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Evaluation of 14-day Concentration-time Curves of Vitamin D_3 and 25-Hydroxyvitamin D_3 in Healthy Adults With Varying Body Mass Index

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Abstract. *Background/Aim: To analyze the concentration-time curves of single-dose oral 25(OH)D_3 in comparison with vitamin D_3 in healthy adults. Patients and Methods: The pharmacokinetics observed over two weeks after orally administering single 900 μg doses of vitamin D_3 and 25(OH)D_3 to six otherwise healthy vitamin D insufficient/deficient adults participating in a broader randomized, double-blind, crossover, single center trial was analyzed. The study protocol was approved by the institutional review board (H-37167). Results: Individual concentration-time curves revealed that vitamin D_3 took longer than 25(OH)D_3 to reach its maximal concentration after ingestion in five participants. After 25(OH)D_3 ingestion, 25(OH)D_3 reached its maximal concentration, dropped rapidly, and plateaued before starting to decrease slowly. There were observable inter-individual variations in the bioavailability of vitamin D_3 and 25(OH)D_3 and the pattern of changes in 25(OH)D_3 concentration after their ingestion. Conclusion: Pharmacokinetics of 25(OH)D_3 in comparison with vitamin D_3 was illustrated and described in this study.*

Vitamin D plays a crucial role in regulating calcium and phosphate metabolism (1). Vitamin D exists in two forms, vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Vitamin D_3 is synthesized by the skin and found naturally in cod liver oil and oily fish. Vitamin D_2 is synthesized from ergosterol and found in yeast and mushrooms exposed to ultraviolet B radiation from sunlight or an artificial source. Once vitamin D_2 and vitamin D_3 enters the circulation, they are converted by the enzyme vitamin D-25-hydroxylase in the liver to 25-hydroxyvitamin D_2 [25(OH)D_2] and 25-hydroxyvitamin D_3 [25(OH)D_3], which are the major circulating metabolites of vitamin D that are clinically measured for determining vitamin D status (1, 2).

Vitamin D is a non-polar and highly lipophilic substance that is easily incorporated into lipid bilayers. Due to its lipophilic character, the gastrointestinal absorption of vitamin D takes place predominantly *via* the lymphatic pathway. Thus, vitamin D gets packed into micelles and chylomicrons and enters the lymph before being delivered to the bloodstream. It is estimated that 60% of the absorbed vitamin D binds with vitamin D-binding protein, while the rest 40% is cleared in the lipoprotein bound fraction (3). Once entering the circulation, vitamin D either binds with the vitamin D-binding protein, gets distributed mostly into the fat tissue, or gets metabolized by the liver to become the more hydrophilic form of 25(OH)D. It has been suggested that if one ingests 25(OH)D, its absorption takes place predominantly *via* the enterohepatic pathway, and, in addition, 25(OH)D is distributed more evenly throughout the body in fat, muscle, serum and other tissues (4).

Previous clinical studies have shown that 25(OH)D is markedly superior to vitamin D in raising and maintaining serum levels of 25(OH)D when being orally administered as either single dose or continuous daily doses (4-13). Consequently, 25(OH)D has been proposed to be an alternative to vitamin D for management of vitamin D deficiency or insufficiency in patients with obesity or fat malabsorptive conditions who are unable to raise serum 25(OH)D efficiently after ingesting high-dose vitamin D supplement (4). However, little is known about absorption and distribution of oral 25(OH)D compared with vitamin D in

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Table I. *Characteristics of the studied participants and pharmacokinetic parameters of oral 900 μg vitamin D_3 and 900 μg 25-hydroxyvitamin D_3.*

ID	Sex	Race	Age		BMI900 μg vitamin D_3 arm						900 μg 25-hydroxyvitamin D_3 arm					
					Baseline 25(OH)D	AUC	C_{max}	T_{max}	$T_{1/2}$	C_{trough}	Baseline 25(OH)D	AUC	C_{max}	T_{max}	$T_{1/2}$	C_{trough}
1	Female	White	25	22.8	24	3,463	42	8	46	0	20	3,357	25	6	44	14
2	Male	White	32	26.4	18	2,317	43	8	29	0	13	3,222	22	8	84	5
3	Female	Asian	27	28.2	17	3,708	64	8	32	0	15	3,155	25	6	56	7
4	Female	White	25	25.1	20	5,169	68	12	46	0	23	2,575	19	8	51	3
5	Female	White	29	26.3	19	4,125	62	12	29	3.13	22	2,276	9	6	113	4
6	Male	White	25	22.2	23	4,080	64	12	33	0	18	6,172	42	8	68	11

AUC: Area under the concentration-time curve; C_{max}: maximal change in serum concentration from baseline; T_{max}: time to maximal concentration; $T_{1/2}$: elimination half-life; C_{trough}: trough level at day 14.

human, as no previous studies have evaluated concentration-time curve of 25(OH)D at early hours after ingestion. Thus, the objective of this study was to qualitatively and quantitatively analyze the concentration-time curves of single-dose orally administered $25(OH)D_3$ in comparison with vitamin D_3 in healthy adults with the aim to gain more insights into the pharmacokinetics of $25(OH)D_3$.

Patients and Methods

Data were selected from a randomized, double-blind, crossover, single center trial aiming to investigate the pharmacokinetics of healthy adults and patients with intestinal malabsorption. The study protocol was registered on ClinicalTrials.gov (NCT03401541) and approved by the Boston University Medical Campus Institutional Review Board (H-37167). We obtained written informed consent from all participants. This study was conducted at Boston University Medical Campus at a latitude of 42.2°N during November 2018 – March 2019, when endogenous vitamin D_3 production is absent or minimal.

Participants. Among the participants enrolled in the study, we selected healthy adults who met the following inclusion criteria: age ≥18 years; healthy adults without any history of fat malabsorption; body mass index (BMI) 18.5-30 kg/m^2; and being vitamin D deficient or insufficient defined by serum total 25(OH)D <30 ng/ml. We excluded participants with the following conditions: conditions known to affect calcium and vitamin D, which include history of primary or tertiary hyperparathyroidism, hypercalcemia, chronic kidney disease, chronic liver disease, use of certain medications such as corticosteroids, antiretroviral medications, anticonvulsants, and use of tanning bed within one week before study enrollment; and history of allergy or adverse reaction to oral 25(OH)D or vitamin D.

Study intervention. We randomized all participants in a double-blinded manner (to the investigators and participants) to receive two oral doses of 450 μg of soft gel capsules (taken together) of either vitamin D_3 or $25(OH)D_3$. The capsules for both vitamin D_3 and $25(OH)D_3$ were formulated identically. After oral administration of each form of vitamin D, all participants underwent a cycle of pharmacokinetic evaluation. For each cycle, we collected blood samples of 15 ml at baseline and at 2, 4, 6, 8, 12 h and days 1, 2, 3, 7, and 14 for evaluation of serum vitamin D (D_2 and D_3) and 25(OH)D [$25(OH)D_2$ and $25(OH)D_3$] using liquid chromatography-tandem mass spectrometry by Quest Diagnostics (San Juan Capistrano, CA, USA). After at least 14 days of wash-out period (28 days after the first administration), we invited each participant to return to take either 900 μg of $25(OH)D_3$ or 900 μg vitamin D_3 (depending on the randomization) and undergo another cycle of pharmacokinetic evaluation.

Pharmacokinetic analysis. Changes from baseline in serum vitamin D_3 and serum $25(OH)D_3$ concentration were plotted to obtain concentration-time curve for each participant. Trapezoidal method was applied to calculate area under the concentration-time curve (*AUC*) from 0 to 336 h (14 days). Maximum observed changes in serum vitamin D_3 (ΔD_3) and $25(OH)D_3$ ($\Delta 25(OH)D_3$) (C_{max}), time to C_{max} (T_{max}), elimination half-life ($T_{1/2}$), and trough levels of ΔD_3 and $\Delta 25(OH)D_3$ at day 14 (C_{trough}) were determined. Collective data including age, BMI, and pharmacokinetic parameters *AUC*, C_{max}, T_{max}, $T_{1/2}$, and C_{trough} were summarized using arithmetic means, standard deviation (SD) and range. The Wilcoxon signed-rank test was applied to compare means of T_{max} and $T_{1/2}$ for vitamin D_3 and $25(OH)D_3$. Statistical significance was defined as $p<0.05$. Statistical analysis was performed using the SPSS version 23 (SPSS Inc., Chicago, IL, USA). Data illustrations were generated using the GraphPad Prism software 9.4.0 (GraphPad, La Jolla, CA, USA).

Results

We included a total of six participants into this study. Characteristics of the studied participants and pharmacokinetic variables of oral 900 μg vitamin D_3 and 900 μg $25(OH)D_3$ are shown in Table I. The mean±SD (range) age and BMI were 27.2±2.6 (25-32) years and 25.2±2.1 (22.2-28.0) kg/m^2, respectively. The mean±SD (range) baseline serum 25(OH)D levels before ingestion of vitamin D_3 and $25(OH)D_3$ were 21.3±3.2 (17-26) and 18.5±3.6 (13-23) ng/ml, respectively.

Individual concentration-time curves of orally administered single dose of 900 μg of vitamin D_3 are shown in Figure 1 [*AUC*: 3,810±854 (2,317-5,169) ng×h/ml; C_{max}: 57.4±10.7 (41.9-68.4) ng/ml; T_{max}: 10.0±2.0 (8-12) h; $T_{1/2}$:

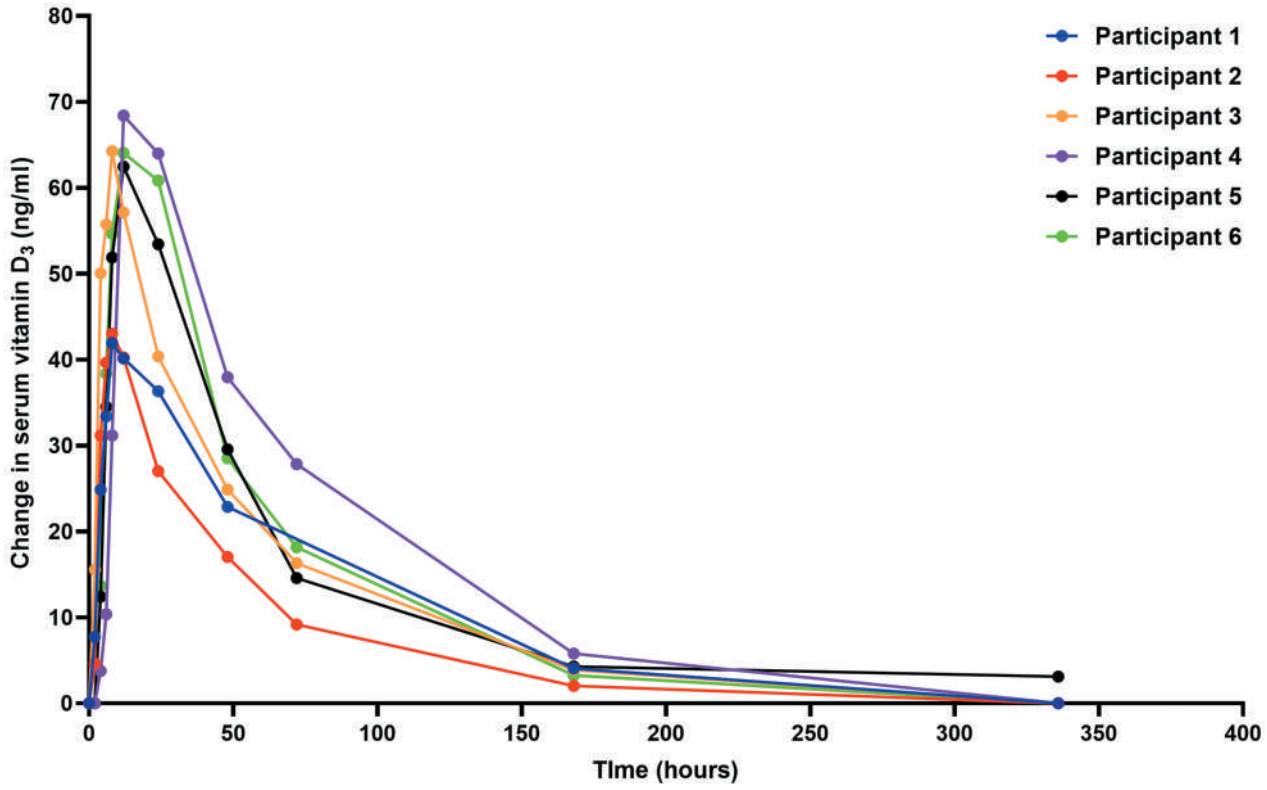

Figure 1. *Changes in serum vitamin D_3 after oral administraion of single-dose 900 μg of vitamin D_3.*

35.8±7.3 (29-46) h; C_{trough}: 0.52±1.17 (0-3.13)]. Individual changes in serum 25(OH)D_3 levels after vitamin D_3 are shown in Figure 2. Individual concentration-time curves of orally administered single dose of 900 μg of 25(OH)D_3 are shown in Figure 3 [*AUC*: 3,460±1,272 (2,276-6,172) ng×h/ml; C_{max}: 23.7±9.8 (9-42) ng/ml; T_{max}: 7.0±1.0 (6-8) h; $T_{1/2}$: 69.3±23.4 (44-113) h; C_{trough}: 7.3±3.9 (4-14)]. Serum 25(OH)D_3 reached C_{max} more rapidly after ingestion of 25(OH)D_3 compared with vitamin D_3 (p<0.05). In addition, the $T_{1/2}$ of 25(OH)D_3 was significantly higher than that of vitamin D_3 (p<0.05). According to Figure 3, participants 1, 2, 4, 6 had a small increase in serum 25(OH)D_3 after an initial peak at 48 h, whereas participants 3 and 5 had slight elevation of serum 25(OH)D_3 at 72 and 168 h, respectively. No serum vitamin D_3 was detectable in any of the participants after given oral 25(OH)D_3.

Discussion

We reported concentration-time curves of orally administered single dose of 900 μg vitamin D_3 and 25(OH)D_3 at early hours in healthy adults with normal BMI, aiming to evaluate the pharmacokinetics of vitamin D_3 and 25(OH)D_3. According to the results, there are some observations that are worth noting. First, we observed in five of six participants that vitamin D_3 takes longer than 25(OH)D_3 to reach its maximal concentration after ingestion. This is in line with the notion that, unlike vitamin D_3 that is absorbed slowly into the lymphatic system, 25(OH)D_3 is absorbed *via* the enterohepatic system. Moreover, the finding that oral administration of 25(OH)D_3 raised serum 25(OH)D_3 rapidly within 8-12 h while vitamin D_3 took 1-3 days to increase serum 25(OH)D_3 to a concentration above 30 ng/ml, suggests that oral 25(OH)D_3 or the combination of 25(OH)D_3 and vitamin D_3 may be treatment of choice in conditions that may require rapid correction of vitamin D deficiency such as symptomatic osteomalacia, hypocalcemia, or severe proximal muscle weakness due to severe vitamin D deficiency (1).

Second, after reaching its maximal concentration, 25(OH)D_3 level drops rapidly and reaches a plateau within approximately 16 h. before it starts to decrease very slowly (essentially with the $T_{1/2}$ of the DBP complex) (14). This supports that once entering the circulation, 25(OH)D_3 is likely equilibrated into different tissues such as the fat, muscles, breast, colon, prostate, and skin, which have the ability to convert 25(OH)D_3 into 1,25$(OH)_2D_3$ (15). Then, it slowly gets catabolized by the enzyme 25-hydroxyvitamin D-24-hydroxylase (CYP24A1), which exists in multiple tissues

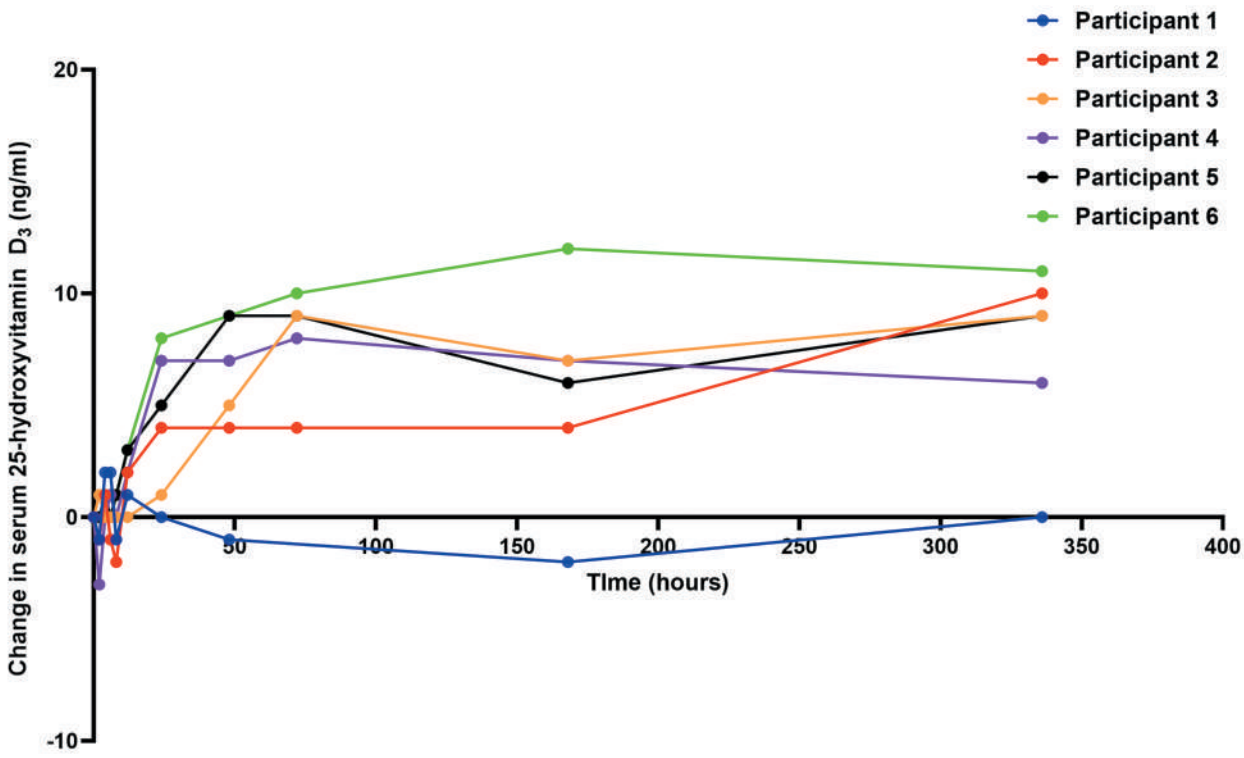

Figure 2. *Changes in serum 25-hydroxyvitamin D_3 after oral administraion of single-dose 900 μg of vitamin D_3.*

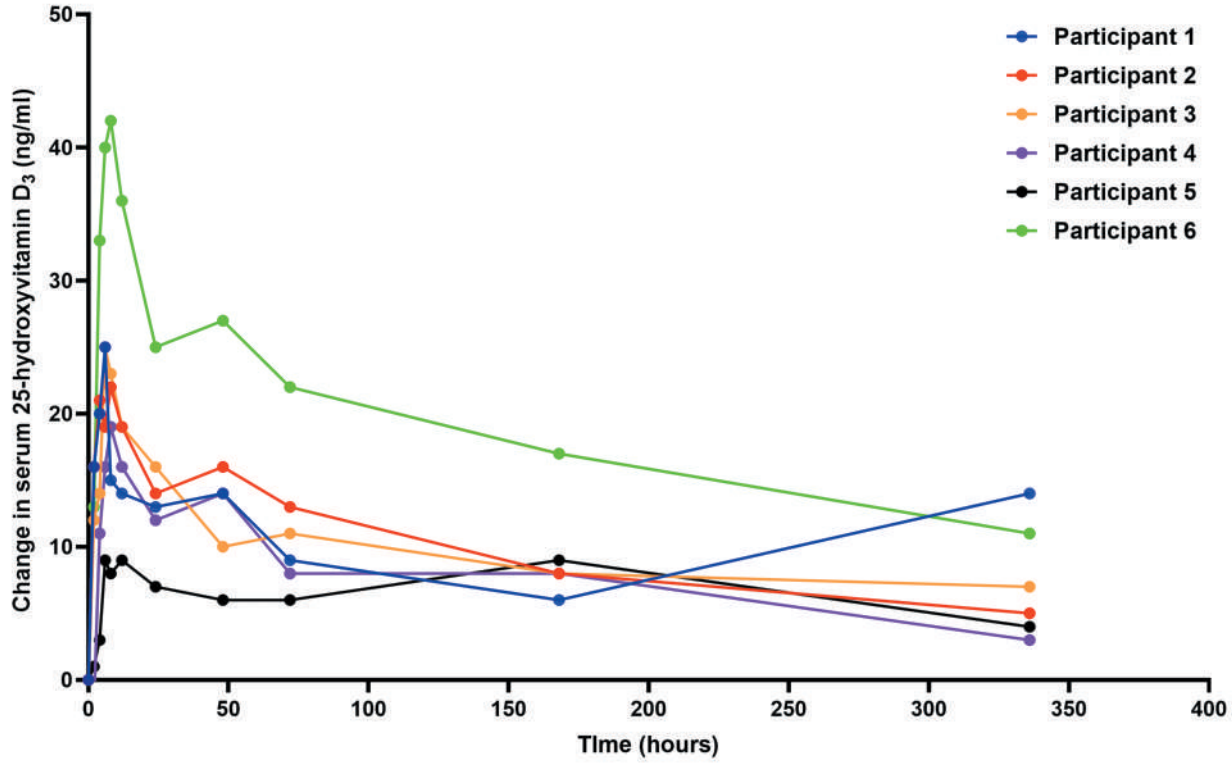

Figure 3. *Changes in serum 25-hydroxyvitamin D_3 after oral administraion of single-dose 900 μg of 25-hydroxyvitamin D_3.*

expressing the vitamin D receptor (16). Interestingly, the second increase in 25(OH)D level was observed at 48 h in four participants (participants 1, 2, 4, 6) and later in the other two participants. Although the explanation for this observation is still undetermined, it is possible that this small increase is due to the delayed release of $25(OH)D_3$ from tissues to the serum after the initial rapid uptake. However, after reaching its maximum, the tissue concentration of vitamin D_3 decreases in essence continuously until undetectable whereas serum $25(OH)D_3$ increases. This together with the significantly longer $T_{1/2}$ of $25(OH)D_3$ than vitamin D_3 suggests that the conversion of vitamin D_3 into $25(OH)D_3$ by the liver 25-hydroxylase is at a significantly higher kinetic activity than CYP24A1.

It is of particular interest that there is some inter-individual difference in the bioavailability of $25(OH)D_3$ despite relatively similar ability to absorb vitamin D_3. For example, participants 5 and 6 had similar pharmacokinetic curves for vitamin D_3. However, after ingestion of $25(OH)D_3$ participant 5 had approximately 2.7 times higher AUC and 4.7 times higher C_{max} than participant 6. This finding supports the inter-individual difference in response to vitamin D supplementation reported by previous studies, which may be in part explained by genetic variations in the vitamin D metabolic pathway (17, 18). Additionally, it strengthens the hypothesis that absorption of vitamin D and 25(OH)D may depend on different mechanisms.

It is also worth noting that there was variation in serum vitamin D_3 concentration as participants 1 and 2 had approximately 40% lower maximal change concentration than the others. The exact explanation of this variation is unknown but could be due to variation in the ability to absorb vitamin D or silent malabsorptive conditions such as celiac disease (19, 20).

This study has a number of strengths including the frequent measurements of serum $25(OH)D_3$ and vitamin D_3 that allow demonstration of concentration-time curves and the randomized crossover design, which enables comparison of the two interventions within the same individual. However, it carries certain limitations one should be aware of. First, the sample size is relatively small, and therefore further studies with a larger number of participants are required to confirm our findings. Second, this study included only healthy non-obese adults. Thus, the results may not be generalizable in patients with obesity or those with different types of malabsorptive conditions. Still, it is noteworthy that the average serum $25(OH)D_3$ levels 1 week and 2 weeks after administering vitamin D_3 increased by 1.8 ng/ml compared to a decrease by 2.0 ng/ml after administering $25(OH)D_3$ arm. If one disregards the two individuals with the lowest BMI (participants 1 and 6), the respective differences become even more significant: an average increase by 2.4 ng/ml for the vitamin D_3 arm and an average decrease by 3.4 ng/ml for the $25(OH)D_3$ arm. This points to accumulation of vitamin D_3, but not $25(OH)D_3$, in fatty tissues, an effect which is probably even more significant in obesity. Finally, serum $25(OH)D_3$ levels beyond 14 days after $25(OH)D_3$ were not measured. Further studies with longer follow-up time are warranted to determine the late elimination kinetics of $25(OH)D_3$.

Conclusion

We reported the concentration-time curves of orally administered single dose of 900 μg vitamin D_3 and $25(OH)D_3$ in healthy adults. We found that oral $25(OH)D_3$ was absorbed faster, stayed longer in the circulation, and caused a more rapid increase in serum $25(OH)D_3$ than oral vitamin D_3. These results imply that 25(OH)D is absorbed *via* the enterohepatic system unlike vitamin D that is absorbed *via* lymphatic system. Therefore, oral 25(OH)D may be a useful choice of treatment in conditions that benefit from rapid correction of vitamin D deficiency such as symptomatic osteomalacia, hypocalcemia, or proximal muscle weakness due to severe vitamin D deficiency. In the concentration-time curves of $25(OH)D_3$, there is a somewhat delayed increase in serum $25(OH)D_3$ level after its rapid peak, which may represent tissue re-equilibration of $25(OH)D_3$. Finally, we observed some inter-individual difference in the bioavailability of $25(OH)D_3$ in participants with otherwise similar bioavailability of vitamin D_3.

Conflicts of Interest

Michael F. Holick has served as a consultant for Biogena Inc., Ontometrics Inc. and Solius Inc, and has grants from Carbogen Amcis BV and Solius Inc. Peter M. Mueller has had high ranking positions in R&D and general management of Carbogen Amcis AG. He has no financial involvement with the company and is retired from all his managerial positions while still having a mail address with it and acting as scientific consultant for it and for a number of additional companies. The Authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Authors' Contributions

Conceptualization: N. Charoenngam, P.M. MUELLER, M.F. Holick; Collecting data: N. Charoenngam, M.F. Holick: Data analysis: N. Charoenngam; Writing – original draft: N. Charoenngam; Visualization: N. Charoenngam; Writing - review & editing: N. Charoenngam, P.M. Mueller, M.F. Holick.

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