ORIGINAL

# Necessity of daily 1000-IU vitamin D supplementation for maintaining a sufficient vitamin D status

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**Abstract : The changes in the serum 25-hydroxyvitamin D (25(OH)D) concentrations after daily 1000-IU vitamin D intake for 3 months (3-month-VD), 6 months (6-month-VD) and then 6-month cessation of vitamin D intake (6-month-VD cessation) were examined. The serum 25(OH)D levels in 11 male and 16 female subjects were 12.1 ± 3.5 ng/mL at baseline, increased to 27.1 ± 4.7 ng/mL at 3-month-VD, 28.5 ± 5.1 ng/mL at 6-month-VD and decreased to 16.4 ± 4.0 ng/mL at 6-month-VD cessation. The present study suggested that a vitamin D intake of 1000 IU/day is required to maintain the 25(OH) D concentration at 30 ng/mL or higher without vitamin D intoxication. J. Med. Invest. 69 : 135-140, February, 2022**

***Keywords*** **:** *Vitamin D supplementation, Serum 25-hydroxyvitamin D concentration*

## INTRODUCTION

Sufficient vitamin D status is important to human health and there is a consensus that the serum 25-hydroxyvitamin D (25(OH)D) concentration is the best indicator of vitamin D status, as it reflects combined oral intake and dermal production (1). Vitamin D deficiency (VDD)/insufficiency (VDI) is known to cause secondary hyperparathyroidism, which adversely affects bone metabolism in the elderly (2). As a phenomenon occurring highly frequently worldwide across all age groups, VDD/VDI is becoming a major global health problem (3, 4). In previous study, patients with a serum 25(OH)D below 75 nmol/L had pathologic mineralization defects of bone, and it was strongly argued whether the dose of vitamin D supplementation should ensure that circulating levels of 25(OH)D reach this minimum threshold (75 nmol/L : 30 ng/mL) to maintain skeletal health (5). Furthermore, the Endocrine Society in the US suggested that a serum concentration of 75 nmol/L is optimal for skeletal and possibly some non-skeleta1 health outcomes (6). As a general consensus in Japan, serum 25(OH)D concentrations of < 20 ng/mL, 20 to < 30 ng/mL and ≥ 30 ng/mL indicate VDD, VDI and sufficiency (VDS), respectively (7).

A study using a fortification model, stating that an average individual needs approximately 23.7 μg (948 IU) a day to reach a concentration of 75 nmol/L, is consistent with other observations (8, 9). Other publications were similar, proposing 25 μg (1000 IU) to obtain a sufficient serum 25(OH)D in the absence of UVB irradiation (10).

In contrast, the vitamin D intake in Japanese adolescents was approximately 10 μg/day (400 IU/day). This is higher than the adequate intake (AI) according to the Dietary Reference Intakes for the Japanese population in 2020 (8.5 μg/day (340 IU/day) for 18-29-year-olds) (11). The effects of daily 1000 IU vitamin D intake for 6 months on skeleta1 muscle mass and muscle strength were investigated, revealing an increase in serum 25(OH)D level from 13.4 ± 0.8 ng/mL to 29.6 ± 0.9 ng/mL, and the improvement of muscle strength and physical functions (12).

In this study, the changes in the serum 25(OH)D concentrations after daily 1000-IU vitamin D intake for 3 months (3-month-VD), 6 months (6-month-VD) and 6-month cessation of vitamin D intake after 1000-IU vitamin D intake for 6 months (6-month-VD cessation), and the association between serum 25(OH)D levels at 6-month-VD and VDDQ-J score developed by Kuwabara (13) were examined.

## MATERIALS AND METHODS

### *Subjects*

The basic characteristics, such as stay at the nursing home, work presence, exercise status, and smoking habits and drinking habits, of 27 subjects are presented in Table 1. The number of male and female subjects in age groups of 21-50 years, 51-75 years and 76 years and older were 7 and 5, 4 and 7, and 0 and 4, respectively. Among them, male and female residents in nursing homes accounted for 4 and 0 in the group of 51-75 years and 0 and 4 in the group of 76 years and older, respectively. The other 19 subjects aged between 21-75 years old were healthy registered care workers and physical and occupational therapists in nursing homes, and teaching and administration staff of Kenshokai Gakuen College for Health and Welfare. None of the healthy subjects were engaged in high levels of exercise training or taking any medications just before or during the study.

### *Dietary assessment*

Daily energy intake, water intake and the habitual vitamin D intake was calculated from food intake records for 3 consecutive days (Table 2). Vitamin D-fortified milk was provided to all subjects daily for 3 months and 6 months. The milk was

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Table 1. Characteristics of subjects

| Groups | 20-50 years | | 51-75 years | | ≥ 76 years | | Total | |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | Male | Female |
| Number (%) | 7 (25.9%) | 5 (18.5%) | 4*(14.8%) | 7 (25.9%) | 0 | 4**(14.8%) | 11 (40.7%) | 16 (59.3%) |
| Work presence | | | | | | | | |
| full time | 7 (25.9%) | 4 (14.8%) | 0 | 7 (25.9%) | 0 | 0 | 7 (25.9%) | 11 (40.7%) |
| part time | 0 | 1 (3.7%) | 0 | 0 | 0 | 0 | 0 | 1 (3.7%) |
| Exercise status | | | | | | | | |
| 2-3 times/week | 3 (11.1%) | 0 | 0 | 3 (11.1%) | 0 | 0 | 3 (11.1%) | 3 (11.1%) |
| 2-3 times/month | 1 (3.7%) | 1 (3.7%) | 4 (14.8%) | 1 (3.7%) | 0 | 4 (14.8%) | 5 (18.5%) | 6 (22.2%) |
| None | 3 (11.1%) | 4 (14.8%) | 0 | 3(11.1%) | 0 | 0 | 3 (11.1%) | 7 (25.9%) |
| Smoking habits | | | | | | | | |
| yes | 4 (14.8%) | 0 | 2 (7.4%) | 1 (3.7%) | 0 | 0 | 6 (22.2%) | 1 (3.7%) |
| no | 3 (11.1%) | 5 (18.5%) | 2 (7.4%) | 6 (22.2%) | 0 | 4 (14.8%) | 5 (18.5%) | 15 (55.6%) |
| Drinking habits | | | | | | | | |
| yes | 3 (11.1%) | 2 (7.4%) | 1 (3.7%) | 1 (3.7%) | 0 | 0 | 4 (14.8%) | 3 (11.1%) |
| no | 4 (14.8%) | 3 (11.1%) | 3 (11.1%) | 6 (22.2%) | 0 | 4 (14.8%) | 7 (25.9%) | 13 (48.1%) |

* : stay at the Nursing Home for 1 year and 9 months to 2 years and 7 months
** : stay at the Nursing Home for 2 years and 4months to 4 years and 5 months

Table 2. Dietary assessment

| Groups | 20-50 years | | 51-75 years | | ≥ 76 years | | Total | |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | Male | Female |
| Number (%) | 7 (25.9) | 5 (18.5) | 4 (14.8) | 7 (25.9) | 0 | 4 (14.8) | 11 (40.7) | 16( 59.3) |
| Age (years) | 36.7 ± 6.4 | 42.2 ± 4.7 | 67.3 ± 4.3 | 54.4 ± 2.0 | – | 86.5 ± 5.3 | 47.8 ± 16.3 | 58.6 ± 17.4 |
| Body weight (kg) | 77.9 ± 16.7 | 60.9 ± 16.0 | 60.5 ± 7.4 | 62.6 ± 9.8 | – | 47.1 ± 5.8 | 71.6 ± 16.2 | 58.2 ± 12.6 |
| Body Height (cm) | 169.2 ± 4.7 | 158.4 ± 3.4 | 154.6 ± 2.1 | 161.3 ± 5.3 | – | 147.3 ± 3.8 | 163.9 ± 8.3 | 156.9 ± 7.2 |
| Energy intake | | | | | | | | |
| (kcal/day) | 2778 ± 543 | 1974 ± 491 | 1550 ± 88 | 1868 ± 310 | – | 1428 ± 124 | 2331 ± 750 | 1790 ± 394 |
| (kcal/kg/day) | 35.9 ± 3.9 | 32.5 ± 3.3 | 25.9 ± 3.0 | 29.9 ± 2.3 | – | 30.4 ± 1.9 | 32.2 ± 6.1 | 30.8 ± 2.7 |
| Water intake | | | | | | | | |
| (ml/day) | 3318 ± 165 | 3046 ± 715 | 2706 ± 23 | 3114 ± 576 | – | 2445 ± 121 | 3096 ± 601 | 2926 ± 596 |
| (ml/kg/day) | 43.1 ± 7.0 | 50.4 ± 3.7 | 45.2 ± 5.2 | 50.7 ± 12.6 | – | 52.3 ± 5.0 | 43.9 ± 6.2 | 51 ± 8.5 |
| Vitamin D intake (IU/day) | 331 ± 290 | 353 ± 97 | 349 ± 19 | 348 ± 247 | – | 323 ± 29 | 337 ± 225 | 343 ± 165 |
| Serum 25-hydroxy | | | | | | | | |
| vitamin D level (ng/ml) | 14.4 ± 3.8 | 13.2 ± 5.0 | 9.7 ± 3.6 | 11.4 ± 1.9 | – | 10.8 ± 1.1 | 12.6 ± 4.3 | 11.8 ± 3.1 |

manufactured by Meiji Co., Ltd., Tokyo, Japan, and it contains 137 kcal, 9.9 g of carbohydrates, 6.8 g of protein, 7.8 g fat, 227 mg of calcium and 1000 IU of vitamin D.

*Clinical laboratory tests*

Blood samples were collected from subjects who underwent a more than 8-hour overnight fast, immediately refrigerated, transported in cold storage to the SRL Laboratory in Tokyo, and analyzed within 24 hours. Serum levels of calcium, phosphorus, intact parathyroid hormone (PTH) and 25(OH)D were measured. Serum 25(OH)D levels were measured by an electro- chemiluminescent immunoassay (ECLIA) as previously reported (14, 15). VDDQ-J, a questionnaire for easily identifying the risk of VDD developed by Kuwabara *et al*. (13), was used to assess the association between VDDQ-J and serum 25(OH)D levels.

*Statistical analysis*

The Wilcoxon signed rank test with Bonferroni correction was used for the changes in serum levels of calcium, phosphorus, PTH and 25(OH)D at baseline, 3-month-VD, 6-month-VD and 6-month-VD cessation, and for the association between VDDQ-J score and serum 25(OH)D level at 6-month-VD cessation or the difference in 25(OH)D level at 6-month-VD and 6-month-VD cessation. Data were expressed as the mean ± SD of total different age groups, and male and female subjects at 3-month-VD, 6-month-VD and 6-month-VD cessation.

*Ethical considerations*

Ethics approval was obtained from the clinical research ethics committee at Tokushima University Hospita1 (approval number

384). Informed consent to participate in the study was also received from participants or from an authorized surrogate. This trial was registered as UMIN000038105.

## RESULTS

*1. Serum 25(OH)D levels, habitual dietary vitamin D intake and exercise status in the subjects*

Exercise status with weekly 2-3 times, monthly 2-3 times and no habit were 22.2%, 40.7% and 40.7%, respectively (Table 1). Their 25.9% (22.9% in male and 3.7% in female) had smoking habit and 25.9% (14.8% in male and 11.1% in female) had drinking habit. Mean daily energy intake and water intake were 32.2 kcal/kg and 43.9 ml/kg in male and 30.8 kcal/kg and 51.0 ml/kg in female, respectively (Table 2). The habitual vitamin D intake from food and serum 25(OH)D concentrations were 337 ± 225 IU/day and 12.6 ± 4.3 ng/mL in 11 male subjects, respectively, and 343 ± 165 IU/day and 11.8 ± 3.1 ng/mL in 16 female subjects, respectively. Neither the basic characteristics including exercise status of subjects nor the daily vitamin D intake affected serum 25(OH)D concentrations. Serum 25(OH)D levels did not differ between sexes or duration of the nursing home stay.

*2. Changes in serum 25(OH)D, PTH, calcium and phosphorus concentrations at baseline, 3-month-VD, 6-month-VD and 6-month-VD cessation (Table 3)*

At baseline, the serum 25(OH)D levels in 11 male and 16 female subjects were 12.1 ± 3.5 ng/mL. Two participants (7.4%) had VDI and 25 (92.6%) had VDD. After vitamin D-fortified milk intake daily for 3 and 6 months, there was a significant increase in the serum 25(OH)D level to 27.1 ± 4.7 ng/mL and 28.5 ± 5.1 ng/mL, respectively, in April. The serum 25(OH)D levels markedly decreased to 16.4 ± 4.0 ng/mL at 6-month-VD cessation. The rates of VDS, VDI and VDD at 3-month-VD, 6-month-VD and 6-month-VD cessation were 5 (18.5%), 21 (77.8%) and 1 (3.9%), 11 (40.7%), 14 (51.9%) and 2 (7.4%), and 0 (0%), 7 (25.9%) and 20 (74.1%), respectively. The response of 25(OH)D levels to intake and cessation of vitamin D in different age groups, such as 20-50 years, 51-75 years, 76 years and older, and in different sexes did not differ.

After vitamin D-fortified milk intake for 6 months, serum calcium and phosphorus levels significantly increased and the PTH level significantly decreased in these subjects. Serum phosphorus and PTH levels at 6-month-VD cessation slightly decreased from 6-month VD to reach baseline levels, but the decreases at baseline, 6-month-VD and 6-month-VD cessation were not significant. Of note, the serum 25(OH)D level was inversely associated with the PTH level.

*3. Changes in serum 25(OH)D levels in different age groups at baseline, 3-month-VD, 6-month-VD and 6-month-VD cessation (Table 4)*

The serum 25(OH)D concentrations in the 20-50 years group, 51-75 years group, and 76 years and older group were similar at baseline, 3-month-VD, 6-month-VD and 6-month-VD cessation. In addition, the 25(OH)D levels at baseline in the 76 years old and older group markedly increased and reached similar levels to those in the 20-25 years and 51-75 years groups.

*4. Association between VDDQ-J score and serum 25(OH)D level*

The 25(OH)D levels (16.4 ± 4.0 ng/mL, 15.2 ± 3.4 ng/mL and 17.3 ± 4.3 ng/mL) in males and females at 6-month-VD cessation and the difference in serum 25(OH)D levels (12.1 ± 5.0 ng/mL, 12.7 ± 4.9 ng/mL and 17.3 ± 4.3 ng/mL) at 6-month-VD and 6-month-VD cessation were not associated with the VDDQ-J score (31.0 ± 7.0, 24.9 ± 4.8 and 35.1 ± 4.8).

Table 3. Changes of serum 25-hydroxyvitamin D, parathyroid hormone, calcium and phosphorus levels at baseline, 3-month-VD, 6-month-VD and 6-month-VD cessation

| | Baseline (n = 27) | | 3-month-VD (n = 27) | | 6-month-VD (n = 27) | | 6-month-VD cessation (n = 27) | |
|---|---|---|---|---|---|---|---|---|
| 25-Hydroxy vitamin D (ng/mL) | 12.1 ± 3.5[#] | a[##] | 27.1 ± 4.7 | b | 28.5 ± 5.1 | b | 16.4 ± 4.0 | c |
| VDS | 0 (0%) | | 5 (18.5%) | | 11 (40.7%) | | 0 (0%) | |
| VDI | 2 (7.4%) | | 21 (77.8%) | | 14 (51.9%) | | 7 (25.9%) | |
| VDD | 25 (92.6%) | | 1 (3.9%) | | 2 (7.4%) | | 20 (74.1%) | |
| Parathyroid hormone (pg/mL) | 43.5 ± 14.0 | a | – | | 33.7 ± 9.7 | b | 40.2 ± 16.8 | ab |
| Calcium (mg/dL) | 9.1 ± 0.4 | a | – | | 9.3 ± 0.4 | b | 9.4 ± 0.4 | b |
| Phosphorus (mg/dL) | 3.5 ± 0.4 | a | – | | 3.9 ± 0.4 | b | 3.7 ± 0.4 | ab |

VDS : Vitamin D sufficiency, VDI : Vitamin D insufficiency, VDD : vitamin D deficiency
# : Mean ± SD, ## : Different letters indicate significantly different ($p < 0.05$)

Table 4. Changes of serum 25-hydroxyvitamin D levels in different age groups at baseline, 3-month-VD, 6-month-VD and 6-month-VD cessation

| Groups | n | Baseline | | 3-month-VD | | 6-month-VD | | 6-month-VD cessation | |
|---|---|---|---|---|---|---|---|---|---|
| 20-50 years | 12 | 13.9 ± 4.2[#] | a[##] | 27.3 ± 4.6 | b | 27.7 ± 4.2 | b | 16.9 ± 3.4 | a |
| 51-75 years | 11 | 10.8 ± 2.6 | a | 25.6 ± 4.6 | b | 27.5 ± 5.7 | b | 14.9 ± 3.8 | a |
| ≥ 76 years | 4 | 10.8 ± 1.1 | a | 30.4 ± 4.3 | b | 33.9 ± 0.8 | b | 19.4 ± 5.3 | a |

# : Mean ± SD (ng/mL), ## : Different letters indicate significantly different ($p < 0.05$).

## DISCUSSION

The mean serum 25(OH)D level (range) in Japanese subjects aged 78.6 ± 2.8 (75-90) years was 22.1 ± 6.7 ng/mL (6-81 ng/mL) (16). The prevalence of the lowest tertile of serum 25(OH)D (< 20 ng/mL) in all subjects was 35.2% and a total of 13.4% had a sufficient level of 30-100 ng/mL (6). In addition, 296 young Japanese women aged 21.2 ± 2.3 years old had a mean vitamin D intake of 12.4 ± 8.1 μg/day (496 ± 324 IU/day), and 82.4% of the subjects had a higher intake of vitamin D than the AI recommended in the DRIs 2015 (5.5 μg/day, 220 IU/day) (17), which was therefore assumed to be sufficient vitamin D intake. The subjects had a mean serum 25(OH)D concentration of 18.4 ± 4.9 ng/mL, with approximately 64% of the subjects having VDD (< 20 ng/mL), and only 0.7% subjects (n = 2) had VDS (18). Thus, 99.3% of the subjects had VDI, with overt VDD (defined as serum 25(OH)D concentrations < 10 ng/mL) found in 3% of the subjects (n = 9).

In this study, a negative correlation was observed between serum 25(OH)D and PTH levels. Lower intake of vitamin D is related to an insufficient 25(OH)D level and causes secondary hyperparathyroidism. Guillemant *et al*. reported that a 25(OH)D concentration > 82.5 nmo1/L is needed to reach the plateau PTH concentration in adolescent boys (19), whereas Hills *et al*. reported that a 25(OH)D concentration of approximately 60 nmo1/L can reach the plateau PTH concentration in girls, but not in boys (20). Although Priemel *et al*. was unable to establish a minimum 25(OH)D level that was inevitably associated with mineralization defects, they found no pathological accumulation of osteoid in any patient with a circulating 25(OH)D above 75 nmo1/L(5). Moreover, mineralization defects of bone were present in approximately 20% of the population analyzed, but these mineralization defects were absent in all individuals who presented with serum 25(OH)D levels above 75 nmol/L (5).

To date, professional societies have similarly defined the RDA for vitamin D intake as 15-20 μg/day (600 - 800 IU/day) in the 2015 Guidelines for the Prevention and Treatment of Osteoporosis (21), 15 μg/day (600 IU/day) for individuals younger than 75 years old, and 20 μg/day (800 IU/day) for those 75 years old or older (the US and Canadian guidelines for the treatment and prevention of vitamin D deficiency) (6) or 20 μg/day (800 IU/day) for older adults (IOF position statement) (22). For people with little or no sun-exposure, including those aged > 75 years, an intake of 20 μg/day (800 IU/day) is recommended (23). The German Nutrition Society vitamin D recommendations for the DACH (Germany, Austria and Switzerland) region (20 μg/day (800 IU/day) for children adolescents and adults, based on serum 25(OH)D > 50 nmo1/L) were established in the context of endogenous synthesis being lacking (24). They suggested that UVB exposure of un-covered skin areas plays a role in attaining required levels of vitamin D, but that individuals who spent little time outside in the sun or fully cover their skin when outside, those of darker skin types and individuals > 65 years may be in need of vitamin D supplementation (24). Neither the amounts of daily vitamin D intake nor exercise status affected serum 25(OH)D concentrations. Thus, vitamin D intake and sun exposure may interactively affect the serum 25(OH)D concentration. Therefore, vitamin D supplementation is recommended as a method of bridging the gap between the current vitamin D intake and recommendations. Kuwabara *et al*. developed a simple questionnaire for easily identifying the risk of VDD, the VDD questionnaire for Japanese score : VDDQ-J score (13). The VDDQ-J score at 6-month-VD cessation in this study was not associated with the serum vitamin D level at 6-month-VD or the difference in 25(OH)D levels between 6-month-VD and 6-month-VD cessation. This suggested that the VDDQ -J score is useful to identify VDD under natural food intake without vitamin D supplementation, but not to identify VDD with vitamin D supplementation or cessation of vitamin D supplementation.

Although many benefits of vitamin D are ubiquitously known, the recommended intake (RI) and more importantly upper limits (UL) must be considered in order to prevent adverse effects such as vitamin D intoxication. Intoxication may occur at 25(OH) D concentrations above 500 nmo1/L (25), whereas 75 nmo1/L is considered adequate (8, 26). The Institute of Medicine set a tolerable upper intake level for vitamin D of 4000 IU (100 μg) per day (27) and the European Food Safety Agency Panel on Dietetic Products, Nutrition and Allergies set a no observed adverse effect leve1 of 10 000 IU (250 μg)per day (28). In addition, hypercalcemia, the hallmark of vitamin D intoxication, has only been consistently observed with anecdotal evidence when 25(OH)D concentrations are between 375-500 nmo1/L (29).

After one year of supplementation of 1000 IU (25 μg) of cholecalciferol/day to healthy persons aged 58.2 ± 6.9 years, serum 25(OH)D concentrations increased from 24.6 ng/mL to 30.9 ng/mL (30). Furthermore, serum 25(OH)D levels significantly increased from 12.1 ± 3.5 ng/mL to 27.1 ± 4.7 ng/mL at 3-month-VD and remained similar at 28.5 ± 5.1 ng/mL at 6-month-VD in this study (Table 2). Thus, the 1evels at 3-month-VD, 6-month-VD and even after 1 year of daily 1000-IU vitamin D supplementation did not reach vitamin D intoxication levels. Furthermore, the increases in 25(OH)D level from baseline to 3-month-VD and 6-month-VD in the 76 years or older group were not attenuated compared with those in other younger 20-50 years and 51-75 years groups (Table 4). As a defect in intestinal absorption of vitamin D in the elderly was suggested (31), this result may be explained by synergy between vitamin D and some constituents in the milk.

The concentration of 25(OH)D in plasma is largely unregulated and it has a relatively long half-life of 2-3 weeks (32, 33). The mean 25(OH)$D_3$ half-life was l4.7 ± 3.5 days and 15.6 ± 2.5 days in Gambia and UK, respectively, and was not significantly different between countries (34). The present study has several limitations. The number of subjects recruited, particularly 76 years and older male and female subjects, was small. Both the lack of control group and randomization are weak points related to the effects of daily 1000-IU vitamin D intake for 3 and 6 months. These factors may weaken the conclusion of this study. However, the present study revealed that the serum 25(OH)D levels markedly decreased from 28.5 ± 5.1 ng/mL to 16.4 ± 4.0 ng/mL and PTH levels slightly increased at 6-month-VD cessation.

The vitamin D intake for 6 months and cessation of vitamin D intake were reflected in serum concentra-tions. Both the AUC of serum 25(OH)D concentrations in the period of vitamin D intake for 6 months that in the period of cessation of vitamin D intake for 6 months were approximately the same. Age and race were not associated with the response to supplementation in previous studies (35, 36). Therefore, it was concluded that daily 1000-IU (25 μg) vitamin D intake is required to maintain the serum 25(OH)D leve1 at 30 ng/mL or higher without vitamin D intoxication.

## CONFLICT OF INTEREST AND ACKNOWLEDGEMENT

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