Improvement of Vitamin D Status in Japanese Institutionalized Elderly by Supplementation with 800 IU of Vitamin D₃

Akiko KUWABARA¹, Naoko TSUGAWA², Kiyoshi TANAKA¹*, Minori FUJI³, Nobuko KAWAI³, Sachiko MUKAE³, Yuzuru KATO³, Yasuko KÔJIMA³, Kaori TAKAHASHI³, Kazumasa OMURA³, Reiko KAGAWA³, Akira INOUE³, Toshiaki NOIKE⁶, Shoko KIDO¹ and Toshio OKANO⁷

¹Department of Food and Nutrition, Kyoto Women's University, 35, Imakamono-kitahiyoshicho, Higashiyama, Kyoto 605–8501, Japan
²Department of Hygienic Sciences, Kobe Pharmaceutical University, Kobe 658–8558, Japan
³Nursing Home, Kajyu-Shirakawa, Kyoto 606–8414, Japan
⁴Nursing and Rehabilitation Institution, Ginka, Inabe 511–0428, Japan
⁵Nursing Home, Jo-ran Home, Kyoto 612–8446, Japan
⁶Nursing Home, Nishishichijo, Kyoto 600–8888, Japan
⁷Takara Healthcare Inc., Kyoto 612–8061, Japan

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Summary To study the adequate intake (AI) for vitamin D in the elderly, we have performed an intervention study with 800 IU/d of vitamin D₃ in the institutionalized elderly. Sixty-two institutionalized elderly were randomly assigned to two groups; receiving either supplements of 200 mg calcium plus 800 IU vitamin D₃/d (Ca + VD group), or supplements of 200 mg calcium/d (Ca group) for 30 d in October. Serum concentrations of 25-hydroxyvitamin D (25OH-D), parathyroid hormone (PTH), and bone turnover markers were measured before and after intervention. Average dietary vitamin D intake during the intervention period was approximately 300 IU/d in both groups, exceeding the AI in Dietary Reference Intakes for Japanese 2005 of 200 IU/d. In both groups, mean serum 25OH-D level at baseline fell into the hypovitaminosis D range (9.7 ng/mL), despite apparently adequate vitamin D intake. Serum PTH level at baseline was within the reference range. Mean serum 25OH-D concentration significantly increased to 19.3 ng/mL in the Ca + VD group and to 11.1 ng/mL in the Ca group. Post intervention serum 25OH-D level was significantly higher in the Ca + VD group than in the Ca group (p<0.001). In 53 subjects (85.5%) who took more than 80% of their supplements for 30 d, serum PTH level in the Ca + VD group was significantly lower than in the Ca groups (p=0.05). Bone turnover markers were not significantly changed after intervention in either group. Daily supplementation of 800 IU vitamin D₃ was considered effective in the institutionalized elderly with minimal chance of sun exposure, but further studies with longer duration are necessary.

Key Words vitamin D, adequate intake, institutionalized elderly, hypovitaminosis D

Vitamin D deficiency causes skeletal mineralization defect, rickets and osteomalacia, since its fundamental physiological role is to enhance the intestinal absorption of calcium and phosphorus (1, 2). It is now recognized that even vitamin D insufficiency, which is milder than vitamin D deficiency, is associated with increased risk of fracture (2, 3).

The Adequate Intake (AI) for vitamin D was uniformly decided to be 200 IU (5 μg)/d for subjects over 30 y old in the Dietary Reference Intakes (DRI) issued in 2005 in Japan (DRI 2005) (4). It was determined to avoid elevated serum parathyroid hormone (PTH) concentration. In DRI for Japanese 2010, AI for vitamin D was decided to be 5.5 μg/d (5). Since this work was done in 2008, consideration is made basically on DRI 2005. In contrast, AI for vitamin D in the United States and Canada is 5 μg (200 IU)/d for subjects between 30 and 50 y, 10 μg (400 IU)/d for those between 51 and 70 y old, and 15 μg (600 IU)/d for those over 71 y old (6). Since elderly people are much more prone to fracture, it is possible that the AI for vitamin D in the elderly in Japan would be higher. Institutionalized elderly have been our special concern, since they are at even higher risk of fracture (7–9) and have been reported to have a high prevalence of vitamin D deficiency or insufficiency (10–14).

For the determination of AI, intervention studies as well as epidemiological ones are required. However, they have seldom been done in Japan (15, 16). In our recent study, we have studied the effectiveness of 200 IU/d supplementation of vitamin D₃ in the institutionalized elderly, since the AI for vitamin D in DRI 2005 was 200 IU daily (17). The endpoints were serum concentrations of 25-hydroxyvitamin D (25OH-D) and PTH concentration, which are considered to be a reli-
Supplementation with 200 IU/d of vitamin D significantly increased serum 25OH-D concentration, whereas that with a placebo did not. Mean serum 25OH-D (ng/mL) was 45.4 ± 4.2 in the vitamin D supplementation group and those in the Ca group were done by Student’s t-test or the Mann-Whitney test, depending on normality.

Subjects and methods

Subjects and intervention protocol. Sixty-eight institutionalized elderly subjects from four institutes, Nursing Home, Kayu-Shirakawa, Nursing and Rehabilitation Institution, Ginka, Nursing Home, Jo-nan Home and Nursing Home, Nishishichijyo were recruited to participate in the study. Exclusion criteria were routine medication that has potential interference with vitamin D and bone metabolism. Detailed information was given and written consent was obtained. The study protocol was approved by the ethical committee of Kyoto Women’s University. Participants were randomly assigned to two groups, to receive either supplements containing 200 mg calcium plus 800 IU of vitamin D₃/d (Ca+VD group), or supplements with 200 mg calcium/d (Ca group) for 30 d between October 1st and October 30th 2008. These supplements were added to their usual diet. Each supplement in gelatinous form was manufactured by Takara Healthcare and contained 48 kcal of energy, 40.8 g of water, 0.1 g of protein and fat, 12.0 g of carbohydrate, and 15.2 mg of sodium. Both Ca+VD and Ca supplements appeared and tasted the same. Each supplement could be identified only by the serial number at the bottom. The subjects as well as the staff were blinded about the content of the supplements until the completion of the study. Baseline measurements were performed at the end of September. Post intervention measurements were performed during October 31st to November 3rd.

Dietary intake. The intake of each nutrient was calculated by multiplying the amount of nutrient supplied from the institution with the average percentage intake of their usual diet. Each supplement in gelatinous form was manufactured by Takara Healthcare and contained 48 kcal of energy, 40.8 g of water, 0.1 g of protein and fat, 12.0 g of carbohydrate, and 15.2 mg of sodium. Both Ca+VD and Ca supplements appeared and tasted the same. Each supplement could be identified only by the serial number at the bottom. The subjects as well as the staff were blinded about the content of the supplements until the completion of the study. Baseline measurements were performed at the end of September. Post intervention measurements were performed during October 31st to November 3rd.

Table 1. Background profiles of the study subjects.

<table>
<thead>
<tr>
<th></th>
<th>Ca+VD (n=32)</th>
<th>Ca (n=30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>83.8±7.6</td>
<td>85.9±8.5</td>
<td>0.311</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>8/24</td>
<td>4/26</td>
<td></td>
</tr>
<tr>
<td>Care level</td>
<td>3.5±1.0</td>
<td>3.4±0.9</td>
<td>0.885</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>149.1±9.6</td>
<td>145.7±10.8</td>
<td>0.280</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>45.0±7.5</td>
<td>40.9±5.0</td>
<td>0.041</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>20.2±2.9</td>
<td>19.4±2.4</td>
<td>0.292</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>110.0±35.7</td>
<td>109.3±53.3</td>
<td>0.952</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>191.4±45.4</td>
<td>187.1±45.2</td>
<td>0.712</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD.

Comparison of indices between subjects in the Ca+VD group and those in the Ca group were done by Student’s t-test or the Mann-Whitney test, depending on normality.

Table 2. Daily dietary energy and nutrients intake during the intervention in the study subjects.

<table>
<thead>
<tr>
<th></th>
<th>Ca+VD (n=32)</th>
<th>Ca (n=30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>1,352±114</td>
<td>1,342±138</td>
<td>0.825</td>
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<tr>
<td>Protein (g)</td>
<td>53.8±4.1</td>
<td>53.2±4.8</td>
<td>0.994</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>35.4±2.8</td>
<td>35.1±3.5</td>
<td>0.870</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>201.4±27.4</td>
<td>201.3±31.1</td>
<td>0.819</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>556±79</td>
<td>573±72</td>
<td>0.295</td>
</tr>
<tr>
<td>Vitamin D (μg)</td>
<td>7.3±1.1</td>
<td>7.6±3.6</td>
<td></td>
</tr>
<tr>
<td>Calcium/diet+supp. (mg)</td>
<td>756±102</td>
<td>777±93</td>
<td>0.593</td>
</tr>
<tr>
<td>Total calcium/diet+supp. (mg)</td>
<td>27.3±5.6</td>
<td>7.6±3.6</td>
<td></td>
</tr>
<tr>
<td>Total vitamin D/diet+supp. (IU)</td>
<td>1,090±223</td>
<td>303±143</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD.

Comparison of indices between subjects in the Ca+VD group and those in the Ca group were done by the Mann-Whitney test.
Biochemical measurement. Blood was obtained after overnight fasting. After centrifugation, serum was kept frozen at \(-30^\circ C\) until analysis. Both serum concentrations of 25OHD and intact PTH were measured by Diasorin automated immunoassay (Diasorin, Stillwater, MN, USA). Serum levels of tartrate-resistant acid phosphatase (TRACP-5b) and bone specific alkaline phosphatase (BAP) were measured by enzyme immunoassay (EIA) (DS Pharma Biomedical, Osaka, Japan) and chemiluminescence enzyme immunoassay (CLEIA) (Beckman Coulter Inc, Tokyo, Japan), respectively. TRACP-5b and BAP are markers of bone resorption and bone formation, respectively. The reference range of serum TRACP-5b was 170–590 mU/dL in males and 120–420 mU/dL in females, and that of serum BAP was 3.7–20.9 \(\mu g/L\) in males and 3.8–22.6 \(\mu g/L\) in females.

Statistical analyses. Efficacy data were analyzed in two ways. One was the analysis from data of all randomized 62 subjects who provided post intervention measurement. The other was from 53 subjects (85.5%) who consumed more than 80% of supplements (per-protocol analysis). Statistical analyses were performed with SPSS 15.0J for Windows (SPSS Japan Inc., Tokyo, Japan). Comparison of two independent groups was done with Student’s \(t\)-test or Mann-Whitney test depending on normality. A one-way between groups analysis of covariance (ANCOVA) was conducted to compare the effect of vitamin D supplementation. Multiple regression analyses were performed to determine significant predictor(s) for the changes in serum 25OHD or PTH concentration.

RESULTS

Participant flow and follow-up

Of the 68 subjects randomized into the study, 62 (91.2%) completed the 30-d study, with good supplement compliance based on the record (median 92.9; 95% CI 89.2–96.6). The reasons for discontinuation were illness unrelated to the study (\(n=3\)), and personal reasons (\(n=1\)). No other adverse events were observed during the study. Two additional subjects were excluded from the analyses, since the correlation between serum 25OHD and PTH concentrations in these subjects was judged as outliers based on Mahalanobis distance. Therefore, the Ca+VD group consisted of 32 subjects (8 males, 24 females), and the Ca group consisted of 30 subjects (4 males, 26 females). As shown in Table 1, there was no difference between the two groups in the age, body height, body mass index (BMI) or the level of care needed except for slight body weight and gender differences. The level of care needed is a 5-grade score in the long-term care insurance in Japan. Serum triglyceride and total cholesterol concentrations did not significantly differ between the two groups.

Dietary intake

The nutrient intake in the two groups at baseline was not statistically different, as shown in Table 2. The intake of macronutrients such as protein, fat and carbohydrates appeared appropriate for their age and sex. Although average calcium intakes were lower than the AI in DRI 2005 (600 mg/d for men and 550 mg/d for women), average daily vitamin D intake was 7.3 \(\mu g\), which is approximately 150% of the AI in DRI 2005 in both groups. Average total vitamin D and calcium intake (usual diet plus supplements) were 1,090 IU/d and 756 mg/d in the Ca+VD group.

Changes in vitamin D status and bone turnover marker

Mean serum 25OHD concentrations at baseline were 9.7 \(\pm\) 2.8 and 9.7 \(\pm\) 3.7 ng/mL in the Ca+VD and Ca groups, respectively. In general consensus, a serum 25OHD concentration less than 20 ng/mL falls into the hypovitaminosis D range (2). None of the subjects had a baseline serum 25OHD concentration exceeding 20 ng/mL in either group. At baseline, there was no significant difference in serum 25OHD or PTH levels between the Ca+VD and Ca groups. The post interven-
tion serum 25OH-D level was significantly higher in the Ca+VD group (19.3±4.1 ng/mL) compared with the Ca group (11.1±4.5 ng/mL) \( (p<0.001) \) (Fig. 1a). The number of subjects in the Ca+VD group with a serum 25OH-D level higher than 20 ng/mL increased to 13 (41%) after supplementation with statistical significance. In contrast, only one circulating 25OH-D level exceeding 20 ng/mL existed in the Ca group after supplementation (data not shown).

In both groups, mean serum PTH concentration was within the reference range at baseline and post intervention, and only 15% of the subjects were above its cutoff level (65 pg/mL) at baseline despite hypovitaminosis D. The serum PTH level after supplementation was significantly decreased by both Ca+VD and Ca supplementation. The post intervention serum PTH level was lower in the Ca+VD group than in the Ca group, but not statistically significant as a whole \( (p=0.077) \) (Fig. 1b). However, the serum PTH level was significantly lower in the Ca+VD group compared with the Ca group in subjects with good compliance (per protocol analysis) \( (p=0.05) \).

Data for serum calcium, BAP and TRACP-5b at baseline and post intervention are shown in Table 3. In both groups, no significant change was observed for serum calcium, BAP and TRACP-5b levels. Serum PTH level was significantly lower in the Ca+VD group compared with the Ca group both in all subjects and in those with good compliance (per protocol analysis) \( (p=0.011) \). Multiple regression analyses revealed that supplementation with vitamin D3 was a significant determinant of changes in serum 25OH-D or PTH level corrected by each baseline concentration in per protocol analyses (supplementation with or without vitamin D3 was expressed as 0 or 1. \( r^2=0.825; \beta=0.906, p<0.001 \) or \( r^2=0.399; \beta=-0.229, p=0.052 \).

**Table 3. Biochemical parameters of bone metabolism in the Ca+VD and Ca groups at baseline and post intervention.**

<table>
<thead>
<tr>
<th></th>
<th>Ca+VD (n=32)</th>
<th>Ca (n=30)</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.8±0.4</td>
<td>8.8±0.3</td>
<td>0.011</td>
</tr>
<tr>
<td>BAP (( \mu )g/L)</td>
<td>20.4±10.0</td>
<td>18.6±10.8</td>
<td>0.706</td>
</tr>
<tr>
<td>TRACP-5b (mU/dL)</td>
<td>354.6±122.1</td>
<td>356.0±122.3</td>
<td>0.186</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD.

The \( p \) values were obtained by one-way analysis of covariance (ANCOVA).

**DISCUSSION**

In this study, we have studied the effectiveness of 800 IU/d of vitamin D3 supplementation on serum levels of 25OH-D, PTH, and bone turnover markers in the institutionalized elderly. Vitamin D deficiency is common in the elderly, especially institutionalized people, due to various factors such as low dietary intake, avoidance of sun exposure, and inadequate supplementation \( (12–14) \). In the present study, the average serum 25OH-D concentrations at baseline were only 9.7 ng/mL in both groups. It is similar to the previous data that Japanese physically disabled elderly living in nursing homes had low serum 25OH-D levels \( (12.0±5.2 \text{ ng/mL}) \) \( (11) \). Average dietary intake of vitamin D was around 300 IU/d, which is approximately 150% of the AI in DRI 2005 in both groups in the present study. Thus, most subjects in the present study had hypovitaminosis D, although their vitamin D intake was apparently sufficient. These results suggested that AI for vitamin D in DRI 2005 would not be high enough to avoid hypovitaminosis D in elderly subjects who have minimal chance for sun exposure.

Daily supplementation with 800 IU vitamin D3 for 30 d markedly increased circulating 25OH-D concentrations from 9.7±2.8 to 19.3±4.1 ng/mL. Serum 25OH-D levels also slightly increased in the Ca group. Since this study was done in October, increased production of vitamin D in the skin is quite unlikely to have occurred. At present, we have no clear explanation for the above finding, but the post intervention serum 25OH-D level was significantly higher in the Ca+VD group compared to the Ca group both in all subjects and in those with good compliance. Serum 25OH-D concentration exceeded 20 ng/mL in approximately 40% of the subjects, which is quite different from our previous results that serum 25OH-D levels were above 20 ng/mL in only 2 out of 33 subjects after intervention with 200 IU/d of vitamin D3 supplementation \( (17) \).

It is generally considered that vitamin D deficiency causes secondary hyperparathyroidism, resulting in high bone turnover and bone loss \( (1, 2) \). In the present study, only 15% of all subjects had elevated serum PTH levels at baseline, and the rest of them had normal to low serum PTH levels in spite of hypovitaminosis D. Sabota et al. reported that elderly subjects with hip fracture had a high prevalence of hypovitaminosis D \( (25OH-D<12 \text{ ng/mL}) \), but secondary hyperparathyroidism occurred in only about half of them \( (21) \). They also suggested the possibility that magnesium deficiency was involved in the above results, since magnesium deficiency is known to be associated with impaired PTH secretion \( (22) \). Serum PTH level was significantly correlated with serum 25OH-D concentration in the current study \( (r=-0.279, p=0.041; \text{data not shown}) \), which suggested that negative feedback regulation of PTH by 25OH-D was not disturbed. The likely explanation for the low percentage of subjects with elevated serum PTH levels would be the large inter-individual...
variation in the threshold of serum 25OH-D level to avoid serum PTH concentration.

Serum PTH concentration after intervention was significantly lower than that at baseline in both the Ca+VD and Ca groups. In the previous intervention studies, only calcium supplementation exhibited some beneficial effects such as suppression of serum PTH concentration or fracture prevention (23, 24). Therefore, the decrease in serum PTH level in the Ca group is likely to be partially due to the calcium supplementation. Serum PTH level after intervention was significantly lower in the Ca+VD group than in the Ca group in subjects with good compliance. The difference was not statistically significant in all subjects. This result suggests that vitamin D3 supplementation with good compliance effectively decreased serum PTH concentration and compliance is the important determinant of the intervention efficacy. Post intervention serum calcium concentration was significantly higher in the Ca+VD group compared to the Ca group, probably through enhanced intestinal calcium absorption.

Although supplementation with 800 IU/d of vitamin D3 for 30 d exhibited beneficial effects, serum 25OH-D levels did not reach 20 ng/mL in approximately 60% of the subjects. One of the reasons for that would be the short duration of the intervention. Chel et al. (25) studied the effects of daily supplementation with 600 IU vitamin D3 for 4 mo in elderly nursing home residents. The serum 25OH-D level increased from 9.2 to 28.0 ng/mL and the percentage of subjects with serum 25OH-D below 20 ng/mL was only 10.9%. Furthermore, Chapuy et al. (26) reported that daily supplementation with 800 IU vitamin D3 in combination with 1,200 mg calcium increased serum 25OH-D from 9.2 to above 30.0 ng/mL after 6 mo. These reports suggest that several months’ intervention is required for the correction of low vitamin D status in institutionalized elderly by the daily administration of a modest dose of vitamin D.

Bone turnover markers were not affected by vitamin D3 supplementation in the current study. One possible reason would be the short duration of the current study. Przybelski et al. studied the effects of the administration of vitamin D3 50,000 IU three times weekly for 4 wk, in elderly nursing home residents. Serum 25OH-D concentration markedly increased from 17.3 to 63.8 ng/mL, but serum bone turnover markers did not change significantly (14). In contrast, Chapuy et al. reported that daily supplementation with 1,200 mg calcium and 800 IU vitamin D3 decreased serum BAP level after 6 mo intervention (26). Although another issue to be considered is the possible effect of co-supplementation of calcium, for the correction of bone turnover, long-term intervention with a smaller dose seems to be more effective than short-term treatment with higher doses. It is likely that 800 IU/d vitamin D3 supplementation for 30 d intervention is not long enough for improving bone turnover.

Although there is a general consensus that the serum 25OH-D concentration must be at least 20 ng/mL, recent studies suggest the possibility that it must be much higher. There have been several papers to show that the level of serum 25OH-D required for complete PTH suppression was 30–32 ng/mL (27, 28). In recent articles, a serum 25OH-D concentration of 21–29 ng/mL was described to indicate insufficiency, and that exceeding 30 ng/mL was considered to be sufficient. These researchers indicated that the optimal 25OH-D level for preventing fracture was 28–32 ng/mL (3, 29, 30). Therefore, longer-term or even higher-dose supplementation would be necessary for more improvement of vitamin D status in subjects of the present study.

The limitation of the present study is that the subjects were limited to the institutionalized elderly with minimal chance of sun exposure. Considering that sunlight exposure and dietary intake are the major sources of vitamin D (2, 29), their serum 25OH-D level is likely to be much lower than that in the community-dwelling elderly. Since vitamin D3 supplementation was reported to exert more effects on serum 25OH-D and PTH levels in subjects with lower baseline serum 25OH-D concentration (31), the results may have been different in those with greater chance of sun exposure.

In conclusion, daily supplementation of 800 IU vitamin D3 was considered to be effective for improving vitamin D status in the institutionalized elderly with minimal chance of sun exposure, and further studies of longer duration are necessary for the consideration of AI for vitamin D in this population.

Acknowledgments

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REFERENCES


