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A Physiologically Based Pharmacokinetic (PBPK) Model of Vitamin D

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Abstract

Despite the plethora of studies discussing the benefits of vitamin D on physiological functioning, there are few mathematical models of vitamin D that predict the response of the body on low-concentration supplementation of vitamin D in a sunlight-restricted diet. This study developed a physiologically based pharmacokinetic (PBPK) model utilizing published human data on the metabolic cascade of orally-derived, low-concentration (placebo, 5 μg , and 10 μg) supplementation of vitamin D over the course of 28 days in the absence of sunlight. Vitamin D and its metabolites are highly lipophilic and binding assays of these compounds in serum may not account for binding by lipids and additional proteins. To compensate for the additional bound amounts, this study allowed the effective adipose:plasma partition coefficient to vary dynamically with the concentration of each compound in serum utilizing the well-known Hill equation for binding. Through incorporating the optimized parameters with the adipose partition coefficient adaptation to the PBPK model, this study was able to fit serum concentration data for circulating vitamin D at all three supplementation concentrations within confidence intervals of the data.

Short Abstract

This study developed a PBPK model utilizing published human data on the metabolic cascade of orally-derived, low-concentration supplementation of vitamin D in the absence of sunlight. In addition, this study allowed the effective adipose:plasma partition coefficient to vary with serum concentration, allowing for a fit to serum concentration data at all supplementation levels.

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Keywords

Vitamin D; PBPK

Introduction

Optimal vitamin D status has been linked to everything from cardiovascular and musculoskeletal health to protection against several types of cancer and autoimmune diseases [Pludowski *et al.*, 2013]; yet, roughly 33% of the population of the United States is either deficient or at risk for vitamin D deficiency [Looker *et al.*, 2011] and similar results can be seen worldwide [Holick, 2007; Lips, 2010]. Current recommendations suggest a minimum threshold level of 30 ng/mL for Vitamin D for sufficiency [Ross *et al.*, 2011]. Changes in lifestyle and increased time indoors are believed to be responsible for the observed increase in vitamin D deficiency. As vitamin D production occurs in the skin in response to sunlight, coupled with the average American spending an estimated 93% of every 24-hour period indoors [Wagner *et al.*, 2008], individuals are increasingly turning to oral supplements as an alternative way of obtaining vitamin D.

The primary source of vitamin D for the human body is through synthesis in the skin in response to ultraviolet (UV) exposure. However, as the strength of UV exposure is highly dependent on latitude and season, reduced sunlight exposure is correlated with lower vitamin D concentrations in the body [Krzy cin *et al.*, 2011]. Furthermore, production of vitamin D is self-limiting [Schuster, 2011], and individual response to UV exposure varies with ethnicity as well as duration of exposure [Webb, 2006]. These factors increase the difficulty of quantifying vitamin D concentration via sunlight exposure. Confounding this variation in vitamin D concentration is the oral supplementation of vitamin D. Beginning in the early 20th century, a majority of milk and cereal products in the United States have been fortified with vitamin D to stave off the effects of vitamin D deficiency [Holick, 2010]. In addition, an increasing number of adults are consuming multivitamins containing vitamin D [Gahche *et al.*, 2011]. Diet-derived vitamin D becomes a major consideration with populations in low UV-exposure environments, such as dark winters or submarines. Here, lack of exposure makes synthesis of vitamin D virtually negligible [Gilman *et al.*, 1982; Webb, 2006]. Data gathered from studies completed in these environments indicate that variation in vitamin D concentration is directly correlated to diet supplementation [Gasier *et al.*, 2014; Zwart *et al.*, 2011]. Despite the rising interest in vitamin D and its influence on biological functions in the human body, current mathematical models that describe the absorption, distribution, metabolism, and excretion, much less the biological effects of vitamin D, are limited [Ramakrishnan *et al.*, 2016]. The three main types of available mathematical models for humans describe: the interplay between vitamin D and other compounds [Chun *et al.*, 2012; Dunn, 1988]; seasonal variation of vitamin D concentrations based only on sunlight exposure [Diffey, 2010; Krzy cin *et al.*, 2011] or the combination of diet and sunlight [Brown *et al.*, 2013; Diffey, 2013]; and lastly, kinetics of solar absorption and its implication on overall vitamin D concentration [Olds *et al.*, 2008]. A notable gap is a mathematical model that can describe variations of the vitamin D metabolic cascade at the individual level for humans. Physiologically based pharmacokinetic (PBPK) models are an

established mathematical tool used to quantify absorption, distribution, metabolism and excretion (ADME) for compounds absorbed via different routes of exposure. The authors selected PBPK modeling to describe human pharmacokinetic data for vitamin D obtained in healthy individuals.

This study constructed a coupled human PBPK model of vitamin D and its primary metabolite 25(OH)D. Our PBPK model focused on low concentration daily oral supplementation used during minimal sunlight exposure during winter in the United Kingdom. Under these conditions, dermal production of vitamin D is assumed to be at a minimum. Both PBPK submodels were initialized to steady-state conditions matching literature serum values for each compound. An additional challenge presented by this compounds is due to its extremely high lipophilicity ($\log K_{ow} = 10.2$) and related impact on the adipose solubility or partition coefficient. A novel component of this model consists of the inclusion of a dynamic change in solubility reflected during quasi-steady state conditions as represented by an equivalent adipose partition coefficient. The authors also discuss potential physiological mechanisms associated with this change in adipose solubility observed in humans during winter months.

Materials and methods

Data sets used for model development and verification

This study utilized published data from [Fry, 2014] to investigate the effects of oral supplementation on vitamin D₃ (notated in this study as D) over the course of 28 days without sunlight as a confounding factor. Participants ($n = 24$) were randomly and equally allocated into three categories according to dosage concentration: a placebo dose, 5 μg (200 IU) vitamin D, and 10 μg (400 IU) vitamin D. Note while Fry [2014] tested an additional hypothesis regarding supplementation of vitamin D₂ (a plant-derived analog of vitamin D), this study focused exclusively on vitamin D to limit the potentially confounding effects of vitamin D₂ on serum metabolite concentrations [Holick *et al.*, 2008; Tripkovic *et al.*, 2012]. Data were segregated by dose and the outer 15% of the data were trimmed to reduce the effects of outliers on error calculations. Model simulations used the trimmed mean and 95% confidence intervals for calibration.

PBPK model for Vitamin D

A seven-compartment PBPK model (adipose tissue, kidney, liver, rapidly perfused tissue, slowly perfused tissue, and venous and arterial blood) was developed for vitamin D and 25(OH)D. A schematic of the PBPK model for each compound is shown in Fig. 1. A recent study suggests protein-mediated uptake of vitamin D from the intestines is possible, but concentration-dependent passive diffusion may also occur [Reboul *et al.*, 2011]. Several other confounding factors, including circulating vitamin D status, may also affect the intestinal absorption of vitamin D [Borel *et al.*, 2015]. As little is known about the protein-mediated routes, this study assumed diffusive, non-saturable, and continuous absorption of vitamin D into the liver [Hollander *et al.*, 1978; Hollis and Wagner, 2013]. Vitamin D was consumed by participants on a daily basis through normal diet (mean consumption 2.4 μg per day) and supplementation; this was incorporated into the PBPK model by pulsing the

initial condition for A_d , the amount of vitamin D effectively in the GI lumen en route to the liver, using (Eq. 1):

$$Day(t) = \begin{cases} 2.4 \mu\text{g} + Sup; & \text{mod}(t, 24) = 0 \\ 0; & \text{else} \end{cases}, \quad (1)$$

where $Day(t)$ is the input of vitamin D to the liver, Sup is one of the three supplementation amounts (0 μg , 5 μg , or 10 μg) and t is in hours. The overall rate of absorption into the liver is given by

$$dAbs/dt = K_{abs} \cdot A_d + Day(t), \quad (2)$$

where K_{abs} is the absorption constant (hr^{-1}). Estimates of vitamin D absorption are highly variable and depend on a myriad of factors, including genetic mutations in enzymes [Borel *et al.*, 2015]. This study estimated the absorption constant by utilizing the half-life of vitamin D ($T_{1/2} = 24$ hours, [Zerwekh, 2008]) to estimate the elimination constant, K_e , and the time to max concentration ($T_{max} = 4$ hours, [Heaney and Armas, 2015]) to solve Eq. (3) for K_{abs} :

$$K_e = \frac{\ln(2)}{T_{1/2}}, \quad T_{max} = \frac{\ln(K_{abs}) - \ln(K_e)}{K_{abs} - K_e}. \quad (3)$$

Physiological parameters for humans were obtained from Brown *et al.* [1997]. The slowly perfused compartment was composed of skin, muscle and bone. The rapidly perfused compartment served as a mass-balance compartment and comprised all tissue not previously accounted. Fractional blood flow rates 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. Physiological values used in this study are found in Table 1.

Partition Coefficients.—Human distribution data for vitamin D and 25(OH)D are limited [Blum *et al.*, 2008; Didriksen *et al.*, 2015; Mawer *et al.*, 1972]. Total amounts of vitamin D and 25(OH)D in each compartment obtained by Heaney *et al.* [2009] were divided by a referent human tissue volume to find concentration. This study followed the assumption in [Heaney *et al.*, 2009] that pigs are sufficiently similar to humans to allow for comparison. Tissue:plasma partition coefficients, $P_{T,p}$, were taken to be the ratio between tissue and serum concentrations. As compound amounts in the kidney were not given, this study assumed the kidney and liver partition coefficients were identical. Calculated tissue:plasma partition coefficients are given in Table 1.

Heaney *et al.* [2009] establishes typical daily intake of vitamin D is not sufficient to allow for accumulation in tissues; rather, additional vitamin D inputs are metabolized into 25(OH)D almost immediately. As this is indicative of a non-steady state situation, traditional

methods of comparing serum and tissue amounts to define a tissue:plasma partition coefficient, particularly in the adipose tissue, may not be appropriate for such a highly lipophilic compound.

The high lipophilicity of vitamin D may allow an increased binding to lipids and the vitamin D binding protein (DBP) in the plasma [Poulin, 2015], with the DBP-bound and lipid-bound fraction of vitamin D and 25(OH)D more important than previously thought [Speeckaert *et al.*, 2010]. This binding may be concentration-dependent and can influence the effective partition coefficient [Poulin and Haddad, 2012], especially in the adipose tissue compartment. To account for binding to DBP, lipids, and other plasma proteins, this study used the following equation to adjust the effective adipose:plasma partition coefficient as a function of the serum concentration of vitamin D or 25(OH)D. The effective adipose:plasma partition coefficient for each compound at time t , $P_{adi}(t)$, is given by Eq. (4):

$$P_{adi}(t) = 2 \cdot P_{adi:p} \cdot \left(\frac{C_v(t)^h}{EC_{50}^h + C_v(t)^h} \right). \quad (4)$$

$P_{adi:p}$ is the calculated partition coefficient using data from [Heaney *et al.*, 2009], $C_v(t)$ is the calculated venous concentration of the compound at time t , EC_{50} is the venous concentration of the compound associated with an effect level of 50%, and h controls the steepness of the venous concentration versus partition coefficient curve.

Metabolic Cascade.—There are four main cytochrome p450 (CYP) enzymes involved in the vitamin D cascade: CYPs 2R1, 27A1, 27B1, and 24A1. A schematic of the cascade can be found in Fig. 2, where each system block is representative of the whole-body PBPK submodel for the associated compound and enzymatic pathways are indicated between organs where appropriate. Enzymes are assumed to follow Michaelis-Menten kinetics. As *in vitro* literature values for the Michaelis-Menten constant for each enzyme are significantly higher than the circulating concentration of vitamin D or 25(OH)D [Chun *et al.*, 2012], each enzyme is operating in the linear portion of the Michaelis-Menten curve and the velocity of the hydroxylation reaction is proportional to the concentration of the substrate in tissue $[S_T]$. (Equation 5) yields the following form for each enzyme:

$$M_T^E = CL_{int}^E \cdot [S_T] \cdot U_s, \quad (5)$$

where M_T^E is the metabolic rate (ng/hr) for enzyme E in tissue T , $CL_{int}^E = V_{max}^E/K_m^E$ is the intrinsic clearance rate for enzyme E , and U_s is the unbound fraction of the substrate. As saturation of each enzyme at low supplementation concentrations is highly unlikely, this study utilizes (Eq. 5) to model the metabolism of each enzyme. *In vitro* estimates of V_{max} are given per unit of enzyme; the conversion factor for the concentration of enzymes in the liver ($P450_{liv}$) and kidneys ($P450_{kid}$) is typically given as the total sum of all enzymes per gram of tissue [Al-Jahdari *et al.*, 2006; Wilson *et al.*, 2003]. This study estimated the fraction

of relevant CYPs by multiplying (Eq. 5) by a scalar CYP_E for enzyme E. This scalar was optimized for each enzyme. Total metabolism in tissue, Met_T , is given by the sum of the metabolic rates for enzymes in that tissue. Values for constant parameters relating to each enzyme are given in Table 1.

Model Equations.—Initial estimates for vitamin D and 25(OH)D in each compartment were generated assuming individuals had achieved a steady-state plasma concentration corresponding to initial trimmed mean 25(OH)D serum measurements using data from [Fry, 2014]. The steady-state serum concentration of vitamin D was 6.7% of the serum concentration of 25(OH)D [Heaney *et al.*, 2003].

As vitamin D and its metabolites are highly lipophilic molecules with low aqueous solubility, binding to plasma proteins, including DBP, is necessary for circulation transport. Greater than 99% of vitamin D and its metabolites are bound to these carrier proteins and have limited access to target cells [Dusso *et al.*, 2005]. Only the remaining unbound (free) portion of each compound is available for metabolism. This study used the following percent estimates for the free amount of each compound: 0.07% vitamin D [Fraser, 1980] and 0.03% 25(OH)D [Bikle *et al.*, 1986]. Total serum amounts of each compound were converted to free amounts using these percentages.

The PBPK model was comprised of conventional mass balance equations assuming well-stirred distribution into each model compartment. As vitamin D and 25(OH)D are primarily bound to DBP which circulates at concentrations 20-fold higher than that of the metabolites [White and Cooke, 2000], this study assumed no binding to red blood cells occurred. The parameters in the equations refer to the arterial blood flow to tissue T , (Q_T), compound amount (A), concentration (C), volume (V), total metabolism in tissue (Met_T), and tissue:plasma partition coefficients ($P_{T:p}$). For notational convenience, Z is the set of non-metabolizing compartments, e.g., not the liver or the kidney, and M is the set of metabolizing compartments (specifically the liver and the kidney). Following the methods of [Ramakrishnan *et al.*, 2016], this study scaled the blood flow to tissues by multiplying the blood flow by BP , the blood:plasma ratio, to model the rate of flow of plasma. Mass balance differential equations are based on Fig. 1 with definitions given in Table 1:

Concentration in each tissue T :

$$C_T = A_T / V_T / P_{T:p} \quad (6)$$

Non-eliminating tissue in the set Z :

$$V_z \cdot dC_z / dt = BP \cdot Q_z \cdot (C_{ap} - C_{vp:Z}) \quad (7)$$

Eliminating tissue in the set M :

$$V_M \cdot dC_M/dt = BP \cdot Q_M \cdot (C_{ap} - C_{vp:M}) - Met_M \quad (8)$$

Arterial plasma:

$$V_{ap} \cdot dC_{ap}/dt = BP \cdot Q_{co} \cdot (C_{vp} - C_{ap}) \quad (9)$$

Venous plasma:

$$V_{vp} \cdot dC_{vp}/dt = \sum_T [BP \cdot Q_T \cdot C_{vp:T}] - BP \cdot Q_{co} \cdot C_{vp} \quad (10)$$

In the case of the liver compartment in the vitamin D submodel, (Eq. 2) is added to account for the movement of orally-obtained vitamin D into the liver. The complete PBPK model diagram, coupled through metabolism, is shown in Fig. 2.

Model simulations and parameter estimation

The model was coded in MATLAB[®] (version 8.5.0.197613 R2015a). Initial sensitivity analysis indicated the importance of *CYP*_{2R1}, *CYP*_{27B1}, *CYP*_{24A1}, %free vitamin D, the hill coefficient *h* and estimates for *EC*₅₀ for both vitamin D and 25(OH)D. All other parameters were held at available literature values. After every iteration of the optimization, model initial conditions were adjusted to account for any difference in the adipose:tissue partition coefficient.

Model evaluation and comparison

As orally-obtained vitamin D appears more rapidly in the body than sunlight- obtained vitamin D [Hollis and Wagner, 2013], the resultant serum concentration shows extreme spikes as vitamin D and its metabolites are processed in the body. The accuracy of measurements of serum 25(OH)D may be dependent of the time difference between participants taking the supplement and the serum draw. To compensate, this study utilized the first moment of the data for each 24-hour period as the model prediction for serum concentration of 25(OH)D for that period. The first moment is calculated as the arithmetic mean of the serum concentrations for each 24-hour period. This results in a smoother curve and more accurately represents the average concentration over the period of a day. Parameters were optimized using the Nelder-Mead search algorithm where the cost of fit was calculated using a weighted linear least squares comparison between the trimmed mean of the data and model predictions utilizing the 95% confidence interval for weighting.

Results

Final model predictions are shown in comparison in Fig. 3. Model predictions have been smoothed using the arithmetic mean of the 25(OH)D serum concentrations for each 24-hour period to generate a curve reflecting the overall average absorption of vitamin D each day.

This eliminates daily spikes from absorption of vitamin D in the liver. Model simulations indicated that changing the absorption rate increases the amplitude of the spikes but not the overall model predictions (results not shown). All relevant parameter values are given in Table 1. Boldface and shaded entries in this table indicate optimized parameters.

Study data contained several outliers; this is especially apparent in Fig. 3, panel (c) where an individual has an initial 25(OH)D serum value of 95.35 nmol/L. This is in severe contrast to six of the other individuals in this dataset with serum 25(OH)D values of less than 27 nmol/L. As a result, data for each dose were trimmed to eliminate the outer 15% of values to reduce the effects of such outliers.

Discussion

PBPK models are useful tools derived from mass balance principles, and used to integrate kinetic, metabolic, and biochemistry data for a series of related chemicals. One of their major advantages is their use to predict compounds that are difficult to measure clinically, in this case the physiologically active metabolite 1,25(OH)₂D, due to its reactive chemistry. A recent trend in PBPK modeling is to assess the ability of a model to describe a particular situation, rather than all possible modifications of that situation [Caldwell *et al.*, 2012]. This vitamin D model is designed to predict the effects of over-the-counter supplementation in winter months, in an attempt to avoid vitamin D deficiency. Although kinetic studies have been designed using single dosing schemes at pharmacological concentrations of vitamin D [Vieth, 1999]; questions still exist about the efficiency of using a single high dose to increase vitamin D to normal values [Chen *et al.*, 2016]. However, at high concentrations, the metabolic pathway may shift from the linear portion of the associated Michaelis-Menten curve to the nonlinear portion of the curve. This shift has implications on the impact substrate concentration has on the velocity of the enzymatic reaction. In addition, the overall health of the patient must be considered when using a high oral dose. In order to avoid potential confounding factors, this study selected a kinetic study that made use of healthy individuals with vitamin D deficiency during winter months.

There are three unique approaches taken in the development of this PBPK model. The first novel contribution consists on the quantification of the metabolic conversion between vitamin D and 25(OH)D. The enzymes capable of this conversion have been identified as members of the CYP450 superfamily. The authors were unable to find any *in vivo* parameters for the metabolic conversion across the two related chemicals. To reduce uncertainty and the number of parameters needed for optimization in the model, this study utilized the metabolic ratios for each pathway. In this case, three metabolic pathway ratios, CYP_E , were optimized for: CYP2R1, CYP27B1, and CYP24A1. The sensitivity of these parameters at physiological concentrations (results not shown) further supports the claim that CYP2R1 may be the key enzyme required for 25-hydroxylation of vitamin D versus CYP27A1, which may be more important at pharmaceutical concentrations [Adams and Hewison, 2010; Cheng *et al.*, 2004]. Future research concentrating on different dose levels can further elucidate the role of different metabolic pathways needed.

A second contribution of this PBPK model consists on its emphasis on the plasma compartment having a central role in distribution due to the importance of plasma binding. Although plasma binding has been considered for other PBPK models, a PBPK model describing a series of metabolically related compounds has unique mathematical constraints [Nestorov *et al.*, 1997] due to the structural link between compounds. The Nestorov *et al.* [1997] approach also included the impact of plasma binding due to the class of drugs selected for their study. Estimates on the amount of plasma unbound 25(OH)D have been known for 30 years [Bikle *et al.*, 1986]. However, estimates for unbound portion of vitamin D (parent compound) are less certain, with an initial estimate of 0.07% [Fraser, 1980]. This parameter was highly sensitive to changes; however, the optimized value (0.083%) for unbound vitamin D was fairly close to the value of [Fraser 1980]. Obtaining a prediction with a value close to a known measurement from the literature increases our confidence in our results.

The third contribution of this PBPK model relates to our novel description of the adipose tissue. Previous PBPK models for highly lipophilic compounds have made use of a membrane-limited description for the adipose tissue, by adding a capillary membrane and a related permeability parameter [Evans and Andersen, 2000]. Although this approach worked well for a quasi steady-state model for dioxin, our assessment for the time course suggested that a different approach was needed. In essence, the time course data indicates that steady-state values may not be achieved within 24 hours. A dynamic adipose partition coefficient using the Hill equation was adopted to reflect the empirically-observed decreased time to steady state. When the Hill coefficient is higher than one, the time course observation shifts to the left, taking less time to achieve a constant value. Our optimized value for the Hill coefficient was 4, making a shorter effective partition coefficient as suggested by the time course data. The application of a dynamic adipose partition coefficient allowed us to preserve the blood flow limited structure for both compounds related to Vitamin D.

In addition, the tissue:plasma partition coefficients for both vitamin D and 25(OH)D were calculated from experimental measurements in pigs [Heaney *et al.*, 2009]. When data for partition coefficients in humans is lacking, it is acceptable to use solubility data from an analog species that is assumed to be similar enough to humans. The use of pig data allowed us to fix the partition coefficient value for all organs, including a base value for adipose partition coefficient. The base adipose value used in our model is 2, and this value is consistent with the estimated adipose value used by [Ramakrishnan *et al.*, 2016]. Partition coefficient values for other organs were comparable to [Ramakrishnan *et al.*, 2016]. This is of importance, due to the sparse number of vitamin D PBPK models available for comparison. Thus, having a base adipose coefficient that is consistent with the other available PBPK model increases our confidence in the approach taken here.

The authors have speculated on the reason for the need for a dynamic adipose partition coefficient and its relation to the studies being performed during the winter months. The existence of brown adipose tissue has been known for some time, but recent research suggesting a possible anti-obesogenic role has contributed to increased understanding of its function in adult humans [Schulz and Tseng, 2013]. Human brown adipose tissue is a combination of white and brown adipose tissue, having distinct properties from white

adipose tissue. There is clinical evidence that brown adipose tissue becomes activated during the winter months, engaging in glucose metabolism needed to generate energy to maintain a constant internal body temperature [Au-Yong *et al.*, 2009]. This metabolism from the brown adipose depot will be constantly drawing vitamin D into the adipose compartment, slightly perturbing steady state. During summer, the brown adipose tissue has been shown to be inactive, making the adipose compartment more homogeneous in nature. Therefore, a separate but similar experiment conducted in the summer should predict a Hill coefficient close to one, where a steady state value for the adipose partitioning would be expected. Data measuring the impact of seasonal variability on the adipose kinetics of very lipophilic compounds is needed for future model refinements, including those of vitamin D like chemicals.

Although the model predictions using the dynamic partition coefficients fit within the confidence intervals of the data (Fig. 3), there is a very large degree of variability in the confidence intervals for the data. This is indicative of the variance in the ability of a human to process and sequester vitamin D. Variability in the data affects the model predictions at latter time points; the optimization process takes the variability into account through the weighted cost function. This flexibility in the optimization procedure allows the model to fit the overall trends of the data rather than just the mean data points which may be skewed by outliers. Since individual clinical data were available from [Fry, 2014], we were able to estimate our own statistics in relation to time course data after oral supplementation.

In summary, this study developed a PBPK model for vitamin D and its metabolites focusing on daily supplementation of vitamin D at physiological levels in the absence of sunlight. A modified form of the Hill equation was utilized to adjust the adipose:plasma partition coefficient dynamically with the concentrations of each metabolite in the venous compartment; this allows the model to compensate for binding of each compound to lipids and different kinds of lipids activated during the winter months. The resulting model was able to predict serum concentration data for circulating vitamin D at all three supplementation concentrations within confidence intervals of the data.

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Figure 1:
Initial PBPK model for vitamin D. Refer to Table 1 for definition of symbols. M_l and M_k are as defined in (Eq. 5)

Figure 2:

Each box is representative of a whole-body PBPK model for a compound, where pathways between boxes indicate metabolic routes with relevant enzymes as labels

Figure 3:

Serum 25(OH)D concentrations for three study doses over a period of 28 days, smoothed using the first moment of the data over each day. Measured geometric mean serum concentration relating to dosage concentrations of 0 μg (placebo) is given by • in panel (a), 5 μg by ▲ in panel (b), and 10 μg by ■ in panel (c). Error bars on the weekly data points signify 95% confidence intervals. Data taken from [Fry, 2014]

Table 1:

Physiological Parameters

Parameter	Description	Value	Units	Source
Volumes				
BW	Body Weight ^a	70	kg	[Brown <i>et al.</i> , 1997]
V_{adi}	Fraction of body weight as adipose	21.4	Unitless	[Brown <i>et al.</i> , 1997]
V_k	Fraction of body weight as kidney	0.4	Unitless	[Brown <i>et al.</i> , 1997]
V_l	Fraction of body weight as liver	2.6	Unitless	[Brown <i>et al.</i> , 1997]
V_{rp}	Fraction of body weight as rapidly perfused ^b	9.6	Unitless	[Brown <i>et al.</i> , 1997]
V_{sp}	Fraction of body weight as slowly perfused ^c	58.0	Unitless	[Brown <i>et al.</i> , 1997]
V_{ap}	Fraction of body weight as arterial blood	2.0	Unitless	[Brown <i>et al.</i> , 1997]
V_{vp}	Fraction of body weight as venous blood	6.0	Unitless	[Brown <i>et al.</i> , 1997]
Flow Rates				
Q_{co}	Cardiac output	312	L/hr	[Brown <i>et al.</i> , 1997]
BP	Blood:plasma ratio	0.55 ^d	Unitless	[Ramakrishnan <i>et al.</i> , 2016]
Q_{adi}	Fraction cardiac output to adipose	5.2	Unitless	[Brown <i>et al.</i> , 1997]
Q_k	Fraction cardiac output to kidney	17.5	Unitless	[Brown <i>et al.</i> , 1997]
Q_l	Fraction cardiac output to liver	22.7	Unitless	[Brown <i>et al.</i> , 1997]
Q_{rp}	Fraction cardiac output to rapidly perfused	25.5	Unitless	[Brown <i>et al.</i> , 1997]
Q_{sp}	Fraction cardiac output to slowly perfused	29.1	Unitless	[Brown <i>et al.</i> , 1997]
Q_{ap}	Fraction cardiac output to arterial blood	1.5	Unitless	[Brown <i>et al.</i> , 1997]
Q_{vp}	Fraction cardiac output to venous blood	4.4	Unitless	[Brown <i>et al.</i> , 1997]
Partition coefficients				
$P_{adi:p}^D$	D adipose/plasma partition coefficient	2.4	Unitless	[Heaney <i>et al.</i> , 2009]
$P_{k:p}^D$	D kidney/plasma partition coefficient ^e	1.02	Unitless	[Heaney <i>et al.</i> , 2009]
$P_{l:p}^D$	D liver/plasma partition coefficient	1.02	Unitless	[Heaney <i>et al.</i> , 2009]
$P_{rp:p}^D$	D rapidly perfused/plasma partition coefficient ^f	0.44	Unitless	[Heaney <i>et al.</i> , 2009]
$P_{sp:p}^D$	D slowly perfused/plasma partition coefficient	0.42	Unitless	[Heaney <i>et al.</i> , 2009]
$P_{adi:p}^{25}$	25(OH)D adipose/plasma partition coefficient	0.11	Unitless	[Heaney <i>et al.</i> , 2009]
$P_{k:p}^{25}$	25(OH)D kidney/plasma partition coefficient	0.23	Unitless	[Heaney <i>et al.</i> , 2009]
$P_{l:p}^{25}$	25(OH)D liver/plasma partition coefficient	0.23	Unitless	[Heaney <i>et al.</i> , 2009]
$P_{rp:p}^{25}$	25(OH)D rapidly perfused/plasma partition coefficient	0.08	Unitless	[Heaney <i>et al.</i> , 2009]
$P_{sp:p}^{25}$	25(OH)D slowly perfused/plasma partition coefficient	0.05	Unitless	[Heaney <i>et al.</i> , 2009]

Parameter	Description	Value	Units	Source
Kinetic Parameters				
K_m^{27A1}	Michelis-Menten constant for CYP27A1	3.2	$\mu\text{mol/L}$	[Sakaki <i>et al.</i> , 2005]
$V_{\max}^{24A1}$	<i>In vitro</i> maximum velocity for CYP27A1	0.269	mol/min/mol p450	[Sakaki <i>et al.</i> , 2005]
K_m^{2R1}	Michelis-Menten constant for CYP2R1	0.45	$\mu\text{mol/L}$	[Shinkyo <i>et al.</i> , 2004]
$V_{\max}^{2R1}$	<i>In vitro</i> maximum velocity for CYP2R1	1.2	mol/min/mol p450	[Shinkyo <i>et al.</i> , 2004]
K_m^{27B1}	Michelis-Menten constant for CYP27B1	0.9	$\mu\text{mol/L}$	[Tang <i>et al.</i> , 2012]
$V_{\max}^{27B1}$	<i>In vitro</i> maximum velocity for CYP27B1	1.3	nmol/min/mg p450	[Tang <i>et al.</i> , 2012]
K_m^{24A1}	Michelis-Menten constant for CYP24A1	0.16	$\mu\text{mol/L}$	[Sakaki <i>et al.</i> , 2005]
$V_{\max}^{24A1}$	<i>In vitro</i> maximum velocity for CYP24A1	0.088	mol/min/mol p450	[Sakaki <i>et al.</i> , 2005]
$P_{450_{liv}}$	Conversion factor for CYP enzymes in liver	11	nmol p450/g liver	[Wilson <i>et al.</i> , 2003]
$P_{450_{kid}}$	Conversion factor for CYP enzymes in kidney	12.8	mg p450/g kidney	[Al-Jahdari <i>et al.</i> , 2006]
CYP_{27A1}	Enzyme fraction for CYP27A1	0.010	Unitless	optimized
CYP_{2R1}	Enzyme fraction for CYP2R1	0.075	Unitless	optimized
CYP_{27B1}	Enzyme fraction for CYP27B1	0.016	Unitless	optimized
CYP_{24A1}	Enzyme scalar for CYP24A1	0.012	Unitless	optimized
Additional Parameters				
K_{abs}	Absorption constant ^g	0.88	hr ⁻¹	estimated
Unb_D	Unbound percentage of vitamin D	0.083	Unitless	optimized
Unb_{25}	Unbound percentage of 25(OH)D	0.03	Unitless	[Bikle <i>et al.</i> , 1986]
h	Hill coefficient	4.0	Unitless	optimized
EC_{50}^D	Effect level for vitamin D ^h	4.08	nmol/L	optimized
EC_{50}^{25}	Effect level for 25(OH)D	4.45	nmol/L	optimized

D, Vitamin D; 25, 25(OH)D

^aReference body weight. Organ density is assumed to be 1 kg = 1 L

^bRichly perfused tissues included adrenal, blood, brain, gastrointestinal tissues, heart, lungs and thyroid. This compartment served as a mass balance compartment, namely 100 — c V C for other compartments c

^cPoorly perfused tissues included muscle, skin, and bone

^dPlasma flow rates assumed a hematocrit value of 0.45 as vitamin D and its metabolites are primarily bound to plasma compounds

^eKidney measurements were not taken in Heaney *et al.* [2009]; this partition coefficient was set equal to the rapidly perfused tissue

^fMeasurements were set to the “remainder” compartment as given in [Heaney *et al.*, 2009]

^gEstimated using the half-life of vitamin D [Zerwekh, 2008] and the time to maximum concentration [Heaney and Armas, 2015]