maximum value of  $V_{max}$ ,  $[D]$  is the total liver concentration of VitD<sub>3</sub>,  $D_{50}$  is the concentration at which 50% of the inhibition occurs, and  $h_V$  is the Hill coefficient for this function. The conversion of VitD<sub>3</sub> to 25D<sub>3</sub> by

CYP2R1 is given by standard Michaelis-Menten kinetics (eq. 4) (Ocampo-Pelland et al., 2016):

$$CL_D = \frac{V_{max} * [D]}{K_m + [D]}. \quad (4)$$

Endogenous production of VitD<sub>3</sub> through incidental exposure to sunlight and additional dietary sources are incorporated into the model using eq. 4. This follows the assumption that the input rate for VitD<sub>3</sub> is equal to the output rate of VitD<sub>3</sub> when there is no external supplementation at low baseline concentrations of VitD<sub>3</sub> (Ocampo-Pelland et al., 2016). Following the approach of Ramakrishnan et al. (2016), the baseline concentration of VitD<sub>3</sub> and its metabolites in tissue were calculated using the relationship between measured total plasma concentrations and partition coefficients as  $C_{T,base} = P_{T,p} * C_{plasma,base}$ , where  $C_{T,base}$  is the baseline concentration in tissue  $T$ ,  $P_{T,p}$  is the calculated tissue:plasma partition coefficient, and  $C_{plasma,base}$  is the baseline concentration of the compound in the plasma compartment.

The metabolite 25D<sub>3</sub> is further converted to either 1,25D<sub>3</sub> by the 1 $\alpha$ -hydroxylase CYP27B1, or deactivated to the metabolite 24,25D<sub>3</sub> by the 24-hydroxylase CYP24A1. Both CYP27B1 and CYP24A1 are renal mitochondrial enzymes which have the capability to demonstrate Michaelis-Menten kinetics (Inouye and Sakaki, 2001; Sakaki et al., 2005). However, since observed concentrations of 25D<sub>3</sub> range roughly 20–50 times smaller than literature values of  $K_m$  for CYP27B1 (Inouye and Sakaki, 2001) and 200–400 times smaller than literature values of  $K_m$  for CYP24A1 (Sakaki et al., 2005), this study assumes linear first-order kinetics for CYP27B1 and CYP24A1 acting on 25D<sub>3</sub>.

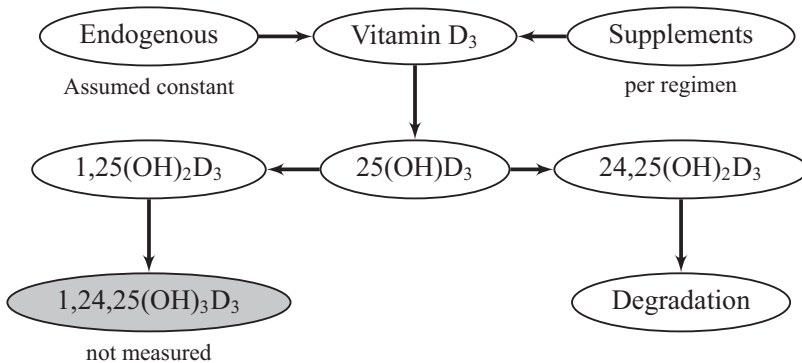
Supplemental doses of VitD<sub>3</sub> during the replenishment period were incorporated into the model through pulsing the initial condition for  $A_{gut}$ , the amount of oral VitD<sub>3</sub> effectively introduced in the intestine en route to the liver. During the initial and final portions of the study where no daily supplements were given, the only source of input to the model is through the endogenous production of VitD<sub>3</sub> throughout sunlight and diet. For the purposes of continuity of the model, all participants were assumed to have 16 weeks of supplementation to achieve sufficient concentrations of 25D<sub>3</sub>.

**Dynamic Adipose Partition Coefficients.** Previous publications have suggested the possibility that partition coefficients in tissues, in particular the kidney, may change with levels of sufficiency (Quach et al., 2015) and the adipose tissue (Sawyer et al., 2017). We chose to incorporate dynamic partitioning for the adipose tissue compartment for VitD<sub>3</sub> and 25D<sub>3</sub> similar to (Sawyer et al., 2017) using the following equations (eq. 5 and 6):

$$AdiPC_D(t) = \frac{AdiD_{max}}{1 + \left(\frac{Adi_{i,50}}{[D]}\right)^{h_D}} \quad (5)$$

$$AdiPC_{25D}(t) = \frac{Adi25_{max}}{1 + \left(\frac{[25D]}{Adi25_{i,50}}\right)^{h_{25}}} \quad (6)$$

Here,  $AdiPC_{i}(t)$  are the adipose partition coefficients at any point in  $t$  related directly to the total plasma concentration of either VitD<sub>3</sub> or 25D<sub>3</sub> at time  $t$  (indicated by  $[D]$  and  $[25D]$ , respectively),  $Adi_{i,50}$  is the concentration of VitD<sub>3</sub> or 25D<sub>3</sub> at which 50% of the inhibition occurs, and  $h_{ij}$  is the Hill coefficient for these functions.



**Fig. 2.** Network diagram for the VitD<sub>3</sub> metabolic cascade model. The network contains compounds with measured concentrations and black arrows define conversion steps with kinetic equations as defined in Table 2. Note that concentrations of 1,24,25(OH)<sub>3</sub>D<sub>3</sub> were not measured in this study.

### Stimulatory and Inhibitory Effects of 1,25D<sub>3</sub>

Circulating and tissue concentrations of 1,25D<sub>3</sub> are tightly regulated through its synthesis by CYP27B1 and degradation by CYP24A1. CYP27B1 expression is tightly regulated by parathyroid hormone and 1,25D<sub>3</sub>, where high levels of 1,25D<sub>3</sub> suppress expression of CYP27B1 (Schuster, 2011). Conversely, the expression of CYP24A1 is enhanced by the presence of 1,25D<sub>3</sub>; upregulation of CYP24A1 by 1,25D<sub>3</sub> serves as feedback control to reduce concentrations of 1,25D<sub>3</sub> (Ramakrishnan et al., 2016). To incorporate the regulatory effects of 1,25D<sub>3</sub> plasma concentrations, this study follows the approach previously outlined (Ramakrishnan et al., 2016) and adjusts the fold change (FC; ratio of changed or basal mRNA levels) of CYP27B1 and CYP24A1 in proportion to total 1,25D<sub>3</sub> plasma concentrations calculated from the partition coefficient in the relevant tissues. The rate of CYP27B1 fold change was expressed as shown in eq. 7:

$$\frac{dCYP27B1_{FC}}{dt} = k_{in,27} \cdot \left(1 - \frac{I_{max}[1,25D_K]^{\gamma_2}}{IC_{50}^{\gamma_2} + [1,25D_K]^{\gamma_2}}\right) - k_{out,27} \cdot CYP27B1_{FC} \quad (7)$$

with  $I_{max}$  the maximum inhibitory effect,  $IC_{50}$  the total plasma concentration of 1,25D<sub>3</sub> in the kidney to achieve 50% of  $I_{max}$ ,  $\gamma_2$  is the Hill coefficient for CYP27B1 in the kidney, and  $[1,25D_K]$  is the total concentration of 1,25D<sub>3</sub> in the kidney (Ramakrishnan et al., 2016). For CYP24A1, eq. 8 gives the fold change in tissue  $T$ , for  $T$  liver, kidney, intestine, or brain:

$$\frac{dCYP24A1_{FC,T}}{dt} = k_{in,24,T} \left(1 + \frac{E_{max,T}[1,25D_T]^{\gamma_{1,T}}}{EC_{50,T}^{\gamma_{1,T}} + [1,25D_T]^{\gamma_{1,T}}}\right) - k_{out,24,T} \cdot CYP24A1_{FC,T} \quad (8)$$

where  $E_{max,T}$  is the maximum stimulatory effect in tissue  $T$ ,  $EC_{50,T}$  is the total plasma concentration of 1,25D<sub>3</sub> in the tissue to achieve 50% of  $E_{max}$ ,  $\gamma_{1,T}$  is the Hill coefficient for CYP24A1 in the tissue, and  $[1,25D_T]$  is the total concentration of 1,25D<sub>3</sub> in the tissue (Ramakrishnan et al., 2016). Following the methods of Noh et al. (2020) and Ramakrishnan et al. (2016),  $k_{in,[1]}$  and  $k_{out,[1]}$  are presumed to have the same value but different (appropriate) units for the differential equation.

### Model Simulations and Parameter Estimations

This study follows the approach outlined by McNally et al. (2011) to explore the global sensitivity of the kinetic parameters for this PBPK model. The model equations were coded in MATLAB (version R2020a, The Mathworks Inc, Natick, MA) and sensitivity analysis was conducted using the SAFE Toolbox (Pianosi et al., 2015). For the initial analysis, the Morris' Method was used to distinguish between the set of influential and noninfluential parameters through ranking (Morris, 1991). Parameters in this study were considered influential if the normalized mean sensitivity measure obtained using Morris' Method was greater than 0.1 (Hsieh et al., 2018). Using this method, the set of influential parameters were chosen for optimization and all other parameters were held at available literature values. If a parameter did not have an available literature value, it was also included in the set chosen for optimization.

This set of parameters was optimized using nonlinear least squares where the cost of fit was calculated using the least squares difference between the observed and predicted model data. To avoid unfairly skewing the model, each compound's data were transformed by subtracting the minimum observed value and dividing by the interquartile range of the observed data for that compound. Data were further weighted by the count of the number of available points for optimization in each phase; this ensured that data below the analytical level of quantification were not considered in and did not negatively affect the optimization routine. This was present primarily in the insufficient VitD<sub>3</sub> data and comprised approximately 15% of the overall data points (3% of points without the insufficient VitD<sub>3</sub> data) used for optimization. The lack of data caused by patient VitD<sub>3</sub> concentrations below the limit of quantification poses some issue for initialization of the model for this compartment; however, the set of influential parameters span the entire timespan of the model, so we used visual inspection of the curve of the model prediction to assess fit in this area for this compartment. The model was initialized to the mean of the first two time points ( $t = 0$  and  $t = 0.5$  hours).

To generate 95% confidence intervals for the optimized parameters, a bootstrapping method was used after initial optimization of the model. For this method, parameters identified as candidates for optimization were allowed to vary twofold from their optimized values, which were based on available literature values, or the best result from multiple iterative guesses. Bootstrapping was performed by systematically running the optimization solver over randomly sampled parameters within these constraints, holding nonoptimized parameters constant at literature values. The resulting parameter output range for each optimized parameter was then subject to standard bootstrapping techniques and the 95% confidence intervals were found for each parameter. To generate a 95% confidence interval around the optimized model, parameters were randomly sampled in a normal distribution from a twofold range of their optimized value for 50 samples, the model run over these parameter sets, and then the 95th percentile of the total model outputs at every half-hour mark was used to generate the total band for the duration of the time-course data.

### Assessment of Prediction Accuracy

The accuracy of the PBPK predictions was evaluated on predicted plasma concentration fit to observations for the three measured compounds. The predicted plasma concentration was formulated using the set of optimized parameters developed from the bootstrapping results. The goodness of prediction for each compound was based on the average fold error (AFE), root mean square error (RMSE), and the normalized root mean square error (NRMSE) (Sheiner and Beal, 1981). The fold error (eq. 9), AFE (eq. 10), RMSE, (eq. 11), and NRMSE (eq. 12) were calculated as follows:

$$\text{fold error} = \frac{\text{Predicted}}{\text{Observed}} \quad (9)$$

$$AFE = \exp\left(\frac{\sum \ln(\text{fold error})}{n}\right) \quad (10)$$

$$RMSE = \sqrt{\frac{\sum (\text{Pred} - \text{Obs})^2}{n}} \quad (11)$$

$$NRMSE = \frac{RMSE}{IQR(\text{Obs})} \quad (12)$$

where  $Pred$  is the predicted value,  $Obs$  is the observed value,  $IQR(Obs)$  is the interquartile range of the observed values, and  $n$  is the number of observed samples.

## Results

A basic PBPK approach was applied to VitD<sub>3</sub> and metabolites using intrinsic clearance parameters determined from the published literature and physiologic distribution parameters (Fig. 1). Data from eleven subjects (demographics given in Table 1) were used to fit the model; two subjects withdrew from the study before the conclusion of the experiment.

Because of limited VitD<sub>3</sub> time course data, the nearly instantaneous conversion of VitD<sub>3</sub> to 25D<sub>3</sub> in VitD deficient individuals (Heaney et al., 2008), and the adipose tissue as a storage compartment that may contribute to circulating levels of VitD<sub>3</sub> (Best et al., 2020), our model was insufficient at predicting available VitD<sub>3</sub> time course data (results not shown). However, clinically, VitD<sub>3</sub> levels are not measured and not likely to be important since therapy decisions are based on 25D<sub>3</sub> levels. All optimized and literature model parameters are reported in Table 2. Bootstrapping was performed and optimized parameters are reported with 95% confidence intervals.

The observed and final model predicted concentrations for 25D<sub>3</sub>, 24,25D<sub>3</sub> and 1,25D<sub>3</sub> during the insufficient and repleted periods are shown in Fig. 3, A–C, respectively, with a 95% confidence interval band around the optimized parameter model as described in section 7.5. Detailed model predictions for the insufficient and repleted periods are shown as insets in Fig. 3. The wavy line in the center of the plots indicate a break in the supplementation period for better clarity on the insufficient and repleted period.

Predictive performance of the PBPK model for the insufficient and repleted periods are shown in Fig. 4. The average fold error (AFE) for

TABLE 2

## Kinetic parameters for the PBPK model

Optimized parameters are given with bootstrapped 95% confidence intervals and all other parameters are held to available literature values or estimates as noted. Note the 95% CIs are not necessarily symmetric about the optimized parameter due to the method of sampling. See the Model simulations and parameter estimations section for details.

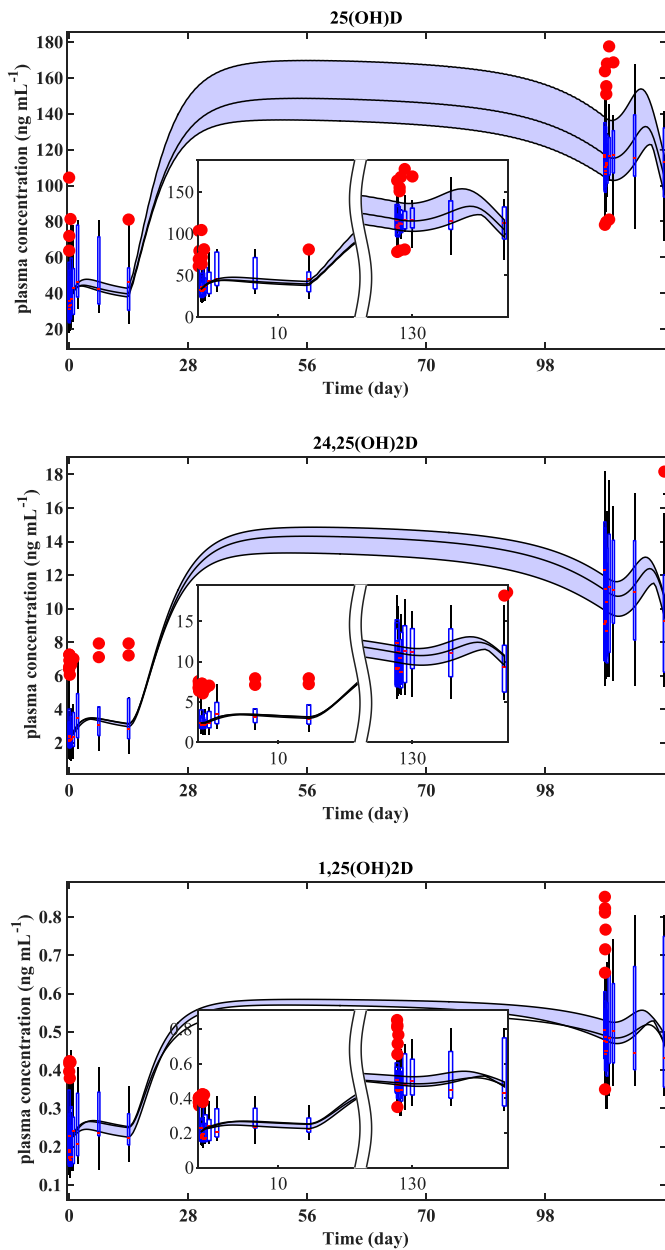
Parameter	Description	Value	Units	Reference
$K_m$	Michaelis-Menten constant for CYP2R1	5.91 <sup>a</sup> (5.09–5.95)	nmol l <sup>-1</sup>	Optimized
$D_{base}$	Endogenous baseline concentration of VitD <sub>3</sub>	2.05 <sup>a</sup> (2.00–2.06)	nmol·l <sup>-1</sup>	Optimized
$k_a$	Absorption constant	0.32	h <sup>-1</sup>	(Ocampo-Pelland et al., 2016)
$R_{27B1}^{25}$	Synthesis of 25D <sub>3</sub> to 1,25D <sub>3</sub> by CYP27B1	17.7 <sup>b</sup> (15.85–18.97)	l·hr <sup>-1</sup>	Optimized
$f_{ub}^{25}$	Percent free unbound 25D <sub>3</sub>	0.03	–	(Bikle et al., 1986)
Synthesis of 24,25D <sub>3</sub> from 25D <sub>3</sub> by CYP24A1 in tissue				
$R_{24A1,L}^{25}$	Liver	10.2 <sup>b</sup> (8.45–10.8)	l·hr <sup>-1</sup>	Optimized
$R_{24A1,K}^{25}$	Kidney	2.43 <sup>b</sup> (2.06, 2.68)	l·hr <sup>-1</sup>	Optimized
$R_{24A1,Br}^{25}$	Brain	13.3 <sup>b</sup> (12.2–13.9)	l·hr <sup>-1</sup>	Optimized
$R_{24A1,I}^{25}$	Intestine	15.4 <sup>b</sup> (13.2–16.8)	l·hr <sup>-1</sup>	Optimized
Clearance of unbound (free) 1,25D <sub>3</sub> by CYP24A1 in tissue $T, CL_{free,int,T}^{125}$				
$CL_{free,int,L}^{125}$	Liver	0.0057	ml·hr <sup>-1</sup> ·g <sup>-1</sup>	(Noh et al., 2020)
$CL_{free,int,K}^{125}$	Kidney	0.066 <sup>a</sup> (0.065–0.082)	ml·hr <sup>-1</sup> ·g <sup>-1</sup>	Optimized
$CL_{free,int,Br}^{125}$	Brain	0.0083	ml·hr <sup>-1</sup> ·g <sup>-1</sup>	(Noh et al., 2020)
$CL_{free,int,I}^{125}$	Intestine	0.0028	ml·hr <sup>-1</sup> ·g <sup>-1</sup>	(Noh et al., 2020)
$CL_{free,int,K}^{2425}$	Clearance of unbound (free) 24,25D	990 <sup>b</sup> (946–1068)	pmol·hr <sup>-1</sup>	Optimized
$I_{max}$	Maximal inhibitory effect of CYP27B1	1	fold change	(Noh et al., 2020)
$IC_{50}$	Total 1,25D <sub>3</sub> concentration when reaching 50% $I_{max}$	672 <sup>a</sup> (680–1068)	pmol l <sup>-1</sup>	Optimized
Maximal stimulatory effect of CYP24A1 in tissue $T, E_{max,T}$				
$E_{max,L}$	Liver	122 <sup>a</sup> (88.7–131.6)	fold change	Optimized
$E_{max,K}$	Kidney	25 <sup>a</sup> (23.9–28.0)	fold change	Optimized
$E_{max,Br}$	Brain	22 <sup>a</sup> (21.5–23.0)	fold change	Optimized
$E_{max,I}$	Intestine	675	fold change	(Noh et al., 2020)
Total tissue 1,25D <sub>3</sub> concentration when reaching 50% $E_{max}, EC_{50,T}$				
$EC_{50,L}$	Liver	2600	pmol l <sup>-1</sup>	(Noh et al., 2020)
$EC_{50,K}$	Kidney	143	pmol l <sup>-1</sup>	(Noh et al., 2020)
$EC_{50,Br}$	Brain	97 <sup>a</sup> (74.–97.5)	pmol l <sup>-1</sup>	Optimized
$EC_{50,I}$	Intestine	2640	pmol l <sup>-1</sup>	(Noh et al., 2020)
Hill coefficient of CYP24A1 in tissue $T, \gamma_{1,T}$				
$\gamma_{1,L}$	Liver	3.05 <sup>a</sup> (2.99–3.47)	–	Optimized
$\gamma_{1,K}$	Kidney	3.59	–	(Noh et al., 2020)
$\gamma_{1,Br}$	Brain	1.12 <sup>c</sup> (0.90–1.15)	–	Optimized
$\gamma_{1,I}$	Intestine	4.16	–	(Noh et al., 2020)
$\gamma_2$	Hill coefficient of CYP27B1	2.7	–	(Noh et al., 2020)
$k_{out,27}$	Turnover rate constant of CYP27B1	0.245	h <sup>-1</sup>	(Noh et al., 2020)
Turnover rate constant of CYP24A1 in tissue $T, k_{out}^{24,T}$				
$k_{out,24,L}$	Liver	0.47	h <sup>-1</sup>	(Noh et al., 2020)
$k_{out,24,K}$	Kidney	0.28	h <sup>-1</sup>	(Noh et al., 2020)
$k_{out,24,Br}$	Brain	0.86	h <sup>-1</sup>	(Noh et al., 2020)
$k_{out,24,I}$	Intestine	0.047	h <sup>-1</sup>	(Noh et al., 2020)
Atypical Kinetic Parameters for $V_{max}$				
$V'$	Maximum value of $V_{max}$ for CYP2R1	5.76 <sup>b</sup> (5.73–5.76)	l hr <sup>-1</sup>	Optimized
$D_{50}$	Concentration at which 50% of $V'$ occurs	8.35 <sup>b</sup> (8.34–8.35)	nmol l <sup>-1</sup>	Optimized
$h_V$	Hill constant for variable $V_{max}$	2.27 <sup>b</sup> (2.26–2.27)	–	Optimized
Dynamic Adipose partition coefficient parameters				
$Adi_{D,max}$	Maximum adipose PC for VitD <sub>3</sub>	9.9 <sup>b</sup> (9.78–10.4)	–	Optimized
$Adi_{25,max}$	Maximum adipose PC for 25D <sub>3</sub>	1.64 <sup>b</sup> (0.90–1.73)	–	Optimized
$Adi_{D,50}$	Concentration at which 50% of $Adi_{D,max}$ occurs	8.96 <sup>b</sup> (4.93–11.98)	nmol l <sup>-1</sup>	Optimized
$Adi_{25D,50}$	Concentration at which 50% of $Adi_{25D,max}$ occurs	117 <sup>b</sup> (61.8–121)	nmol l <sup>-1</sup>	Optimized
$h_D$	Hill constant for $AdiPC_D$	2.12 <sup>b</sup> (2.10–2.30)	–	Optimized
$h_{25}$	Hill constant for $AdiPC_{25}$	0.96 <sup>b</sup> (0.86–1.01)	–	Optimized

D<sub>3</sub>, VitD<sub>3</sub>; 25D<sub>3</sub>, 25(OH)D<sub>3</sub>; 1,25D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub>; 24,25D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub>.

<sup>a</sup>Confidence interval does not contain literature values.

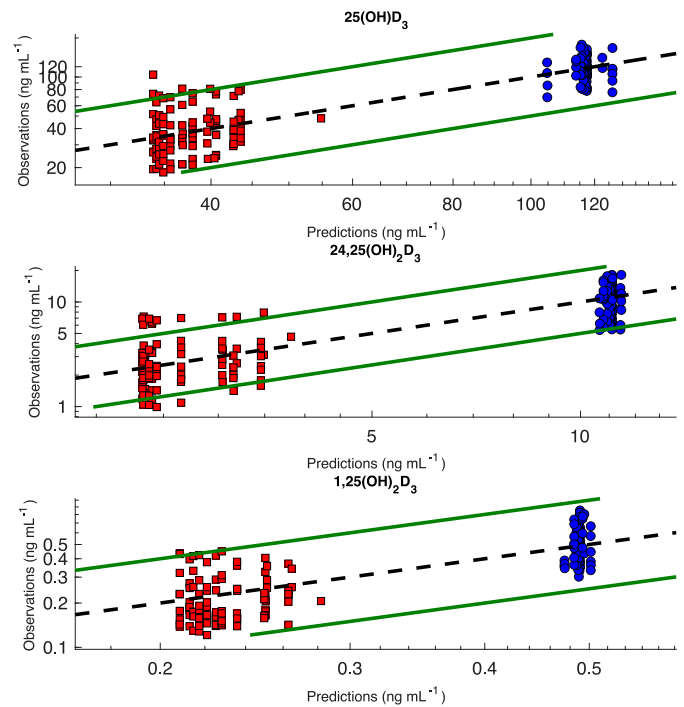
<sup>b</sup>No literature values available for comparison.

<sup>c</sup>Confidence interval contains literature value.



**Fig. 3.** Predicted PBPK plasma concentrations for VitD<sub>3</sub> metabolites (25D<sub>3</sub>, 24,25D<sub>3</sub>, and 1,25D<sub>3</sub>). Box plots indicate the middle 50% of the data with the median data indicated by a line, and the shading indicates the 95% confidence interval of the model predictions. Insets indicate enhanced view of data for predicted PBPK plasma concentrations for VitD<sub>3</sub> metabolites (25D<sub>3</sub>, 24,25D<sub>3</sub>, and 1,25D<sub>3</sub>), clipping out the supplementation period from days 20 through 92 of the experiment, where the wavy line in the center indicates the clipped portion of the model during the supplementation period where no data were observed. (A) 25D<sub>3</sub> serum levels (nmol l<sup>-1</sup>). (B) 24,25D<sub>3</sub> serum levels (nmol l<sup>-1</sup>). (C) 1,25D<sub>3</sub> serum levels (nmol l<sup>-1</sup>).

the VitD<sub>3</sub> metabolites ranges between 1.01 and 1.05 and the normalized root mean square error (*NRMSE*) ranges between 0.26 and 0.38. *AFE* values close to one and *NRMSE* close to zero are indicative of better model fits to data. The correlation of determination (*R*<sup>2</sup>) for each metabolite is 0.77, 0.67, and 0.63, respectively, and 0.92 when considering all data points together. When considering the *fold error* values for the data, the model performed well with 97%, 88%, and 98% of the data for 25D<sub>3</sub>, 24,25D<sub>3</sub>, and 1,25D<sub>3</sub>, respectively, within twofold of unity (*fold error* values between 0.5 and 2.0). When considering a tighter range of fold error (1.5-fold of unity, with *fold error* values between



**Fig. 4.** Observed plasma concentrations versus predicted PBPK plasma concentrations in vitamin and three metabolites. Red x-marks indicate observations from the deficient period, blue circles indicate observations from the replete period, the dotted line represents the identity line, and the green lines represent the twofold interval.

0.67 and 1.5), the model performs adequately with nearly 80% of 25D<sub>3</sub> and 1,25D<sub>3</sub> captured within a 1.5-fold error of unity. 24,25D<sub>3</sub> data were captured less well, with a 1.5-fold error value of 63%.

## Discussion

Vitamin D insufficiency is highly prevalent in the community, afflicting up to 80% of men and women in the United States, Canada, and Europe (Ganji et al., 2012; van Schoor and Lips, 2018). While the definitions of VitD insufficiency (and deficiency) are not standardized across medical organizations, targeted concentrations are generally in the 20 ng/ml to 40 ng/ml range for 25D, the primary metabolite used for classification (Holick et al., 2011; Ross et al., 2011; Ganji et al., 2012; van Schoor and Lips, 2018). Whereas various treatment recommendations have also been proposed according to the IOM (Jernigan and Andress, 2003) and ENDO (Holick et al., 2011), there is variability in success of achieving target concentrations of 25D<sub>3</sub> in patients. There is currently not a clinically established approach to enable prediction of plasma 25D concentrations that might result from a given treatment regimen for a given patient. There is further complication since the plasma concentration of 25D will be impacted by the function of numerous P450 enzymes through activation and deactivation pathways, as well as through sunlight exposure, skin pigment, diet, and clothing. PBPK modeling has a potential to predict expected plasma concentrations of 25D and subsequent metabolites after administration of oral VitD therapy. The current study reports the development of a PBPK model of VitD<sub>3</sub> and metabolites in VitD insufficient subjects in the United States who were treated with moderate doses (5000 I.U.) of daily VitD<sub>3</sub> for up to 16 weeks to achieve replacement as defined by 25D ≥ 30 ng/ml. The comprehensive PBPK model for VitD<sub>3</sub> incorporated dynamic adipose:plasma partition coefficients, atypical kinetics for CYP2R1 using a variable *V*<sub>max</sub> based on the total liver concentration of VitD<sub>3</sub>, and inhibitory and stimulatory effects of 1,25D<sub>3</sub> for CYP27B1 and CYP24A1. The resultant model sufficiently



predicted the concentrations of VitD<sub>3</sub> metabolites well in healthy subjects throughout a multiphase study that incorporated an insufficient phase and replete phase. The model informs an understanding of the disposition of VitD<sub>3</sub> metabolites and could be used to predict plasma concentrations that might result from a given dosing regimen of VitD<sub>3</sub> in human patients.

The developed PBPK model for VitD<sub>3</sub> and its metabolites consisted of nine compartments (adipose tissue, brain, heart, intestines, kidney, liver, rapidly perfused tissue, slowly perfused tissue, and plasma). Partition coefficients were either selected from the literature or calculated as described in the Supplemental Material. Although a previous publication in mice suggested that partition coefficients may differ in states of vitamin D deficiency (Quach et al., 2015), our simulations showed less than a 2% change in liver partition coefficients (as defined by the ratio of liver concentration to plasma concentration) between insufficient and sufficient states for VitD<sub>3</sub> and metabolites, and less than 1% for kidney partition coefficients (data not shown). However, we did find a difference in model outcomes with the inclusion of variable partitioning in the adipose tissue compartment. As subjects in our study were initially at levels of insufficiency, there is evidence to suggest that normal daily inputs and endogenous production of vitamin D is not sufficient for accumulation in tissues (Heaney et al., 2009); however, at higher levels of sufficiency, the body is able to store VitD<sub>3</sub> and 25D<sub>3</sub> in adipose tissue for use at a later time (Mawer et al., 1972; Heaney et al., 2009; Abbas, 2017). The addition of a variable adipose partition coefficient for VitD and 25D, as described in section 7.2.2 and discussed in Sawyer et al. (2017), led to enhanced fits for the model. In addition, our model agrees well with previously published human studies without K<sub>p</sub> values defined (Holick et al., 2008, Fig. 2B).

As VitD<sub>3</sub> is acquired endogenously through the skin from the effects of UV-B light on 7-dehydrocholesterol (7-DHC) and dietary sources including dairy and fish, these contribute to baseline concentrations of VitD<sub>3</sub> in the plasma. The PBPK model incorporated endogenous VitD<sub>3</sub> levels with an assumption of output to 25D<sub>3</sub>. After oral ingestion and absorption of supplemental VitD<sub>3</sub>, the free fraction is taken up in the liver where it is hydroxylated by 25-hydroxylase to form calcifediol or 25D<sub>3</sub>. Subsequently, 25D<sub>3</sub> is hydroxylated by the kidneys to form calcitriol or 1,25D<sub>3</sub>, which is considered the most active metabolite of VitD<sub>3</sub>. The current PBPK model used linear first order kinetics for CYP27B1 and CYP24A1 based on the significantly lower 25D<sub>3</sub> concentrations versus published K<sub>m</sub> values for the respective enzymes. Supplemental dosing of cholecalciferol was incorporated into the model through pulsing the amount introduced into the intestines.

Circulating and tissue concentrations of 1,25D<sub>3</sub> are tightly regulated through the synthesis by CYP27B1 and degradation by CYP24A1. Although CYP27B1 expression is tightly regulated by the binding of 1,25D<sub>3</sub> and parathyroid hormone (Schuster, 2011), the expression of CYP24A1 is enhanced by the presence of 1,25D<sub>3</sub>; upregulation of CYP24A1 by 1,25D<sub>3</sub> serves as a feedback control to reduce concentrations of 1,25D<sub>3</sub> (Ramakrishnan et al., 2016). The current PBPK model incorporated the regulatory effects of 1,25D<sub>3</sub> plasma concentrations by adjusting the fold change of CYP27B1 and CYP24A1 in proportion to 1,25D<sub>3</sub> plasma concentrations in the liver, kidney, intestine, and brain, as previously identified (Ramakrishnan et al., 2016). Whereas the fold-change data were simulated and although the dosing regimen and the supplementation in Ramakrishnan et al. (2016) is different than our dosing regimen and supplementation, we observed the same general behavior for the fold change of each enzyme (results not shown), namely that as the level of 1,25D<sub>3</sub> increases, the fold change of CYP27B1 decreases and the levels of CYP24A1 increase in each compartment. When the supplementation was removed at the repleted portion of the study, we observed an expected increase and decrease, respectively, in the fold changes of the CYP24A1 and CYP27B1 enzymes comparative to the concentration of 1,25D<sub>3</sub> in the

appropriate compartments. This behavior follows the expected directions for CYP27B1 and CYP24A1 given the critical concentration of 1,25D<sub>3</sub> governing these physiologic effects on these enzymes.

Recent publications have discussed the advancement of atypical Michaelis-Menten kinetics for a variety of P450 enzymes, including several enzymes involved in the secondary metabolism cascade for vitamin D. This includes CYP3A4 (Arendse et al., 2013) and CYP2J2 (Leow and Chan, 2019; Leow et al., 2021). In particular, CYP2J2 shares 72.5% sequence similarity with CYP2R1, so it is not unreasonable to assume that CYP2R1 may also exhibit atypical Michaelis-Menten kinetics at varying sufficiency levels. To model this, we chose to use a modification on substrate inhibition kinetics, as shown in eq. 3. This led to strong improvement of model predictions, particularly in latter time points (data not shown).

Since the metabolites versus the parent VitD<sub>3</sub> compound are measured clinically to assist clinicians in determining whether patients are VitD<sub>3</sub> insufficient and/or whether they require changes to dosing regimens, we performed some simulations of predicted concentrations with common regimens. A simulation using the current model was performed inputting a dose of 1000 I.U./daily, which is consistent with dosing recommendations, according to the IOM. Our model predicted 25D<sub>3</sub> plasma concentrations well (Fig. 5) and is consistent with a previous study with this dosing regimen (Holick et al., 2008, Fig. 2B). Note subjects in (Holick et al., 2008) began the study with sufficient levels of VitD; we hypothesize this explains the slight underprediction of our model to participant data at later time points as the ratio of VitD<sub>3</sub> and metabolites may not remain the same across levels of sufficiency for initialization of the model.

Overall, the developed PBPK model of VitD<sub>3</sub> that incorporated data from insufficient participants into a repleted phase after daily administration of 5000 I.U. VitD<sub>3</sub> for 12–16 weeks led to acceptable and satisfactory predictions in healthy human subjects. When considering the *fold error* values for the data, the model performed well with 97%, 88%, and 98% of the data for 25D<sub>3</sub>, 24,25D<sub>3</sub>, and 1,25D<sub>3</sub>, respectively, within twofold of unity (*fold error* values between 0.5 and 2.0). Because of limited VitD<sub>3</sub> time course data, the nearly instantaneous

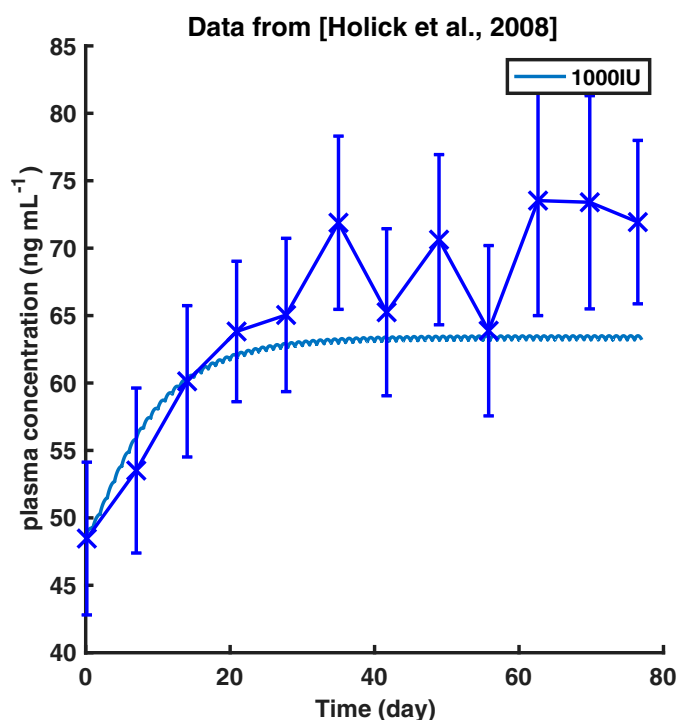


Fig. 5. Simulation of daily 1000 I.U. dosing regimen. Solid line indicates model predictions, data taken from (Holick et al., 2008) are mean  $\pm$  SEM.

conversion of VitD<sub>3</sub> to 25D<sub>3</sub> in VitD deficient individuals (Heaney et al., 2008), and the adipose tissue as a storage compartment that may contribute to circulating levels of VitD<sub>3</sub> (Best et al., 2020), our model was insufficient at predicting available VitD<sub>3</sub> time course data (results not shown). However, this may not be overly relevant since VitD<sub>3</sub> levels are not measured or used clinically. An additional limitation to this model is the lack of available experimental data for comparison of atypical kinetic parameters for CYP2R1; however, the inclusion of the atypical kinetics greatly improved our model behavior and is within the realm of biologic possibility. The model serves to inform an understanding of VitD<sub>3</sub> and metabolite disposition and could be used to predict 25D<sub>3</sub> plasma concentrations that might result from a given dosing regimen in human patients. A beneficial future addition to this study would include incorporation of patient-specific data throughout the supplementation phase to better inform the model predictions. This model has applications in studying the effects of repletion schemes for populations of patients with impaired enzymatic abilities, such as chronic kidney disease, an important diseased population we are currently evaluating.

### Acknowledgments

The authors thank University of Colorado and the University of Pittsburgh for their help in the conduct of the clinical studies. Jarrow Formulas, Inc. (Los Angeles, CA) is gratefully acknowledged for providing study drug (cholecalciferol 5000 I.U.).

### Authorship Contributions

*Participated in research design:* Nolin, Joy.

*Conducted experiments:* Tuey, West, Nolin, Joy.

*Performed data analysis:* Sawyer.

*Wrote or contributed to the writing of the manuscript:* Sawyer, Tuey, Nolin, Joy.

## Appendix

### PBPK Model Equations

The parameters in the PBPK model equations refer to tissue ( $T$ ), arterial blood flow ( $Q$ ), compound amount ( $A$ ), concentration ( $C$ ), volume ( $V$ ), and tissue:plasma partition coefficients ( $P_{T:p}$ ). The concentration in each tissue is given by eq. A1, where the tissue:plasma partition coefficients are given in Supplemental Table 2.

$$C_T = A_T / (V_T \cdot P_{T:p}) \quad (\text{A1})$$

For compartments that have the same equation form across all compounds, general mass balance differential equations are given in eqs. A2 and A3 based on Fig. 1 in the main text. Definitions and values for parameter values for eqs. A2 through A14 are given in Table 2 and Supplemental Tables 1 and 2.

Noneliminating tissue in the set  $Z$  (adipose, heart, rapidly perfused, slowly perfused)

$$V_Z \cdot dC_Z/dt = BP \cdot Q_Z \cdot (C_p - C_{p:Z}) \quad (\text{A2})$$

Plasma:

$$V_p \cdot dC_p/dt = \Sigma_T [BP \cdot Q_T \cdot C_{p:T}] - BP \cdot Q_{CO} \cdot C_p \quad (\text{A3})$$

Equations A4 through A7 describe the mass balance equations for the remaining four compartments (brain [ $Br$ ], intestines [ $I$ ], kidney [ $K$ ], and liver [ $L$ ]) for VitD<sub>3</sub> and each metabolite, where additional components are described in eqs. A8 through A14.

VitD<sub>3</sub> equations:

$$V_{Br} \cdot dC_{Br}/dt = BP \cdot Q_{Br} \cdot (C_p - C_{p:Br}) \quad (\text{A4a})$$

$$V_I \cdot dC_I/dt = BP \cdot Q_I \cdot (C_p - C_{p:I}) + k_a \cdot A_{gut} \quad (\text{A4b})$$

$$V_K \cdot dC_K/dt = BP \cdot Q_K \cdot (C_p - C_{p:K}) \quad (\text{A4c})$$

$$V_L \cdot dC_L/dt = BP \cdot Q_L \cdot (C_p - C_{p:L}) + ENDOG - R_{25D} \quad (\text{A4d})$$

25D<sub>3</sub> equations:

$$V_{Br} \cdot dC_{Br}/dt = BP \cdot Q_{Br} \cdot (C_p - C_{p:Br}) - R_{2425D_{Br}} \quad (\text{A5a})$$

$$V_I \cdot dC_I/dt = BP \cdot Q_I \cdot (C_p - C_{p:I}) - R_{2425D_I} \quad (\text{A5b})$$

$$V_K \cdot dC_K/dt = BP \cdot Q_K \cdot (C_p - C_{p:K}) - R_{125D} - R_{2425D_K} \quad (\text{A5c})$$

$$V_L \cdot dC_L/dt = BP \cdot Q_L \cdot (C_p - C_{p:L}) + R_{25D_3} - R_{2425D_L} \quad (\text{A5d})$$

24,25D<sub>3</sub> equations:

$$V_{Br} \cdot dC_{Br}/dt = BP \cdot Q_{Br} \cdot (C_p - C_{p:Br}) + R_{2425D_{Br}} \quad (\text{A6a})$$

$$V_I \cdot dC_I/dt = BP \cdot Q_I \cdot (C_p - C_{p:I}) + R_{2425D_I} \quad (\text{A6b})$$

$$V_K \cdot dC_K/dt = BP \cdot Q_K \cdot (C_p - C_{p:K}) + R_{2425D_K} - CL_{2425D_{Br}} \quad (\text{A6c})$$

$$V_L \cdot dC_L/dt = BP \cdot Q_L \cdot (C_p - C_{p:L}) + R_{2425D_L} \quad (\text{A6d})$$

1,25D<sub>3</sub> equations:

$$V_{Br} \cdot dC_{Br}/dt = BP \cdot Q_{Br} \cdot (C_p - C_{p:Br}) - CL_{125D_{Br}} \quad (\text{A7a})$$

$$V_I \cdot dC_I/dt = BP \cdot Q_I \cdot (C_p - C_{p:I}) - CL_{125D_I} \quad (\text{A7b})$$

$$V_K \cdot dC_K/dt = BP \cdot Q_K \cdot (C_p - C_{p:K}) + R_{125D} - CL_{125D_K} \quad (\text{A7c})$$

$$V_L \cdot dC_L/dt = BP \cdot Q_L \cdot (C_p - C_{p:L}) - CL_{125D_L} \quad (\text{A7d})$$

Additional differential equations for the PBPK model are given in eqs. A8 to A14, where concentrations in tissue  $T$  are indicated by  $[M^T]$ , where  $M$  is VitD<sub>3</sub> or a metabolite:

Absorption of VitD<sub>3</sub>:

$$dA_{gut}/dt = -k_a \cdot A_{gut} \quad (\text{A8})$$

Endogenous production of VitD<sub>3</sub>:

$$ENDO = \frac{V_{max} \cdot [D_{base}]}{K_m + [D_{base}]} \cdot [D_{base}] \quad (\text{A9})$$

Conversion of VitD<sub>3</sub> to 25D<sub>3</sub> in the liver ( $L$ ):

$$R_{25D} = \frac{V_{max} \cdot [D_L]}{K_m + [D_L]} \cdot [D_L] \quad (\text{A10})$$

Conversion of 25D<sub>3</sub> to 1,25D<sub>3</sub> in kidney by CYP27B1:

$$R_{125D} = R_{27B1}^{25} \cdot [25D_K] \cdot f_{ub}^{25} \cdot CYP_{27B1_{FC}} \quad (\text{A11})$$

Conversion of 25D<sub>3</sub> to 24,25D<sub>3</sub> in tissue  $T$  by CYP24A1:

$$R_{2425D_T} = R_{24A1,T}^{25} \cdot [25D_T] \cdot f_{ub}^{25} \cdot CYP_{24A1_{FC,T}} \quad (\text{A12})$$

Clearance of 1,25D<sub>3</sub> in tissue  $T$  by CYP24A1:

$$CL_{125D_T} = CL_{free,int,K}^{125} \cdot [1,25D_T] \cdot CYP_{24A1_{FC,T}} \quad (\text{A13})$$

Clearance of 24,25D<sub>3</sub> in the kidney:

$$CL_{2425D} = CL_{free,int,K}^{2425} \cdot [24,25D_K] \quad (\text{A14})$$

The initial conditions for the model were generated by taking the mean of the data points to generate an initial estimate for the median plasma concentration.



## References

- Abbas MA (2017) Physiological functions of vitamin D in adipose tissue. *J Steroid Biochem Mol Biol* **165** (Pt B):369–381.
- Abramson FP (1986) Kinetic models of induction: II. decreased turnover of a product or its precursor. *J Pharm Sci* **75**:229–232.
- Arendse L, Blundell TL, and Blackburn J (2013) Combining in silico protein stability calculations with structure-function relationships to explore the effect of polymorphic variation on cytochrome P450 drug metabolism. *Curr Drug Metab* **14**:745–763.
- Armas LA, Hollis BW, and Heaney RP (2004) Vitamin D<sub>2</sub> is much less effective than vitamin D<sub>3</sub> in humans. *J Clin Endocrinol Metab* **89**:5387–5391.
- Baillie GR and Johnson CA (2002) Comparative review of the pharmacokinetics of vitamin D analogues. *Semin Dial* **15**:352–357.
- Battault S, Whiting SJ, Peltier SL, Sadrin S, Gerber G, and Maixent JM (2013) Vitamin D metabolism, functions and needs: from science to health claims. *Eur J Nutr* **52**:429–441.
- Benaboud S, Urien S, Therivet E, Pricé D, Legendre C, Souberbielle JC, Hirt D, Friedlander G, Treliuyer JM, and Courbebaisse M (2013) Determination of optimal cholecalciferol treatment in renal transplant recipients using a population pharmacokinetic approach. *Eur J Clin Pharmacol* **69**:499–506.
- Best CM, Riley DV, Laha TJ, Pflaum H, Zelnick LR, Hsu S, Thummel KE, Foster-Schubert KE, Kuzma JN, Cromer G, et al. (2020) Vitamin D in human serum and adipose tissue after supplementation. *Am J Clin Nutr* **113**:83–91.
- Bikle DD, Halloran BP, Gee E, Ryzyn E, and Haddad JG (1986) Free 25-hydroxyvitamin D levels are normal in subjects with liver disease and reduced total 25-hydroxyvitamin D levels. *J Clin Invest* **78**:748–752.
- Bikle DD, Patzek S, and Wang Y (2018) Physiologic and pathophysiologic roles of extra renal CYP27b1: case report and review. *Bone Rep* **8**:255–267.
- Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, and Russell DW (2004) Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. *Proc Natl Acad Sci USA* **101**:7711–7715.
- Cianferrotti L and Marcocci C (2012) Subclinical vitamin D deficiency. *Best Pract Res Clin Endocrinol Metab* **26**:523–537.
- Davies B and Morris T (1993) Physiological parameters in laboratory animals and humans. *Pharm Res* **10**:1093–1095.
- Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, and Vieth R (2005) Estimates of optimal vitamin D status. *Osteoporos Int* **16**:713–716.
- Fassio A, Adami G, Rossini M, Giollo A, Caimmi C, Bixio R, Viapiana O, Milleri S, Gatti M, and Gatti D (2020) Pharmacokinetics of oral cholecalciferol in healthy subjects with vitamin D deficiency: a randomized open-label study. *Nutrients* **12**:1553.
- Ganji V, Zhang X, and Tangpricha V (2012) Serum 25-hydroxyvitamin D concentrations and prevalence estimates of hypovitaminosis D in the U.S. population based on assay-adjusted data. *J Nutr* **142**:498–507.
- Gao C, Bergagnini-Kolev MC, Liao MZ, Wang Z, Wong T, Calamia JC, Lin YS, Mao Q, and Thummel KE (2017) Simultaneous quantification of 25-hydroxyvitamin D<sub>3</sub>-sulfate and 25-hydroxyvitamin D<sub>3</sub>-3-glucuronide in human serum and plasma using liquid chromatography-tandem mass spectrometry coupled with DAPTAD-derivatization. *J Chromatogr B Analyt Technol Biomed Life Sci* **1060**:158–165.
- German Nutrition Society (2012) New reference values for vitamin D. *Ann Nutr Metab* **60**:241–246.
- Haq A, Wimalawansa SJ, Pludowski P, and Anouti FA (2018) Clinical practice guidelines for vitamin D in the United Arab Emirates. *J Steroid Biochem Mol Biol* **175**:4–11.
- Heaney RP, Horst RL, Cullen DM, and Armas LA (2009) Vitamin D<sub>3</sub> distribution and status in the body. *J Am Coll Nutr* **28**:252–256.
- Heaney RP, Armas LA, Shary JR, Bell NH, Binkley N, and Hollis BW (2008) 25-Hydroxylation of vitamin D<sub>3</sub>: relation to circulating vitamin D<sub>3</sub> under various input conditions. *Am J Clin Nutr* **87**:1738–1742.
- Holick MF (2006) Resurrection of vitamin D deficiency and rickets. *J Clin Invest* **116**:2062–2072.
- Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**:266–281.
- Holick MF, Biancuzzo RM, Chen TC, Klein EK, Young A, Bibuld D, Reitz R, Salameh W, Ameri A, and Tannenbaum AD (2008) Vitamin D<sub>2</sub> is as effective as vitamin D<sub>3</sub> in maintaining circulating concentrations of 25-hydroxyvitamin D. *J Clin Endocrinol Metab* **93**:677–681.
- Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, and Weaver CM; Endocrine Society (2011) Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* **96**:1911–1930.
- Hsieh NH, Reifel-B, Bois FY, and Chiu WA (2018) Applying a global sensitivity analysis workflow to improve the computational efficiencies in physiologically-based pharmacokinetic modeling. *Front Pharmacol* **9**:588.
- Hsu S, Zelnick LR, Lin YS, Best CM, Kestenbaum B, Thummel KE, Rose LM, Hoofnagle AN, and de Boer IH (2021) Differences in 25-hydroxyvitamin D clearance by eGFR and race: a pharmacokinetic study. *J Am Soc Nephrol* **32**:188–198.
- Ilahi M, Armas LA, and Heaney RP (2008) Pharmacokinetics of a single, large dose of cholecalciferol. *Am J Clin Nutr* **87**:688–691.
- Inouye K and Sakaki T (2001) Enzymatic studies on the key enzymes of vitamin D metabolism: 1 $\alpha$ -hydroxylase (CYP27B1) and 24-hydroxylase (CYP24). *Biochemol Annu Rev* **7**:179–194.
- Jemigan P and Andress DL (2003) Vitamin D analogs in uremia: integrating medical and nutritional issues. *Adv Ren Replace Ther* **10**:241–247.
- Jetter A, Egli A, Dawson-Hughes B, Staehelin HB, Stoecklin E, Goessl R, Henschkowsky J, and Bischoff-Ferrari HA (2014) Pharmacokinetics of oral vitamin D(3) and calcifediol. *Bone* **59**:14–19.
- Jones G, Prosser DE, and Kaufmann M (2018) The activating enzymes of vitamin D metabolism (25-and 1 $\alpha$ -hydroxylases), in *Vitamin D*, pp 57–79, Elsevier Publishing Co., Boston.
- Kimura Y, Nakayama M, Kuriyama S, Watanabe S, Kawaguchi Y, and Sakai O (1991) Pharmacokinetics of active vitamins D<sub>3</sub>, 1 $\alpha$ -hydroxyvitamin D<sub>3</sub> and 1 $\alpha$ , 25-dihydroxyvitamin D<sub>3</sub> in patients on chronic hemodialysis. *Clin Nephrol* **35**:72–77.
- Leow JWH and Chan ECY (2019) Atypical Michaelis-Menten kinetics in cytochrome P450 enzymes: a focus on substrate inhibition. *Biochem Pharmacol* **169**:113615.
- Leow JWH, Verma RK, Lim ABH, Fan H, and Chan ECY (2021) Atypical kinetics of cytochrome P450 2J2: epoxidation of arachidonic acid and reversible inhibition by xenobiotic inhibitors. *Eur J Pharm Sci* **164**:105889.
- Levine BS and Song M (1996) Pharmacokinetics and efficacy of pulse oral versus intravenous calcitriol in hemodialysis patients. *J Am Soc Nephrol* **7**:488–496.
- Mawer EB, Backhouse J, Holman CA, Lumb GA, and Stanbury SW (1972) The distribution and storage of vitamin D and its metabolites in human tissues. *Clin Sci* **43**:413–431.
- McNally K, Cotton R, and Loizou GD (2011) A workflow for global sensitivity analysis of PBPK models. *Front Pharmacol* **2**:31.
- Meekins ME, Oberhelman SS, Lee BR, Gardner BM, Cha SS, Singh RJ, Pettifor JM, Fischer PR, and Thacher TD (2014) Pharmacokinetics of daily versus monthly vitamin D<sub>3</sub> supplementation in non-lactating women. *Eur J Clin Nutr* **68**:632–634.
- Morris MD (1991) Factorial sampling plans for preliminary computational experiments. *Technometrics* **33**:161–174.
- Munns CF, Shaw N, Kieley M, Specker BL, Thacher TD, Ozono K, Michigami T, Tiosano D, Mughal MZ, Mäkitie O, et al. (2016) Global consensus recommendations on prevention and management of nutritional rickets. *Horm Res Paediatr* **85**:83–106.
- Noh K, Yang QJ, Sekhon L, Quach HP, Chow ECY, and Pang KS (2020) Noteworthy idiosyncrasies of 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> kinetics for extrapolation from mouse to man: commentary. *Biopharm Drug Dispos* **41**:126–148.
- Ocampo-Pelland AS, Gastonguay MR, French JF, and Riggs MM (2016) Model-based meta-analysis for development of a population-pharmacokinetic (PPK) model for Vitamin D<sub>3</sub> and its 25OHD<sub>3</sub> metabolite using both individual and arm-level data. *J Pharmacokinetic Pharmacodyn* **43**:191–206.
- Ocampo-Pelland AS, Gastonguay MR, and Riggs MM (2017) Model-based meta-analysis for comparing Vitamin D<sub>2</sub> and D<sub>3</sub> parent-metabolite pharmacokinetics. *J Pharmacokinetic Pharmacodyn* **44**:375–388.
- Pérez-López FR, Brincat M, Erel CT, Tremollieres F, Gambacciani M, Lambrinoudaki I, Moen MH, Schenck-Gustafsson K, Vujovic S, Rozenberg S, et al. (2012) EMAS position statement: Vitamin D and postmenopausal health. *Maturitas* **71**:83–88.
- Pianosi F, Sarrazin F, and Wagener T (2015) A Matlab toolbox for global sensitivity analysis. *Environ Model Softw* **70**:80–85.
- Pludowski P, Karczarewicz E, Bayer M, Carter G, Chlebna-Sokół D, Czech-Kowalska J, Dębski R, Decsi T, Dobrzańska A, Franek E, et al. (2013) Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe - recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency. *Endokrynol Pol* **64**:319–327.
- Quach HP, Yang QJ, Chow EC, Mager DE, Hoi SY, and Pang KS (2015) PKPD modelling to predict altered disposition of 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> in mice due to dose-dependent regulation of CYP27B1 on synthesis and CYP24A1 on degradation. *Br J Pharmacol* **172**:3611–3626.
- Ramakrishnan V, Yang QJ, Quach HP, Cao Y, Chow EC, Mager DE, and Pang KS (2016) Physiologically-based pharmacokinetic-pharmacodynamic modeling of 1 $\alpha$ , 25-dihydroxyvitamin D<sub>3</sub> in mice. *Drug Metab Dispos* **44**:189–208.
- Rizzoli R, Boonen S, Brandi ML, Bruyère O, Cooper C, Kanis JA, Kaufman JM, Ringe JD, Weryha G, and Reginster JY (2013) Vitamin D supplementation in elderly or postmenopausal women: a 2013 update of the 2008 recommendations from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Eur Med Res Opin* **29**:305–313.
- Ross AC (2011) The 2011 report on dietary reference intakes for calcium and vitamin D. *Public Health Nutr* **14**:938–939.
- Ross A, Taylor C, Yaktine A, and Del Valle H (2011) *Dietary Reference Intakes for Calcium and Vitamin D*. National Academies Press.
- Roth DE, Al Mahmud A, Raqib R, Black RE, and Baqui AH (2012) Pharmacokinetics of a single oral dose of vitamin D<sub>3</sub> (70,000 IU) in pregnant and non-pregnant women. *Nutr J* **11**:114.
- Sakaki T, Kagawa N, Yamamoto K, and Inouye K (2005) Metabolism of vitamin D<sub>3</sub> by cytochromes P450. *Front Biosci* **10**:119–134.
- Sawyer ME, Tran HT, and Evans MV (2017) A physiologically based pharmacokinetic model of vitamin D. *J Appl Toxicol* **37**:1448–1454.
- Schuster I (2011) Cytochromes P450 are essential players in the vitamin D signaling system. *Biochim Biophys Acta* **1814**:186–199.
- Sheiner LB and Beal SL (1981) Some suggestions for measuring predictive performance. *J Pharmacokinetic Biopharm* **9**:503–512.
- Shinkyo R, Sakaki T, Kamakura M, Ohta M, and Inouye K (2004) Metabolism of vitamin D by human microsomal CYP2R1. *Biochem Biophys Res Commun* **324**:451–457.
- Soullis-Liparota T, Cooper M, Papazoglou D, Clarke B, and Jerums G (1991) Retardation by aminoguanidine of development of albuminuria, mesangial expansion, and tissue fluorescence in streptozocin-induced diabetic rat. *Diabetes* **40**:1328–1334.
- Stubbs JR, Zhang S, Friedman PA, and Nolin TD (2014) Decreased conversion of 25-hydroxyvitamin D<sub>3</sub> to 24, 25-dihydroxyvitamin D<sub>3</sub> following cholecalciferol therapy in patients with CKD. *Clin J Am Soc Nephrol* **9**:1965–73.
- van Schoor N and Lips P (2018) Worldwide vitamin D status, in *Vitamin D* pp 15–40, Elsevier Publishing Co., Boston.
- Wang Z, Schuetz EG, Xu Y, and Thummel KE (2013) Interplay between vitamin D and the drug metabolizing enzyme CYP3A4. *J Steroid Biochem Mol Biol* **136**:54–58.
- Wang Z, Wong T, Hashizume T, Dickmann LZ, Scian M, Koszewski NJ, Goff JP, Horst RL, Chaudhry AS, Schuetz EG, et al. (2014) Human UGT1A4 and UGT1A3 conjugate 25-hydroxyvitamin D<sub>3</sub>: metabolite structure, kinetics, inducibility, and interindividual variability. *Endocrinology* **155**:2052–2063.
- Wong T, Wang Z, Chapron BD, Suzuki M, Claw KG, Gao C, Foti RS, Prasad B, Chapron A, Calamia J, et al. (2018) Polymorphic human sulfotransferase 2A1 mediates the formation of 25-hydroxyvitamin D<sub>3</sub>-O-Sulfate, a major circulating vitamin D metabolite in humans. *Drug Metab Dispos* **46**:367–379.

Address correspondence to: Colton W. Sawyer, 2500 North River Road, Manchester, NH, 03106. E-mail: c.sawyer1@snhu.edu

```
% Supplemental Material: Drug Met Disp, DMD-AR-000609
% Title: Physiologically Based Pharmacokinetic Modeling of Vitamin D3 and Metabolites in
Vitamin D Insufficient Patients
% Authors: Colton W. Sawyer, Stacey M. Tuey, Raymond E. West III, Thomas D. Nolin,
Melanie S. Joy
% Code title: DynamAdi
```

```
function [AdiPC] = DynamAdi(K, venconc)
% K = Kinetic values structure
% Adiconc = concentration of venous in VitD and 25D
% AdiPC = adjusted adipose PC for VitD and 25D

Adimax = [K.Adimax K.Adimax25] ;
hAdi = [K.hAdi K.hAdi25];
Adi50 = [K.Adi50 K.Adi50_25];

AdiPC(1) = Adimax(1) ./ (1 + ( Adi50(1)./venconc(1)).^hAdi(1));
AdiPC(2) = Adimax(2) ./ (1 + ( venconc(2)./Adi50(2)).^hAdi(2));

end
```

```

%
% Supplemental Material: Drug Met Disp, DMD-AR-000609
% Title: Physiologically Based Pharmacokinetic Modeling of Vitamin D3 and Metabolites in
Vitamin D Insufficient Patients
% Authors: Colton W. Sawyer, Stacey M. Tuey, Raymond E. West III, Thomas D. Nolin,
Melanie S. Joy
% Code title: main

```

```

function [serum, A, tout] = main(T, g, varargin)

```

```

% -----
% in:
% T = parameter table
% g = group of interest
% varargin = {doseIU, suppweeks}
% out:
% serum = {D, 25, 125, 2425}
% A = Amt (nmol where appropriate)
% tout = time (days)
% -----

```

```

if nargin==4
    doseIU = varargin{1};
    suppweeks = varargin{2};
else
    doseIU = 5000;
    suppweeks = 16;
end

```

```

WD = 384.64; %weight of vitamin D in g/mol = Da
doseug = doseIU/40; %IU -> ug
dosenmol = doseug*1e3/WD; %ug -> nmol

```

```

[P] = initconcs(T,g); %subfunction to get baseline concentration
%initial conditions for vitD
InitAmts = [P.AmtD; P.Amt25; P.Amt125; P.Amt2425];
initbase = [1; ones(4,1)]; %baseline fold: 27B1; 24A1 for 4 tissues
initrates = zeros(11,1);

```

```

T.Pars = P; %update with initial concentrations.

```

```

t1 = 2*7;
t2 = suppweeks*7;
t3 = 2*7;

```

```

daycount = 1:sum([t1,t2,t3]); %16 week trial\

```

```

tvec = 24.*[0:daycount(end)]; %time markers (hours)

```

```

dailyintake = 0; %average dietary supplement
dailydose = [zeros(t1,1); dosenmol*ones(t2,1); zeros(t3,1);0];
dailydose = dailydose + dailyintake*ones(size(dailydose));
initdose = [dosenmol; zeros(t1-1,1); zeros(t2,1); dosenmol; ...
    zeros(t3-1,1);0];

```

```

intake = initdose + dailydose;

```

```

initamt = [InitAmts(:); %nmol [D, 25, 125, 2425]
    initbase(:); %baseline fold change
    inirates(:); %synthesis and elimination rate tracking
    initdose(1)]; %initial gut dose

```

```

%%%%%%%%%%
refine = 4;

```

```

options = odeset('Refine',refine);

tout = tvec(1);
A = initamt';

%these will be called in multiple times, reduce number of passes
P = T.Pars;
Kin = T.Kinetics(1,:);
Kstruct = table2struct(Kin);

for i = 1:length(tvec)-1 %counts the number of terminal events
    %use vectorized PBPK file with the structure for kinetics
    helper = @(t, amt) PBPK(t,amt,T,Kstruct);
    [t,Aout] = ode15s(helper,[tvec(i) tvec(i+1)],initamt,options);
    nt = length(t);
    vec = 2:nt;
    tout = [fliplr(tout); t(vec)];
    A = [A; Aout(vec,:)]; %nmol in plasma, total amount

    % Set the new initial conditions, introducing input every 24ish hours
    initamt = Aout(nt,:);
    initamt(end) = initamt(end) + intake(i+1); %to GI reserve
end

%% now extrapolate total amount in venous plasma.

serum.time = tout(1:end)/24;

Vplas = P.Vol(9); %volume of plasma
totserum = A(:,9:9:(9*4))./(Vplas); %nmol/L in plasma

serum.vD = totserum(:,1);
serum.v25 = totserum(:,2);
serum.v125 = totserum(:,3);
serum.v24 = totserum(:,4);

end %----- End Main Function -----

%% Initial Concentrations
function [P] = initconcs(T,g)

P = T.Pars;
K = T.Kinetics(1,:);

%P = parameter table
%g = group number

[D] = readdata; % D = table of data

WD = 384.64; %weight of vitamin D in g/mol = Da
W25 = 400.64; %g/mol
W125 = 416.6; %g/mol
W24 = 416.64; %g/mol
MolWeights = [WD; W25; W24; W125];
CF = 1e3./MolWeights; %conversion from ug/L to nmol/L

%now take the average of the first and second time points because there is
%a dip between these points for all cases (0-30min)
mpvD = nanmean(D.medVitD3{g}(1:2)).*CF(1); % nmol/L
mpv25 = nanmean(D.med25{g}(1:2)).*CF(2); %
mpv2425 = nanmean(D.med2425{g}(1:2)).*CF(3); %
mpv125 = nanmean(D.med125{g}(1:2)).*CF(4); %

```

```

%% now use ideas of Ramakrishnan2016 (A2): C_(T,base)/C_(Plas,base) = K_T
%% % % => C_(T,base) = K_T .*C_(Plas,base)
P.ConcD = P.PC_D.*mpvD; %this will be used later in ENDOG in the PBPK file
P.Conc25 = P.PC_25.*mpv25;
P.Conc125 = P.PC_125.*mpv125;
P.Conc2425 = P.PC_2425.*mpv2425;
%
% allow for dynamic adipose partition coefficient based on total 25D conc
% in venous
if K.AdiPC
    venconc = [P.ConcD(end) P.Conc25(end)];
    AdiPC = DynamAdi(K,venconc); %pass in venous concentrations
    P.ConcD(5) = mpvD*AdiPC(1);
    P.Conc25(5) = mpv25*AdiPC(2);
end

P.AmtD = P.ConcD.*P.Vol; % = nmol/L * L = nmol for amt in
P.Amt25 = P.Conc25.*P.Vol;
P.Amt125 = P.Conc125.*P.Vol;
P.Amt2425 = P.Conc2425.*P.Vol;

end

```

```

% Supplemental Material: Drug Met Disp, DMD-AR-000609
% Title: Physiologically Based Pharmacokinetic Modeling of Vitamin D3 and Metabolites in
Vitamin D Insufficient Patients
% Authors: Colton W. Sawyer, Stacey M. Tuey, Raymond E. West III, Thomas D. Nolin,
Melanie S. Joy
% Code title: Pars

```

```
function [T] = Pars
```

```
%output: T = structure of parameters for model, fields:
```

```

%       T.Pars: Physio table
%       T.Kinetics: Kinetic parameter table
%       T.Misc: Misc structure
%       T.C: Conversion structure
%
%-----

```

```
%% Table setup
```

```
% method of plasma rate flow taken from Ramakrishnan2016
```

```

VarNames = {'Vol', 'Rate', 'PC_D', 'PC_25', 'PC_125', 'PC_2425'};
Doublecell = cellfun(@char, repmat({'double'}, 1, length(VarNames)), 'Un', 0);

```

```
VarTypes = [Doublecell(:)];
```

```
compt = {'Liv', 'Kid', 'Brain', 'Int', 'Adi', 'RP', 'SP', 'Heart', 'Plasma'};
```

```
TblSz = [length(compt), length(VarNames)];
```

```

P = table('Size', TblSz, 'VariableNames', VarNames, 'VariableTypes', ...
        VarTypes, 'RowNames', compt);
%-----

```

```
%% physio parameters (Jones 2013)
```

```
BW = 70; %in kg
```

```
CO = 5.600; %L/min %Davies1993; %15*(BW)^(0.74); %L/hr (Brown 1997)
```

```
BP = .55; % blood:plasma ratio = (1-.45)
```

```
%%%%%%%%%% VOLUMES in L (assuming BW = 70kg) (Davies1993)
```

```
VPlasma = 3.000; %
```

```
VL = 1.690; %Liver
```

```
VK = 0.280; %Kidney
```

```
VBr = 1.450; %brain
```

```
VHt = 0.310; %heart
```

```
% VB = 14.3; %bone ----- density = 1.92 g/cm3
```

```
VSP = 42.800; %Muscle + skin
```

```
VAdi = 10.000; %adipose ----- density = 0.916 g/cm3
```

```
VI = 0.637; %Intestine --- 0.91%
```

```
%Brown, Delp, Lindstedt, Rhomberg, & Beliles, 1997 in Noh2020
```

```
VRP = BW - (VPlasma + VL + VK + VBr + VHt + VSP + VAdi + VI); %RP tissue
```

```
P.Vol = [VL; VK; VBr; VI; VAdi; VRP; VSP; VHt; VPlasma]; %this is in L=kg
```

```
%%%% BLOOD FLOW RATES in L/min, adjusted to plasma flow later (Davies1993)
```

```
qL = 0.300; %liver % this is the hepatic flow rate
```

```
qK = 1.240; %kidney
```

```
qBr = 0.700; %brain
```

```
qHt = 0.240; %heart
```

```
qSP = 1.050; %muscle + skin
```

```
qAdi = 0.260; %adipose
```

```
qI = 1.150; %intestine + spleen --- portal vein
```

```
qRP = CO - (qL + qK + qBr + qHt + qSP + qAdi + qI); %rapidly perfused
```

```
qPlasma = CO; %L/min blood flow - will convert to plasma flow rate next
```

```
%plasma flow rates since D binds only in plasma with VDP (vitamin d binding
```



```

%protein) Ramakrishnan2016
% converted to L/hr %% plasma flow rate
P.Rate = [qL; qK; qBr; qI; qAdi; qRP; qSP; qHT; qPlasma].*(60.*BP);

%-----
%% tissue:plasma PCs -- function below
[P,M] = ParCoeffs(P);
%[L;K;Br;Int]
mousewts = [1.75; 0.32; 0.506; 0.36]*1e-3; %mL -> L mouse vol Noh2020
humanwts = P.Vol(1:4); %L human tissue volume
C.manmouse = humanwts./mousewts;
%-----
%% kinetics table

VarNames = {'Kinetic', 'LB', 'UB'};
Doublecell = cellfun(@char, repmat({'double'},1,length(VarNames)), 'Un', 0);

VarTypes = Doublecell(:);

kins = {'VmaxD', 'KmD', ...%clearance of D to 25(OH)D - Ocampo-Pelland2016
'R27B1', ... %ratio for CYP27B1
'Rsyn24L', 'Rsyn24K', 'Rsyn24Br', 'Rsyn24I', ...%rate of synthesis for 24,25 from 25 by 24A1
'fkCL2425', ... %free fraction clearance of 24,25 from kidney
'IC50', 'EC50L', 'EC50K', 'EC50Br', 'EC50I', ... %(Ramakrishnan2016)
'Imax', 'EmaxL', 'EmaxK', 'EmaxBr', 'EmaxI', ... %(Ramakrishnan2016)
'g1L', 'g1K', 'g1Br', 'g1I', ... %gamma 1 (Ramakrishnan2016, Noh2020)%
'kout24L', 'kout24K', 'kout24Br', 'kout24I', ... %kin for 24A1 (Ramakrishnan2016)
'fLCL125L', 'fKCL125K', 'fBrCL125Br', 'fICL125I', ... %CL_int,met,T for 24A1 (Ramakrishnan2016)
'g2', ...%'kin27', .
'kout27', ... %CYP27B1 (Ramakrishnan2016, Noh2020)
'Dbase', ...%(Ocampo-Pelland2016)
'ka', ... %endogenous base, gut absorb (Ocampo-Pelland2016)
'maxVmax', 'hVmax', 'D50', 'VarVmax', ...% variable vmax values
'Adimax', 'hAdi', 'Adi50', ...% variable adipose PC
'Adimax25', 'hAdi25', 'Adi50_25', 'AdiPC' ...% variable adipose PC
};

%
TblSz = [length(kins),length(VarNames)];

K = table('Size', TblSz, 'VariableNames', VarNames, 'VariableTypes', ...
VarTypes, 'RowNames', kins);

%%
K.Kinetic = [%1-2
1.62; %VmaxD %Ocampo-Pelland2016
5.90; %KmD
%% 3
17.7; %R27B1 %endog synth of 1,25 from 25D;
%% 4-7
%% rate of synthesis of 24,25 from 25 by 24A1
1.20; %Rsyn24L
2.43; %Rsyn24K
13.3; %Rsyn24Br
15.4; %Rsyn24I
%% 8
%% free fraction clearance of 24,25 from kidney
990; %fkCL2425; assume units are pmol/hr
%%
%%Inhib/Stim Ramakrishnan2016 fitted values (TM) model
%%UNITs pmol/kg tissue
%% 9-13

```

```

673; %IC50
2600; %EC50L
143;%113; %EC50K
97;%235; %EC50Br
2640; %EC50I
%% Imax and Emax %Noh2020 combined doses
%% 14-18
1; %Imax %upper bound = 1
122; %EmaxL
25; %EmaxK
22; %EmaxBr
675; %EmaxI
%%
%% 19-22
%% Hill constant for indirect response function of 24A1
3.05; %g1L %Ramakrishnan2016 TM model
3.59; %g1K %Ramakrishnan2016
1.12; %g1Br %Ramakrishnan2016
4.16; %g1I %Ramakrishnan2016
%% turnover rates of CYP24A1
%% 23-26
0.047; %kout24L
0.28; %kout24K
0.86; %kout24Br
0.047; %kout24I
%%
%%-----
%% free fraction CL_int,met,T (Noh2020)-- units mL/hr/g tissue
%% tissue - will adjust in PBPK code to account for man
%% vs mouse tissue
%% 27-30
0.00057; %fLCL125L
0.066; %fKCL125K
0.0083; %fBrCL125Br
0.0028; %fICL125I
%% -----
%%
%% 31-32
%% parameters for CYP27B1
2.7; %g2 , %Ramakrishnan2016 TM model
0.245; %kout27b1
%% 33-34
%% Dbase and gut absorption (Ocampo-Pelland2016)
2.05; %Dbase, nmol/L
0.32; %gut absorption 1/hr
%% 35-38
%% Variable Vmax
5.76; %max Vmax (in def state)
2.27; % exponential coeff for dynamic kid PC
8.34;%; %50% concentration of D nmol/L
1;%Vmax switch
%% 39-41
%% variable adipose PC D
9.9; %adimax
2.12; %hadi
8.97; %Adi50 D (nmol/L)
%% 42-45
%% variable adipose PC 25
1.64; %adimax25
.96; %hadi25
117; %Adi50 25 (nmol/L)
1;%adipose switch
];

```

```

%% -----
K = rows2vars(K);

K.Properties.RowNames = K{:,1};
K(:,1) = []; %delete first row which is unnecessary now

K{2,:} = .5*K{1,:};
K{3,:} = 2*K{1,:};
    K{[2 3],14} = 1; %Imax
    K{[2 3],38} = 1; %variable vmax switch
    K{[2 3],45} = 1; %variable adiPC switch

%-----
%% Now package everything into a structure
T.Pars = P;
T.Kinetics = K;
T.Misc = M;
T.C = C;
T.C.optvec = [1:13, 15:37, 39:44]; %leave out imax, vmax switch
T.C.defopt = [3:8 35:37 39:44];
end

%% -- ParCoeffs function -----
function [P,M] = ParCoeffs(P)

% P = parameter table; will update with partition coefficients
% M = structure with misc data

%% Fraction unbound in plasma
%build table M for miscellaneous data here
%convert everything to mol/L
WD = 384.64; %weight of vitamin D in g/mol = Da
W25 = 400.64; %g/mol
W125 = 416.6; %g/mol
W24 = 416.64; %g/mol

MolWeights = [WD; W25; W125; W24];
freefrac = [0.07; 0.03; 0.43; 0.03]*1e-2;

M.fup = freefrac;
M.MolWeights = MolWeights;

%-----
%% Partition coefficients
% %
% compt = {'Liv','Kid','Brain','Int','Adi','RP','SP','Heart', 'Plasma'};
%
% now use comparison of concentrations of D and 25D in different
% compartments
%kid, liv, brain, intestines ratios from Ramakrishnan2016, assume
% heart=liver
BlumSerummol = 7.8;%nmol/kg Blum et al 2008. Endocrine (33)
BlumSerumug = BlumSerummol*WD/1e3; %ug/kg

%data taken from Jakobsen2007, Br J Nutr (98) in ug/kg
P.PC_D([1:4 8]) = (2.67/BlumSerumug)*[1 2 1/3 8/3 1]; %[0.8895]
P.PC_D(5) = (7.47/BlumSerumug); %[2.4885]; adipose
P.PC_D(6) = (1.11/BlumSerumug); %[0.3698]; rapidly (lean)
P.PC_D(7) = (2.99/BlumSerumug); %[0.9961]; Slowly perfused (skin)

JSerum25D = 18.1; %ug/kg
P.PC_25([1:4 8]) = (3.95/JSerum25D) * [1 2 1/3 8/3 1]; %[0.2182]
P.PC_25(5) = (7.47/JSerum25D);% [0.4127]; adipose

```

```
P.PC_25(6) = (0.89/JSerum25D);% [0.0492]; rapidly (lean)
P.PC_25(7) = (3.18/JSerum25D);%[0.1757]; Slowly perfused (skin)

%[L,K,Br,I], liv = heart
P.PC_125([1:4 8]) = [0.15; 0.34; 0.05; 0.4; 0.15]; %Ramakrishnan2016
P.PC_125(5) = P.PC_25(5)./P.PC_25(7) *0.335; %ratio of 25D for adipose/SP
P.PC_125(6) = P.PC_25(6)./P.PC_25(2) *0.34; %assume RP = ratio 25D RP/kid
P.PC_125(7) = 0.335; %SP = peripheral compartment in Noh2020

P.PC_2425 = P.PC_125; %comparable molecular weights

%set plasma PCs = 1 because ratio of plasma/plasma = 1
P.PC_D(9) = 1;
P.PC_25(9) = 1;
P.PC_125(9) = 1;
P.PC_2425(9) = 1;

end
```

```

% Supplemental Material: Drug Met Disp, DMD-AR-000609
% Title: Physiologically Based Pharmacokinetic Modeling of Vitamin D3 and Metabolites in
Vitamin D Insufficient Patients
% Authors: Colton W. Sawyer, Stacey M. Tuey, Raymond E. West III, Thomas D. Nolin,
Melanie S. Joy
% Code title: PBPK

```

```

function [dA] = PBPK(t,A,T,K)

```

```

% A = amt vector
% T = paramter structure
% K = Kinetic structure
% dA = diff eq vector (see below)

```

```

P = T.Pars;
M = T.Misc;

```

```

% differential equations have form:

```

```

% dA = [ vit D (11)
%       25(OH)D (11)
%       1,25(OH)2D (11)
%       24,25(OH)2D (11)
%       CYP27B1 fold change (1)
%       CYP24A1 fold change (4)
% %     Vmax 2R1 eqn (1)
%       synthesis (3)
%       synthesis via 24A1 (4)
%       elimination via 24A1 (4)
%       entry (1)
%     ];
% total equations = 62

```

```

%% Separate out the amounts into compounds

```

```

A_D = A([1:9]+0*9); %ug
A_25 = A([1:9]+1*9);
A_125 = A([1:9]+2*9);
A_2425 = A([1:9]+3*9);

```

```

FC27 = A(4*9+1); %37
FC24 = A([4*9+2]:[4*9+5]); %38-41

```

```

Agut = A(end);

```

```

% free, unbound portion
fup25 = M.fup(2);

```

```

%% build the concentrations

```

```

% C_{vT} = C_T/(Kp) %Kp is the tissue:plasma PC
% Ramakrishnan2016
C_D = (A_D./P.Vol) ./ (P.PC_D); %nmol/L
C_25 = (A_25./P.Vol) ./ (P.PC_25);
C_125 = (A_125./P.Vol) ./ (P.PC_125);
C_2425 = (A_2425./P.Vol) ./ (P.PC_2425);

```

```

%
if K.AdiPC % allow for dynamic adipose partition coefficient based on total 25 conc in
venous

```

```

    venconc = [C_D(end) C_25(end)];
    AdiPC = DynamAdi(K,venconc); %pass in venous concentrations
    C_D(5) = A_D(5)./(P.Vol(5).*AdiPC(1)); %adjust adipose D partition coefficient
    C_25(5) = A_25(5)./(P.Vol(5).*AdiPC(2)); %adjust adipose 25 partition coefficient

```

```

end

```

```

ClivD = C_D(1); % liver D
C25 = C_25(1:4);
C125 = C_125(1:4); %total concentration
Kkid2425 = C_2425(2); %kidney 24,25
R27B = K.R27B1; %L/hr

%rate of synthesis of 24,25 from 25 by 24A1
Rsyn24 = [K.Rsyn24L; K.Rsyn24K; K.Rsyn24Br; K.Rsyn24I];

fkCL2425 = K.fkCL2425; %free fraction clearance of 24,25 from kidney (nmol/hr);

kout27 = K.kout27;
kout24 = [K.kout24L; K.kout24K; K.kout24Br; K.kout24I];
kin27 = kout27;
kin24 = kout24;

%gamma 1 terms for CYP24A1 Ramakrishnan2016
g1 = [K.g1L; K.g1K; K.g1Br; K.g1I];
g2 = K.g2; %hill coefficient

%free fraction unbound clearance terms %Noh2020 (mL/hr in mouse tissue -> L/hr in human
tissue)
freeCLT = [K.fLCL125L; K.fKCL125K; K.fBrCL125Br; K.fICL125I].*P.Vol(1:4); %scaled
according to tissue weight

CFpmolnmol = 1e-3; %conversion factor from pmol/L to nmol/L; 1 pmol = 1e-3 nmol

IC50 = K.IC50.*CFpmolnmol; %CONCENTRATION OF 1,25, now in nmol/L
Imax = K.Imax; %max effect - no units

EC50 = [K.EC50L; K.EC50K; K.EC50Br; K.EC50I].*CFpmolnmol; % now in concentration of 1,25
(nmol/L)
Emax = [K.EmaxL; K.EmaxK; K.EmaxBr; K.EmaxI]; %max effect, no units
%%% -----

ka = K.ka; %absorption into the gut; 1/hr

%%%
%% BASIC EQUATIONS
% rates are all plasma flow rates (already multiplied by BP)

Qlivotot = P.Rate(1) + P.Rate(4); %hepatic artery (liver) plus portal vein (intestine)

%vit D
dAD(1) = P.Rate(1).*C_D(9) + P.Rate(4).*C_D(4) - Qlivotot.*C_D(1); %liver with portal vein
dAD(2:8) = P.Rate(2:8).*(C_D(9) - C_D(2:8)); % main tissues
dAD(9) = Qlivotot.*C_D(1) + sum(P.Rate([2:3 5:8]).*C_D([2:3 5:8])) - C_D(9).*P.Rate(9); %
plasma not including intestine

%25(OH)D
dA25(1) = P.Rate(1).*C_25(9) + P.Rate(4).*C_25(4) - Qlivotot.*C_25(1); %liver with portal
vein
dA25(2:8) = P.Rate(2:8).*(C_25(9) - C_25(2:8)); % main tissues
dA25(9) = Qlivotot.*C_25(1) + sum(P.Rate([2:3 5:8]).*C_25([2:3 5:8])) - C_25(9).*P.Rate
(9); %plasma not including intestine

%1,25(OH)2D
dA125(1) = P.Rate(1).*C_125(9) + P.Rate(4).*C_125(4) - Qlivotot.*C_125(1); %liver with
portal vein

```



```

dA125(2:8) = P.Rate(2:8).*(C_125(9) - C_125(2:8)); % main tissues
dA125(9) = Qlivtot.*C_125(1) + sum(P.Rate([2:3 5:8]).*C_125([2:3 5:8])) - C_125(9).*P.
Rate(9); %plasma not including intestine

%24,25(OH)2D
dA2425(1) = P.Rate(1).*C_2425(9) + P.Rate(4).*C_2425(4) - Qlivtot.*C_2425(1); %liver with
portal vein
dA2425(2:8) = P.Rate(2:8).*(C_2425(9) - C_2425(2:8)); % main tissues
dA2425(9) = Qlivtot.*C_2425(1) + sum(P.Rate([2:3 5:8]).*C_2425([2:3 5:8])) - C_2425(9).
*P.Rate(9); %plasma not including intestine

%%%%%%%%%%%%%%
%% now the fold change terms

%Ramakrishnan2016
%27B1 terms
ICterm = 1 - (Imax.*C_125(2).^g2)./(IC50.^g2 + C_125(2).^g2);
dFC27 = kin27.*ICterm - kout27.*FC27;

%24A1 terms
ECterm = 1 + (Emax.*C125.^g1)./(EC50.^g1 + C125.^g1);
dFC24 = kin24.*ECterm - kout24.*FC24;

%DE fold change terms
dFC = [dFC27; dFC24];
dFC = dFC(:);
%% Now the metabolism terms and other rates
%units are nmol/hr
Gut = ka*Agut; %absorption from the gut based on gut amount

%% This section from Ocampo-Pelland2016

if K.VarVmax %variable Vmax
    [VmaxD] = VaribVmax(K, ClivD); %function below
else
    VmaxD = K.VmaxD; %nmol/h; Ocampo-Pelland2016
end

KmD = K.KmD; %nmol/L; %Ocampo-Pelland2016
Dbase = K.Dbase;

ENDOG = (VmaxD.*Dbase./(KmD + Dbase))*Dbase; %endogenous synth of D in nmol/hr
CLD25 = (VmaxD.*ClivD./(KmD + ClivD))*ClivD;

%% other synthesis terms
S_27B1 = C25(2)* R27B * FC27*fup25; %25-> 1,25 by 27B1 in kidney - first order rate
(Ocampo-Pelland2016)

S_24A1 = C25.*fup25 .*Rsyn24 .*FC24; %free amt

%% elimination terms
E_24A1 = C125 .* freeCLT .* FC24; %free amts

E_2425 = Ckid2425 * fkCL2425.*CFpmolnmol; %free amt %elimination of 24,25 from the
kidney, first order process

%% keep these for tracking purposes
dKin = [ENDOG; CLD25; S_27B1; S_24A1; E_24A1];

dKin = dKin(:);
%% Now adjust basic equations to include metabolism and other rates
% adjust D (1-9)

```

```
dAD(1) = dAD(1) + ENDOG - CLD25; %change in liver compt
dAD(4) = dAD(4) + Gut; %intestine
```

```
% adjust 25 (10-18)
```

```
dA25(1) = dA25(1) - S_24A1(1) + CLD25; %liver
dA25(2) = dA25(2) - S_27B1 - S_24A1(2); %kidney
dA25(3) = dA25(3) - S_24A1(3); %brain
dA25(4) = dA25(4) - S_24A1(4); %intestines
```

```
% adjust 1,25 (19-27)
```

```
dA125(1) = dA125(1) - E_24A1(1); %liver
dA125(2) = dA125(2) + S_27B1 - E_24A1(2); %kidney
dA125(3) = dA125(3) - E_24A1(3); %brain
dA125(4) = dA125(4) - E_24A1(4); %intestines
```

```
% adjust 24,25 (28-36)
```

```
dA2425(1) = dA2425(1) + S_24A1(1); %liver
dA2425(2) = dA2425(2) + S_24A1(2) - E_2425; %kidney
dA2425(3) = dA2425(3) + S_24A1(3); %brain
dA2425(4) = dA2425(4) + S_24A1(4); %intestines
```

```
dAmt = [dAD(:); dA25(:); dA125(:); dA2425(:)];
dAmt = dAmt(:);
```

```
%adjust gut
```

```
dA_gut = -Gut; %absorption from gut
```

```
%% now put it all together
```

```
dA = [dAmt; %1-36
      dFC; %37-41
      dKin; %42-52
      dA_gut]; %53
```

```
dA = dA(:); %ensure column vectors
```

```
end
```

```
%----- End Main Function -----
```

```
%% Variable Vmax function
```

```
function [Vmaxout] = VaribVmax(K, Dconc)
```

```
%
```

```
maxVmax = K.maxVmax;
hVmax = K.hVmax;
D50 = K.D50;
```

```
Vmaxout(1) = maxVmax./( 1 + (Dconc ./ D50).^hVmax);
```

```
end
```

**Supplemental Material: *Drug Met Disp, DMD-AR-000609***

**Title:** Physiologically Based Pharmacokinetic Modeling of Vitamin D<sub>3</sub> and Metabolites in Vitamin D Insufficient Patients

**Authors:** Colton W. Sawyer, Stacey M. Tuey, Raymond E. West III, Thomas D. Nolin, Melanie S. Joy

## **Supplemental Material**

### **S.1 Partition Coefficients**

Human distribution data for vitamin D<sub>3</sub> and metabolites are limited [Blum et al., 2008; Didriksen et al., 2015; Mawer et al., 1972, Ramakrishnan et al., 2016]. To approximate the tissue:plasma partition coefficient (PC) for humans for VitD<sub>3</sub> and 25D<sub>3</sub>, we used the ratio between tissue and plasma concentrations in pigs from [Blum et al., 2008, Jakobsen et al., 2007]. This study further assumed sufficient similarity between pigs and humans [Upton, 2008] and set the human tissue:plasma PCs equal to pig tissue:plasma PCs. These PCs were taken to be the ratio between tissue and serum concentrations. For VitD<sub>3</sub>, as Jakobsen et al. [2007] did not measure serum VitD<sub>3</sub> concentrations, we used the mean serum VitD<sub>3</sub> concentration from [Blum et al., 2008] to find the PCs. Rapidly perfused tissue was set equal to the “lean” compartment and slowly perfused tissue was set equal to the “skin” compartment in [Jakobsen et al., 2007]. As compound amounts in the brain, intestines, or kidney were not given, this study assumed these tissues had PCs equal to the ratio of the PCs in [Ramakrishnan et al., 2016] with respect to the liver. The heart PC was set equal to the liver PC. Note the adipose tissue:plasma PC for

VitD<sub>3</sub> and 25D<sub>3</sub> was allowed to vary in this study based on the concentrations of VitD<sub>3</sub> and 25D<sub>3</sub> using Equations (5) and (6), respectively, in the main text.

For PCs for 1,25D<sub>3</sub> and 24,25D<sub>3</sub>, this study used mouse PCs from [Ramakrishnan, et al., 2016] with the assumption that the heart and liver had identical PCs. The PC for the rapidly perfused compartment was set to the ratio of the PC of 25D<sub>3</sub> in the rapidly perfused to the kidney multiplied by the given PC in the kidney in [Ramakrishnan et al., 2016]. The adipose PC was constant and set to the ratio of the PC of 25D<sub>3</sub> in the adipose to the slowly perfused compartment multiplied by the given PC in the peripheral compartment in [Ramakrishnan et al., 2016]. The slowly perfused compartment for 1,25D<sub>3</sub> was set to the peripheral compartment. Due to the similar molecular size, PCs for 24,25D<sub>3</sub> were assumed equal to 1,25D<sub>3</sub>. All PCs can be found in Supp. Table S2.

## S.2 Supplemental References

- Blum M, Dolnikowski G, Seyoum E, Harris SS, Booth SL, Peterson J, Saltzman E, and Dawson-Hughes B (2008) Vitamin D3 in fat tissue. *Endocrine* 33:90–94.
- Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, and Beliles RP (1997) Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol Ind Health* 13:407–484.
- Davies B and Morris T (1993) Physiological parameters in laboratory animals and humans. *Pharm Res* 10:1093–1095.
- Didriksen A, Burild A, Jakobsen J, Fuskevåg OM, and Jorde R (2015) Vitamin D3 increases in abdominal subcutaneous fat tissue after supplementation with vitamin D3. *Eur J Endocrinol* 172:235–241.
- Heaney RP, Horst RL, Cullen DM, and Armas LA (2009) Vitamin D3 distribution and status in the body. *J Am Coll Nutr* 28:252–256.
- Jakobsen J, Maribo H, Bysted A, Sommer HM, and Hels O (2007). 25-Hydroxyvitamin D3 affects vitamin D status similar to vitamin D3 in pigs—but the meat produced has a lower content of vitamin D. *Brit J Nutr*, 98:908-913.
- Mawer E, Backhouse J, Holman C, Lumb G, and Stanbury S (1972) The distribution and storage of vitamin D and its metabolites in human tissue. *Clin Sci* 43:413–31.
- Ramakrishnan V, Yang QJ, Quach HP, Cao Y, Chow EC, Mager DE, and Pang KS (2016) Physiologically-based pharmacokinetic-pharmacodynamic modeling of 1 $\alpha$ , 25-dihydroxyvitamin D3 in mice. *Drug Metab Dispos* 44:189–208.
- Upton RN (2008) Organ weights and blood flows of sheep and pig for physiological pharmacokinetic modelling. *J Pharmacol Toxicol Methods* 58:198-205.

### S.3 Supplemental Tables

**Table S1:** Physiological Parameters for the PBPK model for vitamin D<sub>3</sub> and metabolites

Parameter	Description	Value	Units	Reference
<b>Miscellaneous physiological parameters</b>				
$BW$	Body weight <sup>a</sup>	70	kg	[Davies and Morris, 1993]
$Q_{CO}$	Cardiac Output	5.6	L·m <sup>-1</sup>	[Davies and Morris, 1993]
$BP$	Blood:plasma ratio	0.55 <sup>b</sup>	Unitless	[Ramakrishnan et al., 2016]
<b>Volumes</b>				
$V_{adi}$	Adipose	10.0	L	[Davies and Morris, 1993]
$V_{br}$	Brain	1.45	L	[Davies and Morris, 1993]
$V_h$	Heart	0.31	L	[Davies and Morris, 1993]
$V_i$	Intestine <sup>c</sup>	0.637	L	[Davies and Morris, 1993]
$V_k$	Kidney	0.28	L	[Davies and Morris, 1993]
$V_l$	Liver	1.69	L	[Davies and Morris, 1993]
$V_{rp}$	Rapidly perfused <sup>d</sup>	9.83	L	[Davies and Morris, 1993]
$V_{sp}$	Slowly perfused <sup>e</sup>	42.8	L	[Davies and Morris, 1993]
$V_p$	Plasma	3.0	L	[Davies and Morris, 1993]
<b>Blood flow rates</b>				
$Q_{adi}$	Adipose	8.58	L·m <sup>-1</sup>	[Davies and Morris, 1993]
$Q_{br}$	Brain	23.1	L·m <sup>-1</sup>	[Davies and Morris, 1993]
$Q_h$	Heart	7.92	L·m <sup>-1</sup>	[Davies and Morris, 1993]
$Q_i$	Intestine	37.95	L·m <sup>-1</sup>	[Davies and Morris, 1993]
$Q_k$	Kidney	40.9	L·m <sup>-1</sup>	[Davies and Morris, 1993]



$Q_l$	Liver <sup>f</sup>	9.9	$L \cdot m^{-1}$	[Davies and Morris, 1993]
$Q_{rp}$	Rapidly perfused	21.78	$L \cdot m^{-1}$	[Davies and Morris, 1993]
$Q_{sp}$	Slowly perfused	34.65	$L \cdot m^{-1}$	[Davies and Morris, 1993]

---

<sup>a</sup> Reference body weight. Organ density is assumed to be 1 kg = 1 L

<sup>b</sup> Plasma flow rates assumed a hematocrit value of 0.45 as vitamin D<sub>3</sub> and its metabolites are primarily bound to plasma compounds

<sup>c</sup> Intestine were assumed to comprise 0.91% of total body weight [Brown et al., 1997]

<sup>d</sup> Richly perfused tissues included adrenal, lungs and thyroid. This compartment served as a mass balance compartment, namely  $BW - \sum_c V_c$  for other compartments  $c$

<sup>e</sup> Poorly perfused tissues were set to the sum of muscle and skin

<sup>f</sup> Hepatic flow rate

**Table S2:** Unitless partition coefficients,  $P_{T:plasma}$ , for the PBPK model of VitD<sub>3</sub> and metabolites.

Partition coefficients were calculated as discussed in the Supplemental Materials.

Partition Coefficients	Description	$D_3$	$25D_3$	$1,25D_3$	$24,25D_3^a$
$P_{adi:p}$	Adipose/plasma	2.49	0.41	0.78	0.78
$P_{br:p}$	Brain/plasma	0.30	0.07	0.05	0.05
$P_{h:p}$	Heart/plasma	0.89	0.22	0.15	0.15
$P_{i:p}$	Intestine/plasma	2.37	0.58	0.4	0.4
$P_{k:p}$	Kidney/plasma	1.78	0.43	0.34	0.34
$P_{l:p}$	Liver/plasma	0.89	0.22	0.15	0.15
$P_{rp:p}$	Rapidly perfused/plasma	0.37	0.05	0.04	0.04
$P_{sp:p}$	Slowly perfused/plasma	1.00	0.18	0.34	0.34

$D_3$ , VitD<sub>3</sub>;  $25D_3$ , 25(OH)D<sub>3</sub>;  $1,25D_3$ , 1,25(OH)<sub>2</sub>D<sub>3</sub>;  $24,25D_3$ , 24,25(OH)<sub>2</sub>D<sub>3</sub>,

<sup>a</sup>Assume  $1,25D_3$  and  $24,25D_3$  partition coefficients are identical