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Original Article

Pharmacokinetics and effects of demographic factors on blood 25(OH)D3 levels after a single orally administered high dose of vitamin D3

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Aim: To examine the biological consequences and demographic factors that might affect the pharmacokinetics of vitamin D3 after a single high dose intervention in a young Chinese population with vitamin D insufficiency status.
Methods: A total of 28 young subjects (25 to 35 years old) with vitamin D insufficiency status [serum 25(OH)D <30 ng/mL] was recruited in Shanghai, China. The subjects were orally administered a single high dose of vitamin D3 (300 000 IU). Baseline characteristics and blood samples were collected at d 0, 1, 2, 3, 7, 28, 56, 84 and 112 after the intervention. The blood biomarker levels were determined with standardized methods.
Results: The intervention markedly increased the blood 25(OH)D3 levels within the first five days (mean T_{max}=5.1±2.1 d) and sustained an optimal circulating level of 25(OH)D3 (≥30 ng/mL) for 56 d. After the intervention, body weight and baseline 25(OH)D3 levels were significantly correlated with circulating 25(OH)D3 levels. No adverse events and no consistently significant changes in serum calcium, creatinine, glucose, parathyroid hormone, vitamin D binding protein, or the urinary calcium/creatinine ratio were observed. However, there was a significant increase in phosphorus after the vitamin D3 intervention. Total cholesterol and triglyceride levels were decreased at the end of the trial.
Conclusion: The pharmacokinetics of vitamin D after intervention were influenced by baseline 25(OH)D3 levels and the body weight of the subjects. The results suggest that a single high oral vitamin D3 intervention is safe and efficient for improving the vitamin D status of young Chinese people with vitamin D insufficiency.

Keywords: vitamin D insufficiency; vitamin D3 intervention; young Chinese people; pharmacokinetics; 25(OH)D3; body weight

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Introduction

Vitamin D has preventive effects against various types of diseases; however, the biological consequences of vitamin D intervention are not fully elucidated. For humans, there are two major sources of vitamin D: (a) conversion of 7-dehydrocholesterol into vitamin D by skin cells exposed to UV light and (b) ingestion of vitamin D-containing foods or supplements. After synthesis or absorption, vitamin D is converted into 25(OH)D by a 25-hydroxylase (CYP2R1) in the liver and then into 1α,25$(OH)_2$D by the renal 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1). The transport of vitamin D and its metabolites among tissues is mediated by their carrier, vitamin D binding protein (DBP, encoded by the *GC* gene), which is primarily produced by liver cells. In cells, 1α,25$(OH)_2$D regulates various types of signaling pathways by binding to its nuclear receptor, vitamin D receptor (VDR), which belongs to the steroid-thyroid-retinoid receptor superfamily of ligand-activated transcription factors. VDR is widely expressed in human cells, and its ligand 1α,25$(OH)_2$D regulates approximately 3%–5% of genes in the genome by stimulating VDR transcriptional activity[1]. One of the target genes for VDR is CYP24A1, the 24-hydroxylase for vitamin D, which is expressed in all types of cells and promotes the degradation of both 1α,25$(OH)_2$D and 25(OH)D in the body. As the major

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storage form of vitamin D in the blood, the circulating level of 25(OH)D is a well-established biomarker for a relatively long exposure of vitamin D status in the body[2]. 25(OH)D has a circulating half-life of 15 d; however, the half-life of 1α,25$(OH)_2$D in blood is only approximately 15 h[3]. In contrast to 25(OH)D, 1α,25$(OH)_2$D is closely regulated by parathyroid hormone (PTH), calcium, and phosphorus, and its levels are typically not decreased unless there is severe vitamin D deficiency[3].

The synthesis of vitamin D by sunlight exposure is affected by geographical regions, race, skin color, clothing, outdoor activities, and sunscreen use. The diet is a minor source of vitamin D because only a small group of foods contain high levels of vitamin D. Therefore, taking supplements containing vitamin D is an alternative method for improving vitamin D status[4]. However, clinical recommendations concerning the optimal dose and frequency of administration of vitamin D supplements to achieve and maintain sufficient vitamin D levels are lacking. To date, many vitamin D intervention trials have been performed, and it has been suggested that the daily administration of low-dose vitamin D (400 to 1000 IU/d) does not increase the circulating 25(OH)D3 levels in a beneficial time frame[5]. A high-dose vitamin D intervention (50 000 to 600 000 IU) has been recommended as a more effective and safe method to increase blood 25(OH)D3 levels[5]. A double-blind, placebo-controlled trial has found that a dose of 250 000 IU of vitamin D3 leads to a significant increase in plasma 25(OH)D3 levels but has no significant effect on the plasma levels of calcium and PTH[6]. Another study has found that a single oral dose of 600 000 IU of vitamin D3 to young participants with vitamin D deficiency leads to a rapid and safe increase in plasma 25(OH)D3 but decreases the PTH level[7]. A systematic review has suggested that a single dose of vitamin D3 (300 000 IU to 500 000 IU) is an effective and safe way to achieve relatively higher 25(OH)D levels and provide a longer benefit for individuals[8]; however, individual responses to supplementation have been found to vary. Although several studies have reported that the intervention dose, body weight, genetic factors, and baseline vitamin D levels may affect the responses to daily, lower levels of supplements (less than 1000 IU) for participants[9], there is a lack of studies investigating the pharmacokinetics of blood 25(OH)D3 levels and related factors after a large oral dose of vitamin D3. Here, we report the results of an interventional study to evaluate the biological consequences of a single oral dose of vitamin D3 (300 000 IU) in young Chinese participants with vitamin D insufficiency/deficiency status. The pharmacokinetics of blood 25(OH)D3 levels and demographic factors that may influence the time-course of 25(OH)D3 levels after the intervention were determined.

Materials and methods

Participants and study design

Healthy young participants (25 to 35 years old) were recruited in Shanghai, China, in October 2013. Eligible men or women were those with a normal body mass index (BMI, 18–25 kg/m^2) and vitamin D deficiency/insufficiency status [total 25(OH)D <30 ng/mL]. Exclusion criteria included people with: (1) clinically diagnosed cardiovascular, gastrointestinal, or mental diseases; (2) self-reported use of any dietary supplements containing vitamin D or calcium within 6 months before enrollment; (3) severe anemia (hemoglobin concentration <70 g/L); (4) serum 25(OH)D ≥30 ng/mL; (5) participation in other clinical research studies within 3 months before enrollment; (6) a history of drug dependence or drug abuse; (7) a history of heavy alcohol consumption (14 servings or more per week, equivalent to 360 mL of beer, 150 mL of white wine, or 45 mL of liquor per serving) or smoking (more than 5 cigarettes per day); (8) abnormal clinical values related to blood biochemistry, hematology, or urine analysis; and (9) current pregnancy or lactation. An invitation to participate in the study was extended to 66 potential participants who were willing to participate and had completed a pre-screening questionnaire. All participants received physical and laboratory examinations, and 38 were excluded because of sufficient vitamin D levels [circulating 25(OH)D >30 ng/mL, n=26], high or low BMI (n=6), use of vitamin D supplements (n=3), arrhythmia (n=2), or detectable kidney stones (n=1). A total of 28 subjects were eligible for the study, including 14 males and 14 females, with a mean time of outdoor activities <1 h per day. All participants provided written consent and completed the study. The Human Research Ethics Committee of the Xuhui District Center Hospital of Shanghai approved the study protocols. The clinical trial is registered in the ClinicalTrial.gov database, under trial ID NCT02158143.

Intervention methods and follow-up timelines

Physicians at the Xuhui District Center Hospital provided the vitamin D3 (Cholecalciferol Cholesterol Emulsion, Shanghai Xinyijinzhu Pharmaceutical Co, Ltd, China), a liquid formulation (300 000 IU of vitamin D3 in 8 mL), to each participant. The supplement was orally administered, and the participants were required not to take other vitamin D and/or calcium-containing supplements during the study period. Participants were followed from October 2013 to February 2014, and the study was performed during the winter, when UV radiation was limited. Clinical and personal assessments of the participants were collected on the day of supplement administration and at 1, 2, 3, 7, 28, 56, 84, and 112 d after administration. Assessments were performed to identify any adverse effects of the supplement related to hypo- and hypercalcemia (decreased appetite; weight loss; vomiting; fever or chills; constipation; abdominal pain; excessive thirst; frequent urination; muscle weakness; back, arm, or leg pain; confusion; or depression). Blood pressure and heart rate were also measured on the follow-up days. For each follow-up day, fasting blood specimens and urine samples were collected, as well as other information, including medical treatments, outdoor activities, and use of vitamin supplements.

Biochemical analyses

For each participant, 10 mL of overnight fasting blood and 15 mL of urine were provided at baseline and on follow-up

days. Blood was collected with EDTA anticoagulation tubes and centrifuged at 3000×*g* for 10 min to obtain plasma. Serum was collected by placing whole blood in tubes without anticoagulation agents for approximately 2 h after blood collection. All plasma, serum, and urine samples were stored in aliquots at -20 °C until use. The primary outcome for the study was the serum concentration of 25(OH)D3, which was determined with a Shimadzu series HPLC (Shimadzu Corporation, Japan) instrument connected to an API 4000 LC-MS/MS system (Applied Biosystems Inc, USA) at the Xuhui District Center Hospital of Shanghai. For all subjects, serum parameters, including total calcium, creatinine, glucose, triglycerides, total cholesterol, and the urinary calcium/creatinine ratio were measured with standard clinical laboratory tests. Plasma PTH was measured with an IMMULITE 1000 Immunoassay System (Siemens Healthcare Diagnostics, Germany), and plasma DBP concentrations were measured with human DBP Quantikine Enzyme-Linked Immuno Sorbent Assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Serum levels of free fatty acids were determined with a Free Fatty Acid Quantitation Kit (Sigma-Aldrich, USA) with standardized protocols. Plasma phosphorus levels were determined with colorimetric assays (Abcam, Cambridge, MA, USA). For biochemical analyses, the within-assay and between-assay coefficients of variation were <15.0%.

Statistical analysis

All continuous variables are presented as mean±SD (standard deviation), and differences among the groups were assessed by Student's *t*-tests. Repeated biochemical measurements for the participants were assessed with the repeated measures ANOVA (analysis of variance) statistical model, and comparisons of variables between baseline and follow-up time points were performed with paired *t*-tests. Multiple linear regressions were performed to determine the effects of demographic factors and baseline vitamin D3 levels on the circulating 25(OH)D3 levels after oral administration of vitamin D3. Variables considered for inclusion in the multiple linear regression were baseline 25(OH)D3 levels, sex, age, and body weight. For each follow-up day, the optimum regression model was selected on the basis of the maximum adjusted R^2 for the multiple linear regression tests. The adjusted R^2 values with 95% confidence intervals (CIs), estimated using percentiles from 500 bootstrap samples, were applied to measure the goodness-of-fit of the multiple linear regressions. Areas under the curve (AUCs) for the serum 25(OH)D3 levels were calculated by using the trapezoidal method individually and aggregated. Other pharmacokinetic parameters [time to reach maximum concentration (T_{max}) and maximum concentration (C_{max})] were calculated individually and aggregated. For all statistical analyses, significance was set at *P*-value <0.05. Statistical analyses were performed with R software (www.r-project.org) and GraphPad Prism version 5 software (GraphPad Inc, La Jolla, CA, USA).

Results

Pharmacokinetics of blood 25(OH)D3 after intervention

The baseline characteristics of the participants are shown in Table 1. No significant difference in baseline vitamin D levels was evident between male and female participants (P>0.05). Nine males and six females were classified as vitamin D insufficient [20 ng/mL < total 25(OH)D <30 ng/mL], and five males and eight females were vitamin D deficient [total 25(OH)D <20 ng/mL]. Males had higher body weight, height, BMI values, and systolic blood pressure values than females (P<0.01; Table 1). After the oral dose of 300 000 IU of vitamin D3, there were increased levels of serum 25(OH)D3 throughout the follow-up period (repeated measure ANOVA test, P<0.01; Figure 1). An increase in 25(OH)D3 levels was evident after one day, and the mean 25(OH)D3 levels peaked at d 3 [mean 25(OH)D3, 71.4±12.9 ng/mL], representing a 2.4-fold increase from the level at d 0 [21.0±4.9 ng/mL, P<0.01]. Subsequently, the mean serum 25(OH)D3 levels decreased, but the mean level at d 112 [mean 25(OH)D3, 25.8±4.5 ng/mL] remained significantly

Table 1. Baseline characteristics for the 28 participants recruited in the intervention study. Mean±SD. $^{**}P$<0.01 *vs* male group.

Characteristic	Total (*n*=28)	Male (*n*=14)	Female (*n*=14)
Age (year)	26.7±1.7	26.9±2.0	26.4±1.3
Height (cm)	166.0±10.9	173.9±7.1	158.2±8.0**
Weight (kg)	60.3±11.2	69.4±7.6	51.1±5.2**
BMI (kg/m^2)	21.7±1.8	22.9±1.4	20.5±1.2**
Systolic pressure (mmHg)	112.9±10.9	121.1±6.3	104.6±7.9**
Diastolic pressure (mmHg)	71.2±7.4	73.8±5.3	68.6±8.3
Baseline 25(OH)D2 (ng/mL)	1.1±0.7	1.0±0.3	1.2±0.9
Baseline 25(OH)D3 (ng/mL)	21.0±4.9	22.3±4.0	19.6±5.3
Baseline 25(OH)D (ng/mL)	22.0±4.7	23.3±4.1	20.8±4.9

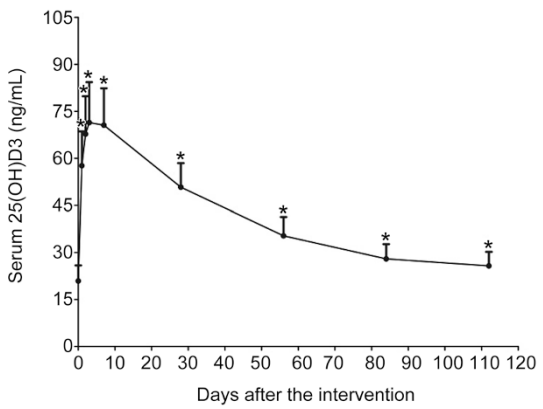

Figure 1. Time-course of mean serum 25(OH)D3 levels after a single oral administration of 300 000 IU vitamin D3 in 28 participants with vitamin D deficiency status at recruitment. Data are presented as mean±standard deviation of 25(OH)D3 level at each follow-up day. $^{*}P$<0.01 *vs* d 0 group of paired *t*-tests.

higher than at baseline (P<0.01).

The pharmacokinetic parameters for blood 25(OH)D3 levels after intervention were determined. The mean C_{max} was 52.1±11.1 ng/mL, and the mean T_{max} was 5.1±2.1 d. After d 7, the serum concentration decreased approximately linearly. The mean $AUC_{0-\infty}$ for 25(OH)D3 was 2445.3±745.1 ng*d/mL. During the entire follow-up period, no intervention-related adverse events were observed.

Influence on PTH, DBP, phosphorus, calcium, glucose, triglyceride, and cholesterol levels after vitamin D3 intervention

After the single high dose of vitamin D3, there was a consistent increase in phosphorus, with the highest level at d 28 (2.23±0.52 mmol/L). Plasma phosphorus decreased after d 28 but remained higher than baseline at d 112 (P<0.01; Table 2). After intervention, no consistent change was observed for blood glucose levels, except for slight increases in blood glucose at d 2 (4.9±0.4 mmol/L, P<0.05) and d 28 (5.0±0.4 mmol/L, P<0.01) relative to baseline (4.7±0.5 mmol/L, Table 2). There were decreased levels of serum triglycerides and total cholesterol at or near the end of the follow-up period but not at the earlier follow-up days after intervention. Serum triglyceride levels were significantly decreased at d 56, 84, and 112 (Table 2), and levels of total serum cholesterol were reduced at d 84 and 112 (P<0.01, Table 2). Consistently with decreased triglyceride levels, the circulating free fatty acids were increased at d 84 (1.55±0.16 mmol/L) and 112 (1.51±0.08 mmol/L) compared with the baseline (1.44±0.12 mmol/L, Figure 2), thus suggesting that the decrease in triglycerides may have been caused by enhanced lipolysis after vitamin D3 intervention.

The vitamin D3 intervention led to decreased serum PTH concentrations at d 2 (P<0.01 *vs* d 0), but an increase in serum PTH was observed at d 84 (P<0.01 *vs* d 0) (Table 3). For the other follow-up days, there was no significant change in PTH (P>0.05 *vs* d 0). The DBP concentrations decreased within the first 2 d after intervention, but no significant change was observed for the other days. At d 28 only, there was a small but significant increase in serum calcium levels (P<0.05 *vs* d 0).

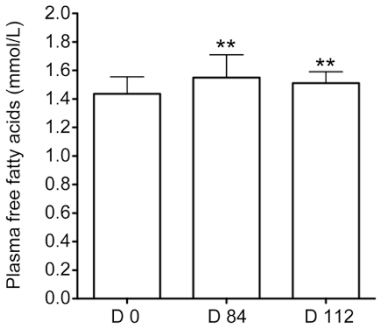

Figure 2. Levels of plasma free fatty acids at d 0, 84, and 112 after oral 300 000 IU vitamin D intervention in 28 participants. $^{**}P$<0.01 *vs* d 0 group of paired *t*-tests.

Serum creatinine increased at d 56 (P<0.01 *vs* d 0), together with a small increase in the urinary calcium/creatinine ratio (P<0.05 *vs* d 0).

Factors influencing blood 25(OH)D_3 levels after intervention

For each follow-up day, we determined the influence of demographic factors and baseline blood parameters, including body weight, sex, and age, and baseline phosphorus, 25(OH)D_3, PTH, DBP, and calcium, on plasma 25(OH)D3 levels after intervention. Only body weight and baseline 25(OH)D3 significantly correlated with levels of circulating 25(OH)D3 (Supplementary Table S1). Because blood DBP, calcium, phosphorus, PTH, and creatinine may be dependent factors for blood 25(OH)D3, the multiple regression model determined only the correlations between demographic factors (body weight, age, and sex) and baseline 25(OH)D3 and circulating 25(OH)D3 levels after intervention. During the first 28 d, demographic factors contributed to the variability of circulating 25(OH)D3 levels among individuals (R^2 range from 0.46 to 0.59; Table 4) but had less influence on this variability from d 56 to 112 ($0.13 \le R^2 \le 0.26$) (Table 4). Multiple regressions showed that body weight and baseline vitamin D3 were significantly correlated with circulating 25(OH)D3 within the first 28 d after

Table 2. Baseline and post-intervention concentrations of serum vitamin D3, plasma phosphorus, fasting plasma glucose, serum triglycerides and serum cholesterol for subjects receiving an oral single dose of 300 000 IU vitamin D3. Mean±SD. $^{**}P$<0.01 for repeated measures ANOVA tests. $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs* d 0 group.

Day	25(OH)D3 (ng/mL)**	Phosphorus (mmol/L)**	Fasting blood glucose (mmol/L)**	Triglycerides (mmol/L)**	Cholesterol (mmol/L)**
Day 0	21.0±5.0	1.51±0.31	4.7±0.5	0.99±0.41	4.45±0.80
Day 1	57.7±11.0$^{\#\#}$	1.81±0.35$^{\#\#}$	4.7±0.4	0.93±0.48	4.35±0.85
Day 2	67.8±12.1$^{\#\#}$	1.83±0.37$^{\#\#}$	4.9±0.4$^{\#}$	0.96±0.44	4.36±0.76
Day 3	71.4±12.9$^{\#\#}$	2.16±0.33$^{\#\#}$	4.6±0.4	0.93±0.35	4.39±0.79
Day 7	70.6±11.8$^{\#\#}$	2.21±0.35$^{\#\#}$	4.7±0.4	0.96±0.40	4.35±0.84
Day 28	50.8±7.7$^{\#\#}$	2.23±0.52$^{\#\#}$	5.0±0.4$^{\#\#}$	0.92±0.44	4.38±0.74
Day 56	35.4±6.0$^{\#\#}$	2.03±0.45$^{\#\#}$	4.6±0.4	0.85±0.29$^{\#}$	4.31±0.75
Day 84	28.0±4.7$^{\#\#}$	2.06±0.37$^{\#\#}$	4.8±0.3	0.80±0.30$^{\#\#}$	4.21±0.71$^{\#\#}$
Day 112	25.8±4.5$^{\#\#}$	1.92±0.28$^{\#\#}$	4.9±0.5	0.79±0.34$^{\#\#}$	3.99±0.73$^{\#\#}$

Table 3. Baseline and post-intervention concentrations of serum PTH, plasma DBP, serum calcium and serum creatinine levels and the urinary calcium/creatinine ratio in subjects supplemented with 300 000 IU vitamin D3. Mean±SD. $^{*}P<0.05$, $^{**}P<0.01$ for repeated measures ANOVA tests. $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* d 0 group.

Day	PTH (pg/mL)**	DBP (μg/mL)*	Ca^{2+} (mmol/L)**	Creatinine (μmol/L)	Urinary calcium/creatinine ratio
Day 0	37.1±9.9	196±93	2.5±0.1	67±16	0.21±0.14
Day 1	36.2±18.8	178±70#	2.4±0.1	67±15	0.17±0.13
Day 2	31.6±11.2##	175±73##	2.5±0.1	67±15	0.18±0.12
Day 3	36.2±15.8	179±65	2.5±0.1	68±15	0.22±0.18
Day 7	41.0±12.8	203±112	2.5±0.1	66±15	0.20±0.14
Day 28	38.1±11.8	188±88	2.6±0.1#	67±14	0.24±0.21
Day 56	39.8±12.5	199±113	2.5±0.1	70±15##	0.30±0.19#
Day 84	47.2±17.4##	199±93	2.5±0.1	68±13	0.32±0.27
Day 112	37.6±14.0	193±92	2.5±0.1	65±15	0.27±0.22

Table 4. Multiple linear regressions for associations between 25(OH)D3 levels after vitamin D3 intervention and demographic factors. Mean±SD. $^{\#}P<0.05$, $^{\#\#}P<0.01$ for the coefficients.

Day	Intercept	Baseline 25(OH)D3 (per 1 ng/mL)	Weight (per kg)	Age (per year)	Sex (female *vs* male)	R^2 (95% CI)
Day 1	116.74±39.3##	0.91±0.38#	-0.56±0.26#	-1.50±1.00	-3.00±6.11	0.46 (0.30–0.77)
Day 2	122.89±37.59##	1.13±0.36##	-0.78±0.25##	-0.97±0.95	-4.14±5.85	0.59 (0.36–0.87)
Day 3	131.13±42.62##	1.26±0.41##	-0.80±0.28#	-1.11±1.08	-6.12±6.64	0.54 (0.32–0.82)
Day 7	145.34±39.72##	1.03±0.38#	-0.74±0.27#	-1.58±1.01	-6.76±6.18	0.52 (0.27–0.83)
Day 28	91.21±24.12##	0.94±0.23##	-0.36±0.16#	-1.09±0.61	-6.50±3.76	0.58 (0.37–0.86)
Day 56	52.34±24.84#	0.50±0.24	0.003±0.166	-1.01±0.63	-0.45±3.87	0.26 (0.12–0.63)
Day 84	39.43±21.40	0.32±0.21	-0.03±0.14	-0.58±0.54	-0.21±3.33	0.16 (0.05–0.56)
Day 112	38.45±20.20	0.23±0.20	-0.02±0.14	-0.62±0.52	0.58±3.18	0.13 (0.04–0.57)

intervention but not on d 56, 84, and 112. No significant correlation was observed between sex or age of the participants and blood 25(OH)D3 levels at any follow-up day (Table 4).

Univariate regression tests demonstrated that baseline 25(OH)D3 correlated with the $AUC_{0\sim\infty}$ of vitamin D3 after vitamin D3 intervention ($P<0.05$; Supplementary Table S2), and sex and body weight were marginally but not statistically correlated with the $AUC_{0\sim\infty}$ of vitamin D3 ($P>0.05$). The baseline blood parameters (DBP, calcium, phosphorus, PTH and creatinine) did not correlate with the $AUC_{0\sim\infty}$ of vitamin D3 after vitamin D3 intervention (Supplementary Table S2).

Discussion

Although relative to daily lower-dose vitamin D supplementation, a single high dose of vitamin D3 increases the circulating 25(OH)D3 levels for those with vitamin D deficiency, potential side effects should be addressed before clinical use. In this study, the safety and efficacy of a single oral dose of vitamin D3 (300 000 IU) were evaluated in young Chinese subjects with vitamin D deficiency or insufficiency. The serum concentrations of 25(OH)D3 increased in the first 5 d after intervention, and a sufficient level of vitamin D was achieved for all participants during the following two months. The circulating levels of 25(OH)D3 were largely affected by the baseline 25(OH)D3 levels, body weight, and genetic factors related to vitamin D metabolism. Blood triglyceride levels (after d 56) and total cholesterol levels (after d 84) were decreased, and free fatty acids were increased. Although high levels of vitamin D may lead to hypercalcemia[10], in the present study, the highest 25(OH)D3 level for the individuals was 107.2 ng/mL, which is significantly below the toxic level of 200 ng/mL[11]. Although plasma phosphorus was significantly increased after the intervention, no significant change for serum calcium or urinary calcium/creatinine was observed during the study period. At the end of follow-up (d 112), the average 25(OH)D3 level remained higher than the baseline level, and 39% of the participants had serum 25(OH)D3 levels >30 ng/mL. These data suggest that a single high dose of vitamin D (300 000 IU) is a safe and efficient method for improving the vitamin D status of young Chinese people.

In the present study, there was a rapid increase in 25(OH)D3 levels after administration of vitamin D, and the mean T_{max} for the blood increase for 25(OH)D3 was 5.1 d. In a previous investigation involving 20 elderly and 10 young participants, the peak 25(OH)D3 level occurred at d 7 after a single dose of 10 000 IU of vitamin D3, and there was a similar metabolic pattern between the two groups[12]. In contrast, another study found a slow increase in serum 25(OH)D3 levels when a single

dose of 300 000 IU of vitamin D3 was administered to elderly subjects, with the peak 25(OH)D3 level at d 30[13]. The basis for such differences is probably due to the decreased capacity of vitamin D absorption and metabolism in elderly subjects. In children and adults, serum calcium and phosphorus levels, but not DBP levels, are higher than those in elderly populations[14]. The major carrier protein for vitamin D metabolites in plasma, DBP, prolongs the half-life for 25(OH)D3 in the blood by acting as a reservoir and promoting the reabsorption of filtered vitamin D in the kidney[15, 16]. In the present study, decreases in circulating DBP levels were found at d 1 and 2 after intervention, but no appreciable change was evident on the other days. We also did not identify any correlation between circulating 25(OH)D3 levels and baseline DBP levels. These results suggest that an immediate reduction of circulating DBP levels after a single high dose of vitamin D3 may lead to a feedback of vitamin D absorption in the body. Another report has indicated that after daily administration of 20 000 IU vitamin D3 for 12 weeks, there was no appreciable change in DBP concentrations[17]; however, these concentrations were measured only at baseline and at the end of the intervention. In the current study, we found that DBP levels were not significantly influenced by the vitamin D3 intervention after d 2 and were not correlated with the $AUC_{0\sim\infty}$ of vitamin D3, thus suggesting that DBP has minimal effects on the pharmacokinetics of single high dose vitamin D3 intervention.

As indicated by multiple linear regressions, participants with greater body weight tended to have relatively lower circulating levels of 25(OH)D3, a result consistent with the observation that drug concentrations in the body usually correlate with weight but not body mass index. From the univariate and multiple variant regression models, we found no significant correlation between sex or age, and circulating 25(OH)D3 levels after intervention. Other baseline blood parameters, including PTH, calcium, phosphorus, and creatinine, were also not correlated with circulating 25(OH)D3 levels, thus suggesting that these blood biomarkers are dependent on blood 25(OH)D3 levels. Because PTH, calcium, and phosphorus regulate the $1\alpha,25(OH)_2D$ level in blood, the influence of these factors on blood levels of $1\alpha,25(OH)_2D$ after an 25(OH)D3 intervention must be addressed in future studies.

After supplementation, there were no appreciable or consistent changes in serum PTH, calcium, or creatinine levels, but there was an increase in plasma phosphorus. High-dose vitamin D interventions may increase phosphorus absorption but not calcium absorption. Epidemiological studies have suggested an inverse relationship between serum 25(OH)D3 levels and glucose levels[18–20]. However, the results of clinical trials studying the associations between vitamin D supplementation and blood glucose levels have been inconclusive[21–24]. Using meta-analysis methods, vitamin D interventions have been determined to have no significant effects on blood glucose levels for the general population, although they may have a small effect on fasting serum glucose levels for those with diabetes or impaired glucose tolerance[25]. The present study did not identify any meaningful change in blood glucose levels after the intervention. There were decreases in serum triglyceride levels from d 56 to 112 after intervention but not in the early follow-up days. A decrease in total cholesterol was observed at d 84 and 112. These data suggest that maintenance of 25(OH)D3 at a high level over an extended period may reduce blood lipids. Consistently with the present results, a previous study has found a reduction in triglycerides but not total cholesterol, low-density lipoproteins or high-density lipoproteins 6 months after the administration of 4000 IU/d of vitamin D[26]. Vitamin D and calcium may increase the oxidation of whole body fat and increase lipolysis[27, 28]. The present investigation found an increase in free fatty acids in blood at d 84 and 112, thus suggesting increased breakdown of lipids after administration of vitamin D. In the current study, a decrease in total cholesterol at d 84 and 112 was observed, consistently with previous findings that after two doses of 50 000 IU vitamin D3, there is a reduction in the concentration of serum total cholesterol in women with gestational diabetes mellitus[29]. Therefore, randomized controlled trials with larger sample sizes are necessary to elucidate the effects of vitamin D on blood lipids and the potential benefits for obesity and the prevention of cardiovascular diseases.

There are several limitations of the current study. First, the sample size is relatively small, and genetic factors that may modulate the responses of individual vitamin D levels after intervention are difficult to determine. Second, all participants were young Chinese people, and whether the intervention methods and biological consequences are applicable to older populations remains to be determined. Third, because this was a one-armed intervention study, it was challenging to determine the effects of other cofactors that may influence the circulating 25(OH)D3 levels and other parameters. Two-armed, double-blinded, randomized clinical trials are necessary to determine the effects of vitamin D supplementation on metabolic factors. Finally, the follow-up time was limited. Although there was a peak level of blood 25(OH)D3 at d 3, the peak levels of vitamin D in individual participants were not determined daily during the first 7 d after the intervention.

In conclusion, the results demonstrate that a single high oral dose of 300 000 IU of vitamin D3 is effective for rapidly and safely improving the vitamin D status of young Chinese people with vitamin D insufficiency or deficiency. Demographic factors and baseline 25(OH)D3 levels may affect the circulating 25(OH)D3 levels after the intervention. However, additional studies with a greater number of participants and different designs are warranted to identify factors that may influence the circulating 25(OH)D levels after vitamin D supplementation.

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Author contribution

Pei-zhan CHEN, Mian LI, Jun-hua HAN, and Hui WANG contributed to study conception and design; Pei-zhan CHEN, Mian LI, Xiao-hua DUAN, Jing-ying JIA, Chen YU, and Hui WANG designed and conducted the statistical analyses; Pei-zhan CHEN, Mian LI, and Hui WANG drafted the manuscript. All authors approved the final version. Hui WANG is the guarantor. All authors were involved in the study performance, data analysis, and interpretation.

Abbreviations

95% CI, 95% confidence interval; ANOVA, analysis of variance; AUC, area under the curve; BMI, body mass index; C_{max}, maximum concentration; DBP, vitamin D binding protein; PTH, parathyroid hormone; SD, standard deviation; SE, standard error; T_{max}, time to reach maximum concentration; VDR, vitamin D receptor.

Supplementary information

Supplementary Tables are available on the website of Acta Pharmacologica Sinica.

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