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SHORT COMMUNICATION

Effect of vitamin D supplementation in the institutionalized elderly

Masako Himeno · Naoko Tsugawa · Akiko Kuwabara · Minori Fujii · Nobuko Kawai · Yuzuru Kato · Noriko Kihara · Tomoko Toyoda · Masami Kishimoto · Yoko Ogawa · Shoko Kido · Toshiaki Noike · Toshio Okano · Kiyoshi Tanaka

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Abstract An intervention study with vitamin D supplementation was conducted in order to study the amount of vitamin D required in the elderly. Sixty-four institutionalized elderly were randomly assigned to two groups: group (A) to take a beverage containing 200 mg calcium daily, and group (B) to take a beverage containing 200 mg calcium and 5 μg vitamin D daily for 30 days. Prior to the study, the subjects' average vitamin D intake was 7.3 μg/day, which is approximately 150% of the current adequate intake (AI), however their mean plasma 25OH-D level at baseline was only 12 ng/mL, strongly indicating hypovitaminosis D. During the study, average plasma 25OH-D concentration significantly increased to 14.7 ng/mL in group (B), but not in group (A). However, group (B) was still in the hypovitaminosis range. Thus, daily intake exceeding the current AI of 5 μg is required for the institutionalized elderly.

Keywords Vitamin D · Elderly · Supplementation · Adequate intake · Hypovitaminosis

M. Himeno · A. Kuwabara · M. Kishimoto · Y. Ogawa · S. Kido · K. Tanaka (✉)
Department of Food and Nutrition, Kyoto Women's University, 35 Imakumano-kitahiyoshicho, Higashiyama-ku, Kyoto 605-8501, Japan
e-mail: tanakak@kyoto-wu.ac.jp

N. Tsugawa · T. Okano
Department of Hygienic Sciences, Kobe Pharmaceutical University, Kobe, Japan

M. Fujii · N. Kawai
Nursing Home, Kayu-Shirakawa, Kyoto, Japan

Y. Kato
Nursing and Rehabilitation Institution, Ginka, Mie, Japan

N. Kihara · T. Toyoda
Nursing Home, Shiozaki-Villa, Hyogo, Japan

T. Noike
Takara Healthcare Inc., Kyoto, Japan

Introduction

Among the diverse actions of vitamin D, enhancement of intestinal calcium absorption is the most essential. Severe deficiency causes of vitamin D classical disorders such as rickets and osteomalacia. Recently, however, a milder form of hypovitaminosis D is known to increase the risk of osteoporotic fracture [1, 2]. Since osteoporosis mainly affects elderly people, it is of great importance to determine how much vitamin D is required for the bone health in the elderly.

Dietary reference intakes (DRIs) refer to a set of nutrient-based reference values [3], which is revised every 5 years in Japan. For vitamin D, adequate intake (AI) is specified, which is a value based on experimentally derived intake levels or approximations of observed mean nutrient intakes by a group of apparently healthy people. In the 6th revision of the DRIs issued in 2000, the recommended dose for vitamin D was 100 IU (2.5 μg)/day [4]. This dose was likely determined for the prevention of rickets or osteomalacia [4]. In the current AI issued in 2005, the recommended dose was raised to 200 IU (5 μg)/day and the significance of avoiding secondary hyperparathyroidism was mentioned, which reflects that the notion of vitamin D insufficiency has become more important [3]. In contrast, 5–10 μg/day was recommended in Guideline for the prevention and treatment of osteoporosis 2006 by the Japan Osteoporosis Society [5]. The AI for vitamin D is much

higher in the United States and in Europe than in Japan, especially for the elderly [6]. In the United States, for example, it is 5 μg/day for subjects between 30 and 50 years, 10 μg/day for those between 51 and 70 years old, and 15 μg/day for those over 71 years old.

Recently, a number of large-scale clinical studies and meta-analyses have been published on the effectiveness of vitamin D supplementation for fracture prevention [7, 8]. As will be detailed in the "Discussion", it has been consistently shown that vitamin D with daily doses higher than 20 μg significantly decrease fracture risk, whereas lesser doses do not [7–9]. In contrast, there have been few intervention studies to determine the AI for vitamin D in Japan [10–12]. In this study, we have examined whether the current AI of 5 μg/day was sufficient by studying the effect of 5 μg supplementation of vitamin D in an elderly population.

Subjects and methods

Subjects

Study subjects were sixty-four institutionalized elderly subjects from three institutes: Nursing Home Kayu-Shirakawa, Nursing and Rehabilitation Institution Ginka, and Nursing Home Shiozaki-Villa. Detailed information was given and written consent was obtained. The study protocol was approved by the ethical committee in Kyoto Women's University. As shown in Table 1, there was no difference between the two groups in the age or the anthropometric data such as body height, body weight, and body mass index (BMI). In addition, there was no difference in the level of care needed, based on a 5-grade score used for long-term care insurance in Japan. Larger score denotes a need for more intensive care.

Evaluation of food intake

In 30 subjects from one institute (Kayu-Shirakawa), data for food intake were available. The intake of each nutrient was calculated by multiplying the amount of nutrient supplied from the institution with the average monthly percentage intake. Based on these records, the subjects' intake of energy and macronutrients was calculated using the software (Healthy Maker Pro 501, Mushroom Software Corp, Okayama, Japan).

Blood tests

Blood samples were obtained after overnight fasting. After centrifugation, serum or plasma was kept frozen at −30°C until analysis. Nutritional indices such as serum albumin or cholesterol levels were not different in the two groups (Table 1). Plasma 25OH-D concentration was measured by radioimmunoassay (RIA) (DiaSorin, Stillwater, MN, USA). Circulating level of intact parathyroid hormone (PTH) was measured by electro chemiluminescent immunoassay (ECLIA) (Roche Diagnostics, Mannheim, Germany).

Test beverage

Participants were randomly assigned to two groups; group (A) where beverage (A) containing 200 mg calcium was given daily, and group (B) where beverage (B) containing 200 mg calcium plus 5 μg of vitamin D_3 was given daily. Beverages were supplied in cans with the volume of 190 ml. Each can contained 91.2 kcal of energy, 166.25 g of water, 1.33 g of protein, 21.66 g of carbohydrate, undetectable level of lipid, and 26.6 mg of sodium.

Beverages (A) and (B) appeared and tasted the same. Each can was identifiable only by the serial number at the bottom. The subjects as well as the staff were blinded about the content of the cans until the completion of the study. These beverages were given for 30 days. Group (A) consisted of 31 subjects (4 males, 27 females), and group (B) consisted of 33 subjects (9 males, 24 females). Although there seems to be some gender difference between the two groups, it was not statistically significant by the chi-square test.

Table 1 Background profiles in subjects with group (A) and (B)

	Group (A) ($n = 31$)	Group (B) ($n = 33$)	P value
Age (years)	86.6 ± 8.0	87.3 ± 7.3	NS
Sex (male/female)	4/27	9/24	NS
Level of care needed	3.1 ± 0.9	3.4 ± 1.0	NS
Body height (cm)	142.3 ± 7.2	146.3 ± 10.1	NS
Body weight (kg)	43.8 ± 9.6	44.2 ± 10.3	NS
Body mass index (kg/m^2)	20.9 ± 4.1	20.3 ± 4.1	NS
Serum albumin (g/dl)	3.8 ± 0.4	3.8 ± 0.4	NS
Serum total cholesterol (mg/dl)	179.3 ± 44.6	183.2 ± 30.9	NS
Serum calcium (mg/dl)	8.8 ± 0.3	8.9 ± 0.4	NS

Data are expressed as mean ± SD. Comparison of indices between subjects with group (A) and those with group (B) were done by Student's t test or Mann–Whitney test depending on normality

NS Non significant difference

Statistical analyses

Statistical analyses were done with SPSS 15.0 J for Windows (SPSS Japan Inc., Tokyo, Japan). Comparison of two independent groups was done with Student's t test. The contingent table was analyzed with the chi-square test.

Results

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 the subjects' age and sex.

Table 2 Daily energy and nutrients intake in the study subjects

	Group (A) ($n = 14$)	Group (B) ($n = 16$)	P value
Energy (kcal)	1345 ± 125	1367 ± 107	NS
Protein (g)	52.0 ± 4.6	52.9 ± 3.8	NS
Fat (g)	33.5 ± 3.2	34.0 ± 2.7	NS
Carbohydrate (g)	180.7 ± 15.6	184.2 ± 12.3	NS
Calcium (mg)	493 ± 41	511 ± 40	NS
Vitamin D (μg)	7.3 ± 1.1	7.3 ± 1.2	NS

Data are expressed as mean ± SD. Comparison of indices between subjects with group (A) and those with group (B) were done by Student's t test

NS Non significant difference

Although average calcium intakes were lower than AI (600 mg/day for men and 550 mg/day for women), average daily vitamin D intake was 7.3 μg, which is approximately 150% of the current AI in both groups.

Plasma 25OH-D levels were 12.6 ± 4.1 ng/mL in group (A) and 11.1 ± 3.2 ng/mL in group (B), and serum intact PTH concentration was 36.4 ± 34.7 ng/mL in group (A) and 37.8 ± 19.0 ng/mL in group (B) before supplementation. These values were not significantly different between the two groups. As shown in Fig. 1, supplementation with beverage (B) significantly increased plasma 25OH-D level (14.7 ± 3.6 ng/mL) and significantly decreased serum intact PTH concentration (30.3 ± 17.2 ng/mL), whereas supplementation with beverage (A) did not.

Concentration below 20 ng/mL is generally considered to indicate vitamin D deficiency [2]. The number of subjects with plasma 25OH-D concentration exceeding 20 ng/mL was only 1 out of 31 in group (A) and 2 out of 33 in group (B) even after supplementation.

Discussion

Vitamin D, either from food intake or production in the skin, is metabolized to 25OH-D by a hepatic 25-hydroxylase, then to the active form, $1,25(OH)_2D$ by renal 1α-hydroxylase [1, 2]. Since the latter activity is under the strict control by factors such as PTH, blood level of

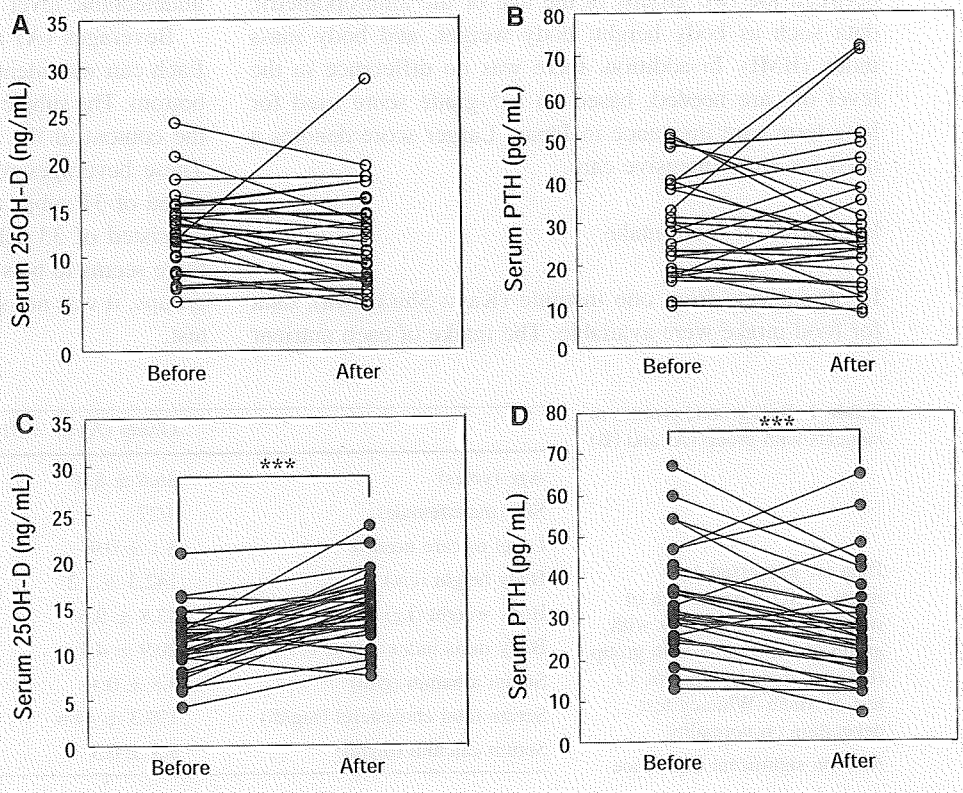

Fig. 1 Blood levels of 25OH-D and PTH before and after supplementation. Each figure represents the following data; **a** plasma 25OH-D concentration in group (A), **b** serum PTH concentration in group (A), **c** plasma 25OH-D concentration in group (B), and **d** serum PTH concentration in group (B). Data before and after supplementation were compared with paired t tests, and the *asterisks* denote the statistical significance ($P < 0.001$)

$1,25(OH)_2D$ is subject to fluctuation. Thus, circulating 25OH-D level best reflects a person's vitamin D status. Concentration below 20 ng/mL is generally considered to indicate vitamin D deficiency [2]. Hypovitaminosis D, even if not so severe as to cause mineralization defects, causes secondary hyperparathyroidism, and enhanced secretion of PTH increases bone resorption [1, 2].

The average intake of vitamin D from food was 7.3 μg/day in the current subjects at baseline. Opportunities for sun exposure were minimal for practically all subjects. In light of minimal contribution of vitamin D production in the skin, their vitamin D intake was approximately 150% of the current AI. Nevertheless, the average plasma 25OH-D concentration was only approximately 12 ng/mL, and exceeded 20 ng/mL in only three subjects out of 64. Thus most subjects in the current study were vitamin D deficient in spite of vitamin D intake far exceeding the current AI.

Since 5 μg of vitamin D was additionally supplied daily by the test beverage to subjects in group (B), their average vitamin D intake was 12.3 μg/day which is almost 250% of current AI. Nevertheless, the mean 25OH-D concentration after vitamin D supplementation was only 14.7 ng/mL, and did not reach 20 ng/mL in most of the subjects. These results indicate that daily supplementation with 5 μg vitamin D has some beneficial consequences to the skeleton, which, however, falls far short of ideal.

Recently, meta-analyses and large-scale clinical trials have been published on the role of vitamin D in fracture prevention [7, 13, 14]. Bischolff-Ferrari et al. [7] reported that daily vitamin D supplementation with 17.5–20 μg achieved a circulating 25OH-D level higher than 20 ng/mL and significantly decreased subjects' fracture risk, whereas supplementation with 10 μg/day did not. In a recent meta-analysis, Tang et al. [13] reported that the relative risk (RR) of fracture was decreased at daily doses higher than 20 μg of vitamin D in combination with 1200 mg/day of calcium supplementation, but not at lower doses. In a large-scale trial by Jackson et al. [14], daily supplementation with 1,000 mg calcium and 10 μg vitamin D_3 for 7 years did not significantly reduce the RR for fracture, except in a subgroup with good compliance. These reports suggest that vitamin D supplementation significantly decrease the risk for fracture at the daily doses of 20 μg, but the results are inconsistent at lower doses.

Recently, Nakamura et al. [15–17] have extensively studied vitamin D nutritional status of the Japanese. Average plasma 25OH-D levels in their data were 22.2 ng/mL in home-dwelling postmenopausal women [16], and 21 ng/mL in non-institutionalized elderly requiring care [17], which is much higher than the data in the present study. They also reported that the prevalence of vitamin D insufficiency was dependent on activity of daily living (ADL) [17]. Indeed, they also reported that the average plasma 25OH-D concentration was 12 ng/mL in physically inactive elderly living in nursing homes [18], which is similar to the current data. Thus caution is necessary not to over-generalize the present finding.

Our study has some limitations. First, information on the participants' food intake was available in only approximately half of the subjects. Second, this study is a preliminary one with a modest dosage of vitamin D and only a one-month duration.

To be further studied is the relative importance of oral vitamin D supplementation and sun exposure. It is well established that sun exposure is a major contributing factor for vitamin D status and circulating 25OH-D concentrations exhibit seasonal changes [1, 2, 15, 19]. One would expect that sun exposure could be an alternative to oral vitamin D supplementation to improve vitamin D status in the elderly. Nakamura et al. [15], however, argued that the dermal production of vitamin D by sun exposure was compromised in elderly subjects, and sun exposure might be a less effective means to improve the vitamin D nutritional status in elderly people. Since the chance for sun exposure was minimal in the current study, and our study purpose was focused on the role of oral supplementation, a detailed discussion on the significance of sun exposure cannot be addressed in the present paper. Further studies, hopefully of intervention type, would be required to clarify the possible role of sun exposure for improving vitamin D status in the elderly.

In conclusion, our data strongly suggest that the current AI of 5 μg/day is suboptimal for the institutionalized elderly. It is quite likely that a much higher dose is required for these subjects. An intervention study with much higher doses of vitamin D is required.

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Improvement of Vitamin D Status in Japanese Institutionalized Elderly by Supplementation with 800 IU of Vitamin D_3

Akiko KUWABARA[1], Naoko TSUGAWA[2], Kiyoshi TANAKA[1,*], Minori FUJII[3], Nobuko KAWAI[3], Sachiko MUKAE[4], Yuzuru KATO[4], Yasuko KOJIMA[5], Kaori TAKAHASHI[5], Kazumasa OMURA[5], Reiko KAGAWA[6], Akira INOUE[6], Toshiaki NOIKE[7], Shoko KIDO[1] and Toshio OKANO[2]

[1]*Department of Food and Nutrition, Kyoto Women's University, 35, Imakumano-kitahiyoshicho, Higashiyama, Kyoto 605–8501, Japan*
[2]*Department of Hygienic Sciences, Kobe Pharmaceutical University, Kobe 658–8558, Japan*
[3]*Nursing Home, Kayu-Shirakawa, Kyoto 606–8414, Japan*
[4]*Nursing and Rehabilitation Institution, Ginka, Inabe 511–0428, Japan*
[5]*Nursing Home, Jo-nan Home, Kyoto 612–8446, Japan*
[6]*Nursing Home, Nishishichijo, Kyoto 600–8888, Japan*
[7]*Takara 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 D_3 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 D_3/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 D_3 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 D_3 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-

*To whom correspondence should be addressed.
E-mail: tanakak@kyoto-wu.ac.jp

Table 1. Background profiles of the study subjects.

	Ca+VD (n=32)	Ca (n=30)	p value
Age (y)	83.8±7.6	85.9±8.5	0.311
Sex (male/female)	8/24	4/26	—
Care level	3.5±1.0	3.4±0.9	0.885
Body height (cm)	149.1±9.6	145.7±10.8	0.280
Body weight (kg)	45.0±7.5	40.9±5.0	0.041
Body mass index (kg/m^2)	20.2±2.9	19.4±2.4	0.292
Triglyceride (mg/dL)	110.0±35.7	109.3±53.3	0.952
Total cholesterol (mg/dL)	191.4±45.4	187.1±45.2	0.712

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.

	Ca+VD (n=32)	Ca (n=30)	p value
Energy (kcal)	1,352±114	1,342±138	0.825
Protein (g)	53.8±4.1	53.2±4.8	0.994
Fat (g)	35.4±2.8	35.1±3.5	0.870
Carbohydrate (g)	201.4±27.4	201.3±31.1	0.819
Calcium (mg)	556±79	573±72	0.295
Vitamin D (μg)	7.3±1.1	7.6±3.6	
(IU)	294±131	303±143	0.824
Total calcium (diet+supplementation) (mg)	756±102	777±93	0.593
Total vitamin D (diet+supplementation) (μg)	27.3±5.6	7.6±3.6	
(IU)	1,090±223	303±143	<0.001

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.

able indicators for vitamin D status and a sensitive marker for vitamin D insufficiency, respectively. There is a general consensus that a serum 25OH-D level below 20 ng/mL strongly indicates hypovitaminosis D (*2*, *3*). Supplementation with 200 IU/d of vitamin D_3 significantly increased serum 25OH-D concentration, whereas that with a placebo did not. Mean serum 25OH-D level after intervention, however, was only 14.7 ng/mL even in the vitamin D supplementation group. The results suggest that daily supplementation with 200 IU vitamin D_3 is not high enough in this study population. It has been indicated that vitamin D supplementation at daily doses of 800 IU significantly decreased the fracture risk, but lower doses did not (*18–20*). Thus we have studied the effectiveness of daily supplementation of 800 IU of vitamin D_3 in the institutionalized elderly in the current study.

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, Nishishichijo 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 D_3/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 during the intervention study. Based on these records, their intake of energy and macronutrients was calcu-

lated using software (Healthy Maker Pro 501, Mushroom Software Corp, Okayama, Japan).

Biochemical measurement. Blood was obtained after overnight fasting. After centrifugation, serum was kept frozen at −30°C until analysis. Both serum concentrations of 25OH-D 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 μg/L in males and 3.8–22.6 μg/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 25OH-D 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 25OH-D 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

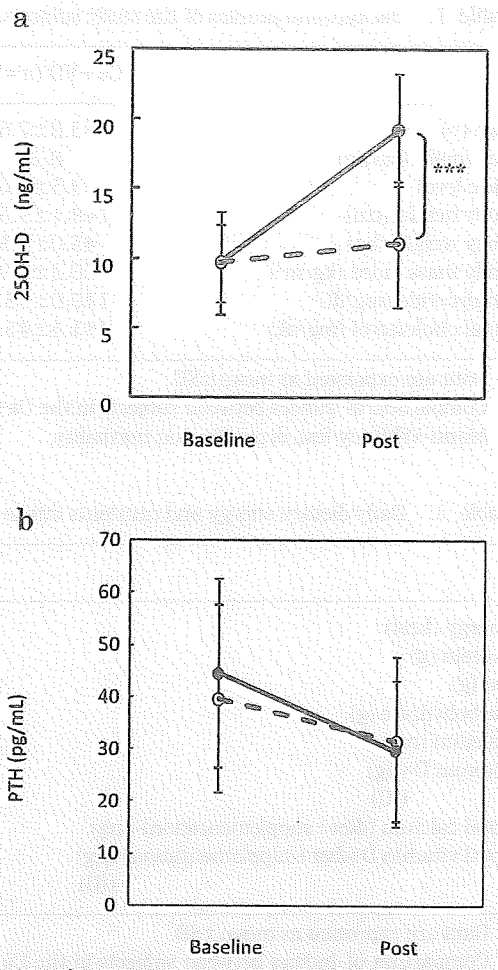

Fig. 1. Changes in serum 25OH-D and PTH concentrations. Data are expressed as mean±SD. (a) serum 25OH-D concentrations, (b) serum PTH concentrations. The solid line and the broken line represent the Ca+VD group and Ca group, respectively. Comparison of post intervention serum 25OHD between the Ca+VD group and Ca group were done by one-way analysis of covariance (ANCOVA). The asterisk indicates the statistical significance (***p<0.001).

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 μg, 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 25OH-D concentrations at baseline were 9.7±2.8 and 9.7±3.7 ng/mL in the Ca+VD and Ca groups, respectively. In general consensus, a serum 25OH-D concentration less than 20 ng/mL falls into the hypovitaminosis D range (2). None of the subjects had a baseline serum 25OH-D concentration exceeding 20 ng/mL in either group. At baseline, there was no significant difference in serum 25OH-D or PTH levels between the Ca+VD and Ca groups. The post interven-

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

	Ca+VD (n=32)		Ca (n=30)		p value
	Baseline	Post	Baseline	Post	
Calcium (mg/dL)	8.8±0.4	9.2±0.4	8.8±0.3	9.0±0.4	0.011
BAP (μg/L)	20.4±10.0	19.5±9.6	18.6±10.8	17.7±9.3	0.706
TRACP-5b (mU/dL)	354.6±122.1	364.2±179.8	356.0±122.3	323.9±143.9	0.186

Data are expressed as mean±SD.
The p values were obtained by one-way analysis of covariance (ANCOVA).

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 BAP and TRACP-5b level. The post intervention serum calcium concentration significantly increased in the Ca+VD compared with the Ca group (p=0.011). Multiple regression analyses revealed that supplementation with vitamin D_3 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 D_3 was expressed as 0 or 1. r^2=0.825; β=0.906, p<0.001 or r^2=0.399; β=−0.229, p=0.052).

DISCUSSION

In this study, we have studied the effectiveness of 800 IU/d of vitamin D_3 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 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 D_3 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 D_3 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. Sahota et al. reported that elderly subjects with hip fracture had a high prevalence of hypovitaminosis D (25OH-D<12 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; 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 D_3 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 D_3 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 D_3 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 D_3 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 the institutionalized elderly by the daily administration of a modest dose of vitamin D.

Bone turnover markers were not affected by vitamin D_3 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 D_2 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 D_3 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 D_3 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 D_3 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 D_3 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.

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ORIGINAL ARTICLE

High prevalence of vitamin K and D deficiency and decreased BMD in inflammatory bowel disease

A. Kuwabara · K. Tanaka · N. Tsugawa · H. Nakase · H. Tsuji · K. Shide · M. Kamao · T. Chiba · N. Inagaki · T. Okano · S. Kido

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Abstract
Summary Vitamin K and D deficiency and decreased bone mineral density (BMD) were highly prevalent in patients with inflammatory bowel disease (IBD), especially Crohn's disease (CD). Dietary intakes of these vitamins, however, were above the Japanese adequate intakes in IBD patients, suggesting that malabsorption is the basis for hypovitaminosis K and D and decreased BMD.
Introduction We have studied the possible involvement of vitamin K and D deficiency in the pathogenesis of decreased BMD in IBD.
Methods Seventy patients with IBD were evaluated for their BMD; plasma levels of vitamin K; phylloquinone (PK), menaquinone-7 (MK-7), and 25OH-D; serum PTH, protein induced by vitamin K absence (PIVKA-II), and undercarboxylated osteocalcin (ucOC) levels; and their food intake.
Results Compared with ulcerative colitis (UC) patients, CD patients had significantly lower plasma vitamin K and 25OH-D concentrations; significantly higher serum levels of PTH, PIVKA-II, and ucOC; and significantly lower BMD scores at almost all measurement sites. More IBD patients were vitamin K deficient in bone than in liver. Multiple regression analyses revealed that low plasma concentrations of vitamin K and 25OH-D were independent risk factors for low BMD and that they were associated with the patients' fat intake, but not with their intake of these vitamins.
Conclusion IBD patients have high prevalence of decreased BMD and vitamin K and D deficiency probably caused by malabsorption of these vitamins.

Keywords Inflammatory bowel disease · Malabsorption · Vitamin K · Vitamin D

A. Kuwabara · K. Tanaka (✉) · S. Kido
Department of Food and Nutrition, Kyoto Women's University,
35, Imakumano-kitahiyoshicho,
Higashiyama, Kyoto 605-8501, Japan
e-mail: tanakak@kyoto-wu.ac.jp

N. Tsugawa · M. Kamao · T. Okano
Department of Hygienic Sciences,
Kobe Pharmaceutical University,
Kobe, Japan

H. Nakase · T. Chiba
Department of Gastroenterology, Graduate School of Medicine,
Kyoto University,
Kyoto, Japan

H. Tsuji · K. Shide · N. Inagaki
Department of Diabetes and Clinical Nutrition,
Graduate School of Medicine, Kyoto University,
Kyoto, Japan

Introduction

Crohn's disease (CD) and ulcerative colitis (UC), collectively termed inflammatory bowel disease (IBD), are often associated with osteoporosis, the pathogenesis of which is considered to be multifactorial including inflammatory disease process, low body weight, calcium and vitamin D deficiency, and glucocorticoid use [1–5]. In this paper, we focused our attention to the possible involvement of vitamin K and D deficiency in IBD-induced osteoporosis based on the following considerations.

Vitamin K has received far less attention than vitamin D in the development of IBD-related osteoporosis [6]. The most fundamental role of vitamin K is to work as the coenzyme of hepatic γ-carboxylation of four of the blood coagulation factors [ ]. Recent evidences suggest that

vitamin K is also essential in the extrahepatic tissues including skeleton and vasculature [ ]. Fracture risk was increased in subjects with low vitamin K intake [,] or increased serum undercarboxylated osteocalcin (ucOC) level, which is a sensitive marker for skeletal vitamin K deficiency [,]. Furthermore, recent metaanalysis has shown that vitamin K treatment decreased fracture incidence [ ]. These findings prompted us to study both vitamin K and D status in IBD patients.

Next, the vitamin K and D status of IBD patients has been studied by evaluating their food intake [,] or by measuring circulating level of these vitamins [, –], but rarely by both [,]. Patients with IBD have been reported to be at high risk of malabsorption of these vitamins due to intestinal inflammation or intestinal resection in some patients [, , –]. Therefore, the patients' intake of these vitamins may be discrepant from their circulating levels. Thus, we considered it mandatory that the vitamin K and D status of IBD patients should be evaluated by studying both the patients' intake and plasma levels.

In this paper, we have studied bone mineral density (BMD) at various sites, measured plasma concentrations of vitamin K and D as well as markers for their deficiency, and evaluated the patients' food intake to clarify the possible involvement of vitamin K and D deficiency in IBD-induced bone loss.

Materials and methods

Subjects

Seventy outpatients with IBD (CD, 29 and UC, 41) attending the Gastroenterology Clinic at Kyoto University Hospital participated in the study. Excluded from the study were patients already treated for osteoporosis with drugs such as bisphosphonates, calcium, vitamin K, or vitamin D. None had history of fragility fractures. Consent to participate in this study was obtained after explanation of the objective and protocol of this study. All subjects except two with CD and one with UC were receiving 5-aminosalicylic acid. Eight patients with CD and 17 with UC were under oral glucocorticoid therapy. Immunosuppressive drug was prescribed to 19 patients with CD and eight patients with UC. Three patients with CD, but none with UC, were on combined therapy of infliximab, oral glucocorticoid, and immunosuppressive drug. None of them were under warfarin therapy.

Measurement

Biochemical measurements

Plasma samples were stored at −30°C with protection from light until analyzed. Plasma vitamin K_1 (phylloquinone [PK]) and K_2 (menaquinone-7 [MK-7]) levels were determined by high-performance liquid chromatography–tandem mass–mass spectrometry with atmospheric pressure chemical ionization (LC-APCI-MS/MS) using a HPLC system (Shimadzu, Kyoto, Japan) and API3000 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) with ^{18}O-labeled vitamin K as the internal standard [ ]. Plasma concentration of 25OH-D was measured by radioimmunoassay (RIA) (DiaSorin, Stillwater, MN, USA). This study was done between September and November to minimize the seasonal variation in serum 25OH-D levels. Serum intact PTH was measured by a fully automated immunochemilumetric assay (Nichols Institute Diagnostics, San Clemente, CA, USA) with 15–55 pg/mL as the reference range in Kyoto University Hospital. Serum protein induced by vitamin K absence (PIVKA-II) and ucOC levels were measured by electrochemiluminescent immunoassay (ECLIA; Sanko Junyaku, Tokyo, Japan) as the markers of hepatic and skeletal vitamin K deficiency, respectively. Serum NTX-I and bone specific alkaline phosphatase (BAP) levels were measured by enzyme immunoassay (EIA) (Mitsubishi Chemical Medience, Tokyo, Japan)

BMD measurement

BMD was measured at the lumbar spine (L1–4), femoral neck, total hip, and distal one-third of nondominant radius with dual-energy X-ray absorptiometry (QDR-2000, Hologic, Waltham, MA, USA). BMD (g/cm^2) values thus obtained were expressed as T or Z score. The diagnosis for osteoporosis was made according to the World Health Organization criteria with T score below −2.5 SD and between −2.5 and −1.0 SD being diagnostic of osteoporosis and osteopenia, respectively [ ].

Dietary intake

Dietary information was obtained from 1-day dietary record completed by the patients [ ]. Based on these records, their intake of energy and nutrients was calculated using a software (Healthy Maker Pro 501, Mushroom Software, Okayama, Japan).

Statistical analyses

Statistical analyses were performed using the SPSS 15.0 J for Windows (SPSS Japan, Tokyo, Japan). The difference between two independent groups was analyzed by unpaired t test or Mann–Whitney test depending on normality. Multiple regression analyses were performed to determine independent risk factors for plasma vitamin K, 25OH-D levels, or BMD.

Results

The baseline characteristics and data from blood examination are shown in Table 1. CD patients were younger, but had longer disease duration than those with UC. Although body mass index (BMI) was not significantly different between these groups, nutritional indices such as serum albumin and total cholesterol levels were lower and serum inflammatory marker, C-reactive protein (CRP), was higher in patients with CD than those with UC. Serum calcium level was not different between the two groups. Plasma concentrations of PK and MK-7 were significantly lower, and serum PIVKA-II and ucOC levels were reciprocally higher in CD patients than those with UC. We have then performed the multiple regression analysis to identify factors affecting serum ucOC level, since it may be subject to altered bone turnover. Serum BAP and plasma PK were both significant predictors for serum ucOC level (R^2= 0.453; β=0.442, p=0.036 and β=0.415, p=0.044). More detailed consideration on plasma vitamin K levels will be done in the "Discussion" section, since no definite reference values are available at present.

Current concept holds that plasma 25OH-D levels of less than 20 ng/mL and between 21 and 29 ng/mL indicate vitamin D deficiency and insufficiency, respectively [28,]. Average plasma 25OH-D concentration was 15.7 ng/mL in IBD patients as a whole, 11.2 and 20.2 ng/mL in CD and UC patients, respectively. Plasma 25OH-D level was below 20 ng/mL in all patients with CD and approximately 60% of patients with UC. Serum PTH concentration was significantly higher in CD than in UC patients, and above the cut-off value of 55 pg/mL in approximately 40% and 20% of patients with CD and UC, respectively. Serum BAP and NTX-I were higher in CD than UC patients, although statistically not significant.

BMD measurement

Considering that CD patients were significantly younger than UC subjects, comparison of BMD in these groups was made principally based on Z scores, which was significantly lower than zero in all measurement sites in CD and distal one-third of radius in UC. Thus, the Z score in the CD group was significantly lower than that in the UC group, except at the distal one-third of radius where Z score was decreased in both groups. Results expressed as T score are basically the same, although the difference between CD and UC was not so marked than expressed as Z score, probably reflecting the younger mean age in the CD group. T scores at the distal one-third of radius were below −2.5 SD in 39%

Table 1 Background profiles and results from blood tests in patients with CD and UC

	IBD (n=70)	CD (n=29)	UC (n=41)	p value
Age (years)	36.4±12.4 (34.0)	32.2±6.7 (31.0)	39.3±14.6 (37.0)	0.008[a]
Sex (male/female)	44/26	20/9	24/17	–
Body mass index (kg/m^2)	20.4±3.0 (20.3)	20.1±2.8 (19.5)	20.7±3.2 (20.8)	0.401[a]
Disease duration (years)	9.8±8.3 (9.0)	12.7±6.6 (12.0)	7.8±8.8 (5.0)	0.001[b]
Glucocorticoid therapy (n)	25	8	17	–
Immunosuppressive therapy (n)	27	19	8	–
Infliximab therapy (n)	3	3	0	–
C-reactive protein (mg/dL)	1.4±2.8 (0.3)	2.4±3.2 (0.8)	0.7±2.2 (0.2)	<0.001[b]
Albumin (g/dL)	4.1±0.6 (4.1)	3.9±0.5 (3.9)	4.3±0.6 (4.4)	0.001[b]
Total cholesterol (mg/dL)	153.0±42.3 (145.5)	126.1±26.3 (120.0)	175.8±40.1 (177.0)	<0.001[b]
Calcium (mg/dL)	8.9±0.4 (9.0)	8.8±0.4 (8.8)	9.0±0.3 (9.1)	0.095[a]
PK (ng/mL)	0.735±0.533 (0.570)	0.462±0.281 (0.470)	0.985±0.591 (0.890)	0.002[b]
MK-7 (ng/mL)	3.282±4.414 (1.369)	1.989±3.824 (0.470)	4.472±4.657 (2.190)	0.001[b]
PIVKA-II (mAU/mL)	22.77±8.54 (22.0)	25.75±9.34 (24.50)	19.79±6.57 (18.50)	0.020[b]
ucOC (ng/mL)	8.52±7.96 (5.84)	12.26±9.65 (9.08)	4.94±3.21 (3.93)	<0.001[b]
25OH-D (ng/mL)	15.69±6.71 (15.5)	11.20±4.20 (11.00)	20.18±5.68 (19.50)	<0.001[a]
PTH (pg/mL)	50.76±21.58 (45.8)	57.00±22.74 (42.90)	44.53±18.80 (41.20)	0.031[b]
Serum BAP (μg/L)	15.0±7.2 (12.5)	16.3±7.7 (12.9)	12.6±5.5 (10.3)	0.190[b]
Serum NTX-I (nmol BCE/L)	15.0±6.8 (14.3)	16.8±7.9 (15.2)	12.8±4.4 (11.9)	0.077[b]

Data are expressed as the mean±SD with the values in parentheses showing the median.
PK phylloquinone, *MK-7* menaquinone-7, *PIVKA-II* protein induced by vitamin K antagonist, *ucOC* under carboxylated osteocalcin, *BAP* bone specific alkaline phosphatase
[a] Comparison of indices between patients with CD and those with UC were done by unpaired t test depending on normality
[b] Comparison of indices between patients with CD and those with UC were done by Mann–Whitney test depending on normality

of CD patients and 18% of UC patients and between −2.5 and −1.0 SD in 50% and 55% of subjects with CD and UC, respectively (Table).

Multiple regression analyses for variables associated with BMD *Z* scores at various sites

Multiple regression analyses were done for BMD including BMI, plasma concentrations of PK, MK-7, and 25OH-D as independent variables. Serum PTH level was excluded since coinclusion of 25OH-D and PTH caused multicollinearity to skew the results. As shown in Table , BMI was a significant predictor of BMD at weight-bearing sites such as the lumbar spine, femoral neck, and total hip. Plasma MK-7 and 25OH-D concentrations were significant predictors of femoral neck BMD. Plasma PK concentration was a significant predictor of BMD at the distal one-third of radius and lumbar spine.

Analysis of food intake in CD and UC patients

Food intake could be evaluated in 25 patients (15 with CD and 10 with UC). Fat intake was significantly lower and protein intake was significantly higher in patients with CD than those with UC. The results were similar when expressed as the percentage of total energy intake. The adequate intakes (AI) for calcium in Japan are 600–650 mg for men and 550–600 mg for women. AI for vitamin K is 75 μg for men and 65 μg for women, respectively, and that for vitamin D is 5 μg [30]. As a whole, although the average calcium intake was below AI, vitamin K and D intakes apparently exceeded AI (Table 4).

Ten patients with CD were on enteral nutrition (EN) with almost fat-free formula; Elental® (Ajinomoto Pharma, Tokyo, Japan) with 18.8%, 1.4%, and 79.8% of total energy contributed by protein, fat, and carbohydrate, respectively. One patient with UC was on total parenteral nutrition. When nutrient intake was compared between CD patients with EN and those without EN, the former had higher protein and carbohydrates intakes and lower fat intake than the latter. Regarding other nutrients intake, there was no significant difference between the two groups except calcium. There were no significant differences in plasma vitamin K and 25OH-D concentrations between these groups (data not shown).

Table 2 BMD in patients with CD and UC

	CD (n=18)	UC (n=22)	p value
BMD (g/cm^2)			
Lumbar spine (L1–4)	0.880±0.072	0.931±0.138	0.152
Femoral neck	0.697±0.105	0.768±0.126	0.064
Total hip	0.801±0.120	0.910±0.136	0.012
Distal one-third of radius	0.634±0.066	0.664±0.084	0.222
Z scores			
Lumbar spine (L1–4)	−0.96±0.57**	−0.14±1.13	0.005
Femoral neck	−1.00±0.78**	−0.09±1.16	0.005
Total hip	−0.85±0.91**	0.27±1.11	0.001
Distal one-third of radius	−2.19±0.94**	−1.29±1.79**	0.064
T scores			
Lumbar spine (L1–4)	−1.18±0.59	−0.79±1.06	0.155
Femoral neck	−1.14±0.85	−0.56±1.05	0.067
Total hip	−0.95±0.97	−0.11±1.08	0.014
Distal one-third of radius	−2.31±1.00	−1.83±1.81	0.055

Values represent the mean±SD, and comparison between CD and UC groups was made with unpaired t test
**p<0.01, statistically significant difference from zero with one-sample t test in the Z score

Table 3 Multiple regression analyses for the determination of independent factors for BMD

Sites	R^2	Variable	β coefficient	p value
Lumbar spine	0.529	BMI	0.663	0.005
		Plasma PK	0.612	0.035
Femoral neck	0.748	BMI	0.363	0.028
		Plasma MK-7	0.295	0.036
		Plasma 25OH-D	0.484	0.037
Total hip	0.731	BMI	0.438	0.012
Distal one-third of radius	0.388	Plasma PK	0.813	0.016

Only significant predictors are shown. Determinants of independent predictors for BMD at each site were analyzed by multivariate analysis with forced entry. Variables included were BMI, plasma 25OH-D, PK, and MK-7

Multiple regression analyses for plasma vitamin K and 25OH-D concentrations

Multiple regression analyses revealed that fat intake was a significant determinant of plasma PK and 25OH-D levels. Vitamin K intake was a significant predictor for plasma MK-7 level (Table).

Discussion

In this study, we have studied the IBD-induced osteoporosis in relation to vitamin K and D status of the patients. Decreased BMD and high-turnover bone was far more pronounced in patients with CD than those with UC.

Although glucocorticoid treatment is one of the postulated pathogenic factors for osteoporosis in IBD [, , 31, 32], current use of glucocorticoid was not associated with decreased BMD in the present study. Unfortunately, the possible involvement of glucocorticoid could not be

Table 4 Food intake in CD and UC patients

	IBD (n=25)	CD (n=15)		UC (n=10)	p value	EN p value
		EN therapy (n=10)	Non-EN therapy (n=5)			
Energy (kcal)	1,707±479 (1,580)	1,961±465 (1,796)	1,412±320 (1,501)	1,602±466 (1,524)	0.338[a]	0.055[a]
Energy intake from EN (kcal)	–	810±318 (750) (min 300–max 1200)	–	–	–	–
Proportion of total energy intake from EN (%)	–	42.0±16.8 (39.1) (min 20–max 77)	–	–	–	–
Protein (g)	68.2±19.3 (62.8)	81.9±21.1 (79.8)	60.3±12.3 (61.9)	58.5±11.8 (57.0)	0.022[b]	0.028[b]
Fat (g)	29.9±13.9 (28.3)	22.1±10.0 (24.0)	29.1±7.5 (30.7)	38.1±15.8 (38.1)	0.030[b]	0.164[b]
Carbohydrates (g)	287.8±98.4 (274.3)	359.0±85.3 (339.3)	223.5±60.3 (242.1)	248.9±85.5 (258.9)	0.098[b]	0.005[b]
Calcium (mg)	483±250 (431.0)	662±230 (675)	380±144 (351)	356±214 (354.5)	0.032[b]	0.014[b]
Vitamin K (μg)	131.1±124.6 (73.0)	96.8±68.8 (66.0)	207.0±220.9 (73.0)	127.5±102.2 (97.0)	0.846[a]	0.337[b]
Vitamin D (μg)	9.6±10.4 (6.9)	9.3±7.4 (7.4)	10.2±13.3 (1.5)	9.6±12.5 (6.6)	0.782[a]	0.893[b]
Macronutrient (% energy)						
Protein	16.2±2.9 (15.6)	16.7±2.0 (16.0)	17.4±2.4 (17.4)	15.2±3.7 (14.4)	0.008[a]	0.617[b]
Fat	16.4±7.7 (14.9)	10.1±4.0 (10.6)	18.8±3.9 (19.0)	21.5±7.6 (21.1)	0.009[b]	0.004[b]
Carbohydrates	66.5±8.7 (66.1)	73.4±6.4 (72.7)	62.7±5.1 (61.3)	61.5±7.8 (61.9)	0.017[b]	0.005[b]

Values represent the mean±SD with values in parentheses being the median. "p value" and "EN p value" represent the comparison between CD and UC patients and the comparison between CD subjects with EN and those without EN, respectively

[a] Comparisons between CD and UC patients and that between CD with EN and without EN were done with unpaired t test depending on normality

[b] Comparisons between CD and UC patients and that between CD with EN and without EN were done with Mann–Whitney test depending on normality

evaluated in more detail, since most of them were referred to the university hospital from another hospital and cumulative dose of glucocorticoid could not be precisely calculated. We believe, however, that glucocorticoid use is unlikely to be mainly responsible for the decreased BMD in the current subjects based on the following consideration. Trabecular bone is mainly affected in glucocorticoid-induced osteoporosis (GIO) [33]. In GIO, decreased BMD is most prominent at the lumbar spine with trabecular predominance [33]. In contrast is the present finding that decreased BMD was most marked at the distal one-third of radius, a site of cortical predominance.

Table 5 Multiple regression analyses for the predictor(s) of plasma 25OH-D, PK, and MK-7 levels

	R^2	Variable	β coefficient	p value
Plasma PK	0.586	Fat intake	0.620	0.030
Plasma MK-7	0.464	Vitamin K intake	0.708	0.036
Plasma 25OH-D	0.452	Fat intake	0.584	0.046

Only significant predictors are shown. Independent predictor for plasma PK, MK-7, or 25OH-D concentrations was analyzed by multivariate analysis with forced entry. Serum CRP level and intakes of protein, fat, and carbohydrates were included in all analyses. Vitamin D intake was additionally included in the analysis for plasma 25OH-D concentration. For plasma PK and MK-7, vitamin K intake was additionally included

Another possible factor includes disease severity. IBD is associated with increased production of inflammatory cytokines, e.g., IL-1, IL-6, and TNF-α which are potent stimulators of osteoclastic bone resorption [34–36]. Although circulating concentration of these cytokines could not be measured, serum level of CRP was evaluated as an inflammation marker. Although serum CRP level was higher in CD patients, it was not associated with BMD (data not shown).

Low BMI is another factor to be associated with IBD-related osteoporosis [5, 37], but the current results that the average BMI was in the normal range and BMD at nonweight-bearing site was also decreased, which make it unlikely that the reduced BMD in these subjects is related to their BMI.

Then, we focused our attention to the possible involvement of vitamin K and D deficiency. Unfortunately, no single measure can represent the vitamin K status with PK and MK-7 being the two major circulating forms. PK is rich in green vegetables, whereas MK-7 content is extraordinarily high in fermented soy "natto," which is a common food in Japan, but not elsewhere [38, 39]. Large standard deviation in plasma MK-7 concentration probably reflects that some Japanese favors, but some dislike "natto." Indeed, a large geographic difference in plasma MK-7 concentration in Japan was reported to be due to the frequency of natto intake [10]. Since most vitamin K intake

comes from green vegetables in America and Europe [,], previous reports on the plasma concentration of vitamin K from outside Japan focused on PK [,]. Although circulating vitamin K levels have been measured with various methods, the present data were obtained with our newly developed LC-APCI-MS/MS procedure with stable isotope-labeled internal standard yielding high sensitivity and specificity [ ]. In our recent report from the Nagano study using the same assay procedure, mean plasma PK level was 1.52 ng/mL, 1.74 ng/mL, and 1.29 ng/mL in healthy women aged 30–49, 50–69, and over 70 years, respectively [ ]. Thus, blood level of vitamin K was much lower in IBD patients than that in the healthy Japanese measured by the same assay. The data in the Nagano study may be higher than those in the average Japanese, since many participants in the Nagano study were farmers with much vegetable consumption, for which further discussion will be made in the next paragraph.

Then, we considered the physiological relevance of the above data. We measured serum levels of PIVKA-II and ucOC as the sensitive markers of vitamin K deficiency in the liver and bone, respectively, with the cut-off values being 28 mAU/mL for PIVKA-II and 4.5 ng/mL for ucOC. Both levels were significantly higher in CD patients than those with UC. These results, together with the decreased plasma levels of PK and MK-7 in CD patients, strongly suggest that circulating vitamin K levels are decreased at least in patients with CD. Decreased plasma levels of 25OH-D, PK, and MK-7 are likely to have physiological significance considering that they were determinants of BMD at some measurement sites as shown in Table , as well as the above-mentioned elevated concentrations of PIVKA-II and ucOC.

The average and median concentration for ucOC, but not for PIVKA-II, was above the cut-off value in these subjects, especially CD patients. Serum PIVKA-II level exceeded the cut-off level in only 25% and 4% of patients with CD and UC, respectively. In contrast, serum ucOC concentration was above the cut-off value in 92% and 36% of patients with CD and UC, respectively. These differences could be explained by a pharmacokinetic feature called "first-pass effect." Vitamin K absorbed from the gastrointestinal tract is transported to the liver via the portal vein where it is used for the γ-carboxylation of clotting factors [42, 43]. Only the vitamin K unutilized in the liver will be available to the bone. Therefore, the bone is likely to be much more susceptible to vitamin K deficiency than the liver. Thus, serum ucOC level well reflects the skeletal vitamin K deficiency, but needs to be interpreted with caution that it is also affected by bone turnover as exemplified with its association with BAP.

The average serum concentration of 25OH-D was 11.5 and 20.2 ng/mL in CD and UC patients, respectively. Serum PTH concentration was reciprocally higher in CD than in UC. Thus, most IBD patients, especially those with CD, were considered to be vitamin D deficient.

The next consideration relates to the factor(s) responsible for the deficiency of these vitamins. As shown in Table , there was no significant difference in vitamin K and D intakes between CD and UC, which suggests that the difference in blood levels of these vitamins could not be ascribed to the difference in their intake. Malabsorption of these vitamins would be the most likely explanation for the apparent discrepancy, which is compatible with the previous report that the absorption of exogenously administered vitamin D_2 was severely disturbed in CD, but not in UC [ ].

As the basis for the malabsorption of vitamin K and D, compromised ability of the intestine to absorb these vitamins would be the most fundamental because of intestinal inflammation or intestinal resection in some cases. In the current study, multiple regression analyses revealed that fat intake was a significant determinant of plasma concentrations of both PK and 25OH-D. Many patients in the current study were under nutritional therapy with restricted fat intake, since excessive fat intake is considered to worsen the intestinal inflammation in IBD patients. These results suggest that restricted fat intake could be another factor responsible for the impaired absorption of vitamin K and D, which, however, is not supported by some previous studies. For example, Tangpricha et al. [44] reported that vitamin D dissolved in fat-free orange juice was effectively absorbed from the intestine and indicated that fat content of the diet little influenced vitamin D absorption. Thus, further studies, favorably the intervention ones, are required on the role of fat restriction on the absorption of fat-soluble vitamins.

Unlike PK, vitamin K intake was the significant predictor for plasma MK-7 level. The difference between two vitamin K analogs may reflect their pharmacokinetic difference such as the far longer half-life of MK-7 than PK [38], although further detailed studies are needed. Actually, this study is a baseline valuation. Follow-up study is now under way to evaluate the patients' vitamin status and BMD with milder food restriction with more use of immunosuppressants and biomodulators.

In the present study, vitamin K and D status of IBD patients was both studied, which was not adopted before. The intake of vitamins and their plasma concentration were simultaneously evaluated, which was not usually the case in the previous studies. These would be the strength of the current study. We have to mention two limitations of this study. First, the number of subjects studied was not so large. Thus, it could not be determined whether vitamin K and D deficiency observed in the current study was associated with increased fracture risk as reported in the

previous report [ ]. Next, the patients were under nutritional therapy with restricted fat intake. Thus, further studies with larger number of subjects with wider variety of background profiles are necessary to generalize the present findings.

In summary, BMD was decreased and plasma concentrations of PK, MK-7, and 25OH-D were quite low in patients with IBD, especially CD, despite apparently sufficient intake of these vitamins. Impaired intestinal absorption of these fat-soluble vitamins is likely to be associated with vitamin K and D deficiency and bone loss in IBD.

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Conflicts of interest None.

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