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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 [7]. Recent evidences suggest that

vitamin K is also essential in the extrahepatic tissues including skeleton and vasculature [8]. Fracture risk was increased in subjects with low vitamin K intake [9, 10] or increased serum undercarboxylated osteocalcin (ucOC) level, which is a sensitive marker for skeletal vitamin K deficiency [11, 12]. Furthermore, recent metaanalysis has shown that vitamin K treatment decreased fracture incidence [13]. 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 [14, 15] or by measuring circulating level of these vitamins [6, 16–18], but rarely by both [19, 20]. 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 [6, 18, 21–24]. 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 [25]. 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 [26].

Dietary intake

Dietary information was obtained from 1-day dietary record completed by the patients [27]. 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, 29]. 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 2).

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 3, 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 5).

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 [1, 3, 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 [40]. Since most vitamin K intake

comes from green vegetables in America and Europe [9, 10], previous reports on the plasma concentration of vitamin K from outside Japan focused on PK [11, 40]. 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 [25]. 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 [41]. 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 3, 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 4, 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 [23].

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 [45]. 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.

References

1. Compston JE, Judd D, Crawley EO et al (1987) Osteoporosis in patients with inflammatory bowel disease. Gut 28:410–415
2. Bjarnason I, Macpherson A, Mackintosh C et al (1997) Reduced bone density in patients with inflammatory bowel disease. Gut 40:228–233
3. Ardizzone S, Bollani S, Bettica P et al (2000) Altered bone metabolism in inflammatory bowel disease: there is a difference between Crohn's disease and ulcerative colitis. J Intern Med 247:63–70
4. Bjarnason I (1999) Metabolic bone disease in patients with inflammatory bowel disease. Rheumatology 38:801–804
5. Bartram SA, Peaston RT, Rawlings DJ et al (2006) Multifactorial analysis of risk factors for reduced bone mineral density in patients with Crohn's disease. World J Gastroenterol 12:5680–5686
6. Schoon EJ, Müller MC, Vermeer C et al (2001) Low serum and bone vitamin K status in patients with longstanding Crohn's disease: another pathogenetic factor of osteoporosis in Crohn's disease? Gut 48:473–477
7. Vermeer C (1990) γ-Carboxyglutamate-containing proteins and the vitamin K-dependent carboxylasa. Biochem J 266:625–636
8. Booth SL (1997) Skeletal functions of vitamin K-dependent proteins: not just for clotting anymore. Nutr Rev 55:282–284
9. Feskanich D, Weber P, Willett WC et al (1999) Vitamin K intake and hip fractures in women: a prospective study. Am J Clin Nutr 69:74–79
10. Booth SL, Tucker KL, Chen H et al (2000) Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr 71:1201–1208
11. Booth SL, Broe KE, Peterson JW et al (2004) Associations between vitamin K biochemical measures and bone mineral density in men and women. J Clin Endocrinol Metab 89: 4904–4909
12. Szulc P, Chapuy M-C, Meunier PJ et al (1993) Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. J Clin Invest 91:1769–1774
13. Cockayne S, Adamson J, Lanham-New S et al (2006) Vitamin K and the prevention of fractures: systematic review and meta-analysis of randomized controlled trials. Arch Intern Med 166:1256–1261
14. Bernstein CN, Bector S, Leslie W (2003) Lack of relationship of calcium and vitamin D intake to bone mineral density in premenopausal women with inflammatory bowel disease. Am J Gastroenterol 98:2468–2473
15. Duggan P, O'Brien M, Kiely M et al (2004) Vitamin K status in patients with Crohn's disease and relationship to bone turnover. Am J Gastroenterol 99:2178–2185
16. Abreu MT, Kantorovich EA, Vasiliauskas EA et al (2004) Measurement of vitamin D levels in inflammatory bowel disease patients reveals a subset of Crohn's disease patients with elevated 1,25-dihydroxyvitamin D and low bone mineral density. Gut 53:1129–1136
17. Driscoll RH, Meredith SC, Sitrin M et al (1982) Vitamin D deficiency and bone disease in patients with Crohn's disease. Gastroenterology 83:1252–1258
18. Jahnsen J, Falch JA, Mowinckel P et al (2002) Vitamin D status, parathyroid hormone and bone mineral density in patients with inflammatory bowel disease. Scand J Gastroenterol 37:192–199
19. Vogelsang H, Klamert M, Resch H et al (1995) Dietary vitamin D intake in patients with Crohn's disease. Wien Klin Wochenschr 107:578–581
20. Silvennoinen J (1996) Relationships between vitamin D, parathyroid hormone and bone mineral density in inflammatory bowel disease. J Intern Med 239:131–137
21. Bikle DD (2001) Osteoporosis in gastrointestinal, pancreatic, and hepatic diseases. In: Marcus R, Feldman D, Kelsey J (eds) Osteoporosis. 2nd edn. Academic, San Diego, CA, pp 237–258
22. Haderslev KV, Jeppesen PV, Sorensen HA et al (2003) Vitamin D status and measurements of markers of bone metabolism in patients with small intestinal resection. Gut 52:653–658
23. Lo CW, Paris PW, Clemens TL, Nolan J, Holick MF (1985) Vitamin D absorption in healthy subjects and in patients with intestinal malabsorption syndromes. Am J Clin Nutr 42:644–649
24. Krasinsk SD, Russell RM, Furie BC et al (1985) The prevalence of vitamin K deficiency in chronic gastrointestinal disorders. Am J Clin Nutr 41:639–643
25. Suhara Y, Kamao M, Tsugawa N et al (2005) Method for the determination of vitamin K homologues in human plasma using high-performance liquid chromatography-tandem mass spectrometry. Anal Chem 77:757–763
26. Kanis JA, Melton LJ 3rd, Christiansen C et al (1994) The diagnosis of osteoporosis. J Bone Miner Res 9:1137–1141
27. Todd KS, Hudes M, Calloway DH (1983) Food intake measurement: problems and approaches. Am J Clin Nutr 37:139–146
28. Davies M, Berry JL, Mee AP (2005) Bone disorders associated with gastrointestinal and hepatobiliary disease. In: Feldman D, Pike JW, Glorieux FG (eds) Vitamin D. 2nd edn. Academic, San Diego CA, pp 1293–1311
29. Holick MF (2007) Vitamin D deficiency. N Engl J Med 357: 266–281
30. Ministry of Health, Labour, and Welfare, Japan (2005) Dietary reference intakes for Japanese. Daiichi Shuppan, Tokyo
31. Silvennoinen JA, Karttunen TJ, Niemelä SE et al (1995) A controlled study of bone mineral density in patients with inflammatory bowel disease. Gut 37:71–76
32. Motley RJ, Clements D, Evans WD et al (1993) A four-year longitudinal study of bone loss in patients with inflammatory bowel disease. Bone Miner 23:95–104
33. Sambrook PN (2006) Glucocorticoid-induced osteoporosis. In: Favus MJ (ed) (2006) Primers on the metabolic bone diseases and disorders of mineral metabolism. 6th edn. American Society for Bone and Mineral Research, Washington DC, pp 296–302

34. Nemetz A, Tóth M, García-González MA et al (2001) Allelic variation at the interleukin 1β gene is associated with decreased bone mass in patients with inflammatory bowel diseases. Gut 49:644–649
35. Moschen AR, Kaser A, Enrich B et al (2005) The RANKL/OPG system is activated in inflammatory bowel disease and relates to the state of bone loss. Gut 54:479–487
36. Sylvester FA, Wyzga N, Hyams JS et al (2002) Effect of Crohn's disease on bone metabolism in vitro: a role for interleukin-6. J Bone Miner Res 17:695–702
37. Héla S, Nihel M, Faten L et al (2005) Osteoporosis and Crohn's disease. Joint Bone Spine 72:403–407
38. Schurgers LJ, Teunissen KJ, Hamulyák K et al (2007) Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. Blood 109:3279–3283
39. Kaneki M, Hedges SJ, Hosoi T et al (2001) Japanese fermented soybean food as the major determination of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. Nutrition 17:315–321
40. Beavan SR, Prentice A, Stirling DM et al (2005) Ethnic differences in osteocalcin γ-carboxylation, plasma phylloquinone (vitamin K1) and apolipoprotein E genotype. Eur J Clin Nutr 59:72–81
41. Tsugawa N, Shiraki M, Suhara Y et al (2006) Vitamin K status of healthy Japanese women: age-related vitamin K requirement for γ-carboxylation of osteocalcin. Am J Clin Nutr 83:380–386
42. Booth SL, Martini L, Peterson JW et al (2003) Dietary phylloquinone depletion and repletion in older women. J Nutr 133:2565–2569
43. Vermeer C, Gijsbers BL, Crācium AM et al (1996) Effects of vitamin K on bone mass and bone metabolism. J Nutr 126:1187S–1191S
44. Tangpricha V, Koutkia P, Rieke SM et al (2003) Fortification of orange juice with vitamin D: a novel approach for enhancing vitamin D nutritional health. Am J Clin Nutr 77:1478–1483
45. Bernstein CN, Blanchard JF, Leslie W, Wajda A, Yu N (2000) The incidence of fracture among patients with inflammatory bowel disease. A population-based cohort study. Ann Intern Med 133:795–799