Table 1
Demographic and physical characteristics, nutritional intakes, bone mineral density (BMD), and serum biochemical profiles of study subjects

	N	Mean	SD
Age (years)	600	63.5	5.8
Menopausal age (years)	598	51.0	8.3
Number of children	599	2.3	0.8
Height (cm)	600	150.7	5.5
Weight (kg)	600	53.1	8.3
Body mass index (kg/m^2)	600	23.4	3.5
Thigh muscle strength (kg)	584	36.0	7.6
Grip strength (kg)	599	23.2	3.9
Timed Up & Go test (s)	593	5.0	1.0
Calcium intake[a] (mg/day)	600	527	160
Vitamin D intake (μg/day)	600	11.7	2.7
BMD at lumbar spine (g/cm^2)	599	0.846	0.147
BMD at femoral neck (g/cm^2)	598	0.668	0.094
Serum 25-hydroxyvitamin D (nmol/L)	600	55.6	14.6
Serum 1,25-dihydroxyvitamin D (pmol/L)	598	130.5	44.5
Serum intact parathyroid hormone (pmol/L)	600	4.24	1.40
Serum osteocalcin (mg/ml)	600	9.93	3.95
Serum type I collagen cross-linked N-telopeptides (nmol BCE/L)	595	21.0	6.5

[a] Calcium intake from dietary source was 518 mg (SD 147).

There may be ethnic differences in the effects of low vitamin D status on bone mass or bone metabolism. African Americans typically have lower vitamin D levels than Caucasian Americans, yet they have a lower prevalence of osteoporosis [3]. Furthermore, the relationship between serum 25-hydroxyvitamin D (25[OH]D, an index of vitamin D status) concentrations and bone mineral density (BMD) may differ between blacks and whites [4]. These findings demonstrate the importance of studies aimed at understanding the effect of vitamin D status on bone in non-white populations.

There have been only a few studies on the association between vitamin D status and bone parameters in Asians; those that have been conducted have typically had small sample sizes. One large population-based study among Japanese elderly women reported a cutoff level of serum 25(OH)D concentration in relation to elevated serum parathyroid hormone (PTH) as low as 40 nmol/L. This is lower than cutoff levels reported recently by several studies among Caucasian patients [5] and is less than current recommended levels (75–80 nmol/L or higher) of serum 25(OH)D [6]. These results suggested a possible ethnic difference between Asians and whites.

The primary aim of this study is to investigate the association between the serum 25(OH)D concentration and bone mass or bone metabolism among Japanese postmenopausal women. Results from this study may inform the appropriate levels of serum 25(OH)D to aim for in preventive vitamin D supplementation programs for these women.

Subjects and methods

Subjects

All 1310 women who lived in Yokogoshi area (Niigata City, Japan) aged between 55 and 74 years on March 31, 2006, were invited to participate in the Yokogoshi Study, a cross-sectional, epidemiologic, community-based investigation of bone health for postmenopausal women. The study was conducted in November 2005. Of the 1310 women, 674 (51.5%) agreed to participate in the study. All participants were non-institutionalized and ambulatory. The following women who had medical histories that may have affected their bone metabolism were excluded from analysis: (1) 13 women with a history of bilateral oophorectomy, (2) 7 women who had undergone corticosteroid therapy, and (3) 54 women treated with bisphosphonates, selective estrogen receptor modulators, active vitamin D analogues, vitamin K (menatetrenone), estrogen, or calcitonin for suspected osteoporosis. Ultimately, 600 of 674 (89%) women agreeing to participate in the study formed the group analyzed. Written informed consent was obtained from all subjects. The protocol of this study was approved by the Ethics Committee of Niigata University School of Medicine.

BMD measurement

BMDs of the lumbar spine (L2–4) and right femoral neck were measured through the dual-energy X-ray absorptiometry (DXA) method using a

Table 2
Results of simple linear regression analyses with bone mineral density (BMD) as the dependent variable

Predictor variable	BMD of the lumbar spine			BMD of the femoral neck		
	Regression coefficient (β)	R^2	P value	Regression coefficient (β)	R^2	P value
Age (years)	−0.00611	0.057	<0.0001	−0.00473	0.084	<0.0001
Years since menopause	−0.00289	0.039	<0.0001	−0.00193	0.042	<0.0001
Number of children	−0.00158	0.000	0.8312	−0.00216	0.000	0.6519
Height (cm)	0.00481	0.033	<0.0001	0.00322	0.036	<0.0001
Weight (kg)	0.00610	0.119	<0.0001	0.00440	0.151	<0.0001
Body mass index (kg/m^2)	0.0118	0.078	<0.0001	0.00854	0.099	<0.0001
Thigh muscle strength (kg)	0.00464	0.058	<0.0001	0.00341	0.076	<0.0001
Grip strength (kg)	0.00914	0.059	<0.0001	0.00541	0.050	<0.0001
TUG test[a] (s)	−0.101	0.013	0.0050	−0.0876	0.024	0.0002
Engage in housework (No, 0; yes, 1)	−0.00392	0.000	0.8908	−0.0179	0.002	0.3289
Engage in light exercise (No, 0; yes, 1)	−0.0110	0.001	0.3705	−0.00537	0.001	0.4936
Engage in farmwork (No, 0; yes, 1)	−0.0159	0.003	0.1864	−0.00164	0.000	0.8323
Calcium intake (mg/day)	0.0000631	0.005	0.0946	0.0000225	0.001	0.3532
Serum 25(OH)D (nmol/L)	0.000622	0.004	0.1322	0.000914	0.020	0.0005
Serum 1,25(OH)$_2$D (pmol/L)	−0.000405	0.015	0.0028	−0.000246	0.013	0.0046
Serum intact PTH[a] (pmol/L)	−0.0183	0.002	0.3298	−0.0357	0.015	0.0029
Serum osteocalcin (ng/ml)	−0.00868	0.054	<0.0001	−0.00532	0.050	<0.0001
Serum NTX (nmol BCE/L)	−0.113	0.043	<0.0001	−0.0655	0.035	<0.0001

Abbreviations: TUG, Timed "Up & Go"; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; NTX, type I collagen cross-linked N-telopeptides.
[a] Logarithmically transformed.

QDR4500a (Hologic Inc., Bedford, MA, USA) by a single, trained X-ray technician. The in vivo coefficients of variation (CVs) of the BMD measurements were 0.3% for the lumbar spine and 0.6% for the femoral neck.

Physical examination

The grip strength of each hand was measured once with a digital hand dynamometer, and the average value of both hands was adopted. Isometric thigh muscle strength of both legs together was measured with a leg muscle dynamometer (T.K.K.5710g, Takei Scientific Instruments, Co., Ltd., Niigata, Japan). Walking ability (walking time) was assessed by the timed "Up & Go" (TUG) test [7]. Body height and weight of the subjects in light underwear were measured to the nearest 1 mm and 100 g, respectively. The body mass index (BMI) was calculated by dividing body weight (kg) by the square of body height (m^2).

Biochemical measurements

A 6-h-fasting blood specimen was drawn in the daytime. The specimen was immediately maintained at 4 °C. The serum was obtained within 1 day of collection by centrifugation at 1613×*g* for 10 min and stored at −80 °C until the biochemical analysis. The serum 25(OH)D concentration was determined by radioimmunoassay (DiaSorin, Stillwater, MN, USA) with an inter-assay CV value of 9.9%. The serum 1,25-dihydroxyvitamin D (1,25$[OH]_2$D) concentration was determined by radioimmunoassay (IDS Ltd., Boldon, England, UK), which has an inter-assay CV value of 12.8%. The serum intact PTH concentration was measured with a two-site immunoradiometric assay (Nichols Institute Diagnostics, San Clemente, CA, USA), which has an inter-assay CV value of 1.5%. The serum osteocalcin (OC) concentration was determined by an immunoradiometric assay (Mitsubishi Kagaku Medical, Inc., Tokyo, Japan) with an inter-assay CV value of 6.6%. The serum type I collagen cross-linked N-telopeptides (NTX) concentration was determined by an enzyme-linked immunosorbent assay (Osteomark NTX Serum, Ostex International, Inc., Seattle, WA, USA), which had an inter-assay CV value of 2.8%.

Interview

Demographic, lifestyle, and nutritional information was obtained through interview. Age, reproductive history, medical history, and current medications were recorded. Current calcium intake was assessed with a previously validated food frequency questionnaire [8]. The correlation coefficient between values measured by this method and the conventional 3-day diet record was 0.668. Physical activity levels were assessed based on whether subjects engaged in the following three activities at least once a week: (1) housework, (2) light exercise, such as gate ball (or croquet), taking walks, and so on, as light activity, and (3) farmwork (or gardening), as moderate activity.

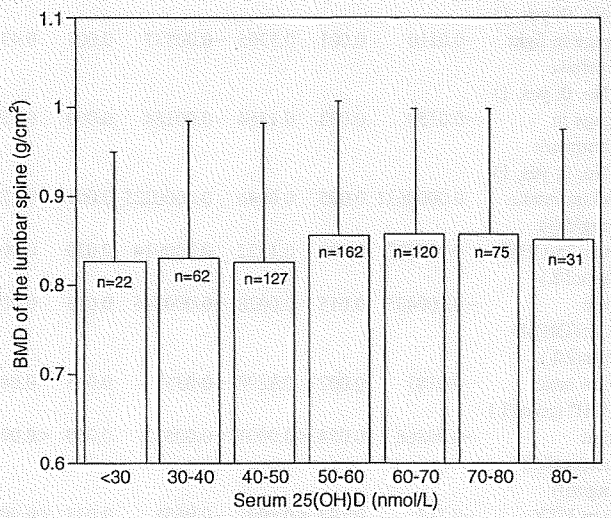

Fig. 1. Mean (plus SD) values of bone mineral density (BMD) of the lumbar spine for each 10-nmol/L increment in the serum 25-hydroxyvitamin D (25[OH]D) concentration. The serum 25(OH)D concentration was not linearly associated with BMD at the lumbar spine ($P=0.1322$), although 50 nmol/L may be an inflection point.

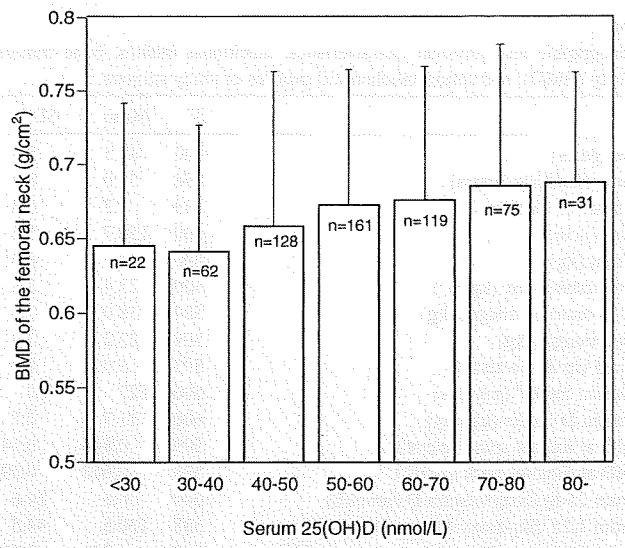

Fig. 2. Mean (plus SD) values of bone mineral density (BMD) of the femoral neck for each 10-nmol/L increment in the serum 25-hydroxyvitamin D (25[OH]D) concentration. BMD becomes higher as the 25(OH)D level becomes higher beginning from the 40- to 50-nmol/L group of serum 25(OH)D.

Statistical analysis

All continuous variables were checked for normality. TUG test, serum intact PTH, and NTX concentrations were skewed to higher values and were transformed logarithmically prior to conducting statistical tests. Categorical variables, such as "housework", "light exercise", and "farmwork" were coded as 0 for "no" and 1 for "yes". Student's *t*-test was used to test a difference in two mean values. Analysis of variance (ANOVA) was used to test differences among multiple mean values. ANOVA with Dunnett's multiple comparison was used to compare

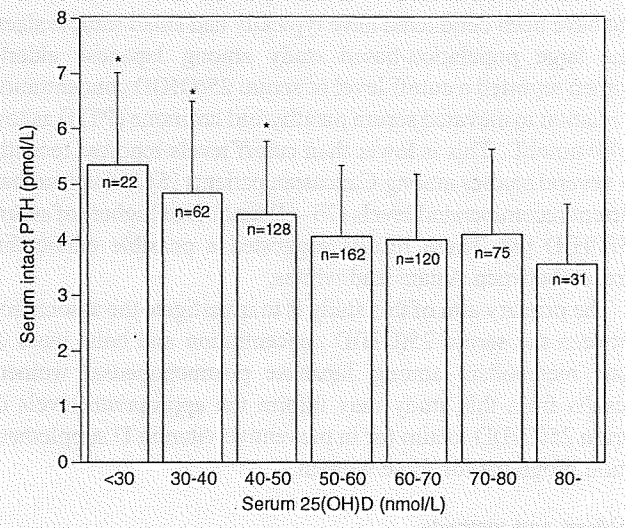

Fig. 3. Mean (plus SD) values of the serum intact parathyroid hormone (PTH) concentration for each 10 nmol/L increment in the serum 25-hydroxyvitamin D (25[OH]D) concentration. Mean serum intact PTH concentrations for 25(OH)D <30 nmol/L, 30–39 nmol/L, and 40–49 nmol/L, indicated with an asterisk (*), are significantly higher than those for serum 25(OH)D concentrations ≥50 nmol/L, as assessed by ANOVA with the Dunnett multiple comparison.

Table 3
Results of a stepwise multiple linear regression analysis predicting bone mineral density (BMD)

Independent variable	Regression coefficient (β)	Standard error	R^2	P value
BMD of the lumbar spine				
BMI (kg/m^2)	0.0115	0.0016	0.099	<0.0001
Age (years)	−0.00487	0.00117	0.058	<0.0001
Serum osteocalcin (ng/ml)	−0.00633	0.00152	0.041	<0.0001
Grip strength (kg)	0.00482	0.00146	0.016	0.0011
Calcium intake (mg/day)	0.0000904	0.0000344	0.012	0.0089
Years since menopause	−0.00142	0.00065	0.005	0.0279
Serum NTX[a] (nmol BCE/L)	−0.0434	0.0224	0.005	0.0535
BMD of the femoral neck				
BMI (kg/m^2)	0.00825	0.00010	0.112	<0.0001
Age (years)	−0.00521	0.00061	0.084	<0.0001
Serum osteocalcin (ng/ml)	−0.00422	0.00085	0.031	<0.0001
Serum 25(OH)D (nmol/L)	0.000705	0.000235	0.020	0.0029
Serum intact PTH[a] (pmol/L)	−0.0292	0.0107	0.013	0.0065
Grip strength (kg)	0.00215	0.00090	0.009	0.0167
Calcium intake (mg/day)	0.0000449	0.0000211	0.008	0.0336

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; NTX, type I collagen cross-linked N-telopeptides.
[a] Log-transformed values.

one mean value with other mean values. Simple linear regression analysis was used to identify predictors of BMD, indices of bone metabolism, including the log-transformed serum intact PTH, OC, and log-transformed NTX, and physical tests, including muscle strength and log-transformed TUG test as outcome variables. A stepwise multiple linear regression analysis was used to identify independent predictors of BMD. Candidate independent variables for the stepwise method were age, menopausal age, BMI, physical tests, lifestyle variables, calcium intake, the 25(OH)D, log-transformed serum intact PTH, OC, and log-transformed NTX concentrations. The serum 1,25(OH)$_2$D concentration was not included in the model because a negative association between serum 1,25(OH)$_2$D concentrations and BMDs was considered to be due to a compensatory increase of serum 1,25(OH)$_2$D concentrations for low bone mass [9,10]. Multiple logistic regression analyses were used to calculate adjusted odds ratios (ORs) of vitamin D insufficiency for "low BMD (t score ≤−2.5 SD)". Test for linear trend was performed by using the logistic regression technique. Computations were performed by using the SAS statistical package (release 8.02, SAS Institute Inc., Cary, NC, USA). A P value less than 0.05 was considered statistically significant.

Results

The demographic and physical characteristics, nutritional intake, bone mass, and serum biochemical profiles are shown in Table 1. The proportion of subjects who had the serum 25(OH)D concentration less than 30 nmol/L and 50 nmol/L were 22/600 (3.7%) and 212/600 (35.3%), respectively. On the physical activity measure, 572 (95.3%) subjects did housework, 250 (41.7%) engaged in light activity, and 298 (49.7%) engaged in farmwork (moderate activity). "Low BMDs" (t score ≤−2.5 SD) were observed in 106/599 (17.7%) of lumbar spines and 82/598 (13.7%) of femoral necks.

The results of the simple linear regression analyses with BMD as the outcome are shown in Table 2. The serum 25(OH)D concentration was not significantly associated with BMD of the lumbar spine but was positively associated with BMD of the femoral neck. Mean BMD at the lumbar spine for each 10-nmol/L increment in the serum 25(OH)D concentration is shown in Fig. 1. The serum 25(OH)D concentration was not linearly associated with BMD at the lumbar spine (P=0.1322). Mean BMDs at the femoral neck for each 10-nmol/L increment in the serum 25(OH)D concentration are shown in Fig. 2. BMD increases as the 25(OH)D concentration increases beginning from the 40- to 50-nmol/L group of serum 25(OH)D.

The serum 25(OH)D concentration was negatively associated with the log-transformed serum intact PTH concentration (β=−0.00543, R^2=0.061, P<0.0001). Mean serum intact PTH concentrations for each 10 nmol/L increment in the serum 25(OH)D concentration are shown in Fig. 3. Mean serum intact PTH concentrations for serum 25(OH)D <30 nmol/L, 30–39 nmol/L, and 40–49 nmol/L were significantly higher than a probable baseline intact PTH concentration, i.e., the mean intact PTH concentration for serum 25(OH)D concentrations ≥50 nmol/L. A linear association between calcium intake and the log-transformed serum intact PTH concentration was of borderline significance (P=0.0611). The log-transformed serum intact PTH concentration was significantly associated with both serum OC (β=1.29, R^2=0.011, P=0.0102) and log-transformed NTX (β=0.0749, R^2=0.008, P=0.0302) concentrations.

Table 4
Odds ratios (OR) and 95% confidence intervals (CI) for "low bone mineral density (BMD) (t score ≤−2.5 SD)" according to levels of serum 25(OH)D

	Levels of serum 25(OH)D (nmol/L)						P for trend
	<30 (n=22)	30–40 (n=62)	40–50 (n=127)	50–60 (n=162)	60–70 (n=120)	≥70 (n=106)	
Lumbar spine							
Prevalence of low BMD (%)	18.2	22.6	25.2	16.1	15.0	11.3	
Unadjusted OR	1.61	1.99	2.23	1.42	1.33	1 (ref.)	0.0109
95% CI	0.57–4.52	0.99–4.03	1.21–4.10	0.75–2.69	0.67–2.62		
Adjusted[a] OR	3.03	2.44	3.02	1.32	1.48	1 (ref.)	0.0173
95% CI	0.57–16.02	0.84–7.12	1.31–6.97	0.59– 2.99	0.61–3.59		
Femoral neck							
Prevalence of low BMD (%)	18.2	21.0	23.4	11.2	9.2	5.7	
Unadjusted OR	3.21	3.70	4.14	1.98	1.63	1 (ref.)	<0.0001
95% CI	0.99–10.44	1.48–9.25	1.79–9.57	0.81–4.81	0.63–4.26		
Adjusted[a] OR	2.86	3.59	7.55	2.07	1.40	1 (ref.)	0.0017
95% CI	0.28–29.03	1.06–12.11	2.45–23.24	0.74–5.80	0.45–4.35		

[a] Adjusted for age, menopausal age, BMI, calcium intake, grip strength, log-transformed intact PTH, OC, and log-transformed NTX.

The results of the stepwise multiple regression analysis are shown in Table 3. BMI was the predominant independent variable, followed by age and serum OC concentration for both BMDs of the lumbar spine and femoral neck. The serum 25(OH)D concentration was independently associated with BMD of the femoral neck, although its R^2 was smaller than those of BMI, age and serum OC concentration.

Table 4 shows ORs for "low BMD (t score ≤ -2.5 SD)" by level of serum 25(OH)D. After adjustment for model covariates, prevalence of low BMD for the lumbar spine was significantly higher in the 40- to 50-nmol/L group compared to the reference group (≥ 70 nmol/L). Similarly, a significantly higher prevalence of low BMD of the femoral neck was observed in the 30- to 40-nmol/L and 40- to 50-nmol/L groups compared to the reference group (≥ 70 nmol/L). The serum 25(OH)D concentration was not significantly associated with the serum OC concentration ($P=0.1715$) or the serum NTX concentration ($P=0.2355$). The lack of these associations remained after subjects were restricted to those with serum 25(OH)D concentrations <50 nmol/L ($P=0.4839$ for serum OC and $P=0.9574$ for serum NTX).

The serum 25(OH)D concentration is generally believed to be associated with physical strength. However, the serum 25(OH)D concentration was significantly associated with neither thigh muscle strength ($P=0.1144$), grip strength ($P=0.3131$), nor the TUG test ($P=0.6140$). Even when comparing in these three physical variables between lower and higher subgroups by using any thresholds, there were no significant differences in any variables between them.

Discussion

This is the first large-scale epidemiologic study exploring a possible association between vitamin D status and bone mass, bone metabolism, or physical strength in postmenopausal Asian women. The mean serum 25(OH)D concentration (55.6 nmol/L) and prevalence of vitamin D insufficiency observed in this population were similar to those of other populations of ambulant Japanese elderly women [11,12]. The vitamin D status of ambulant elderly Japanese, including this study population, is well maintained even in winter, due in part to high dietary intake of vitamin D from fish [11,13]. This study demonstrated that the serum 25(OH)D concentration was linearly associated with BMD of the femoral neck in subjects with a serum 25(OH)D concentration of 30 nmol/L or higher. This finding is in accordance with the result of a large epidemiologic study recently conducted [4] and supports a rationale that the serum 25(OH)D levels should be maintained 75–80 nmol/L or higher [6,14]. By contrast, an association between the serum 25(OH)D concentration and BMD of the lumbar spine was not significant. This discrepancy has not been frequently reported in the literature, but may be explained by the fact that vitamin D status affects cortical bone more than spongy bone. This hypothesis is supported by Stone et al.'s [15] finding that lower 25(OH)D levels are associated with hip but not calcaneal bone loss. Regarding the association between the serum 25(OH)D concentration and BMD of the lumbar spine, 50 nmol/L appears to be an inflection point (Fig. 1). This study may have failed to detect a true association due to the relatively small number of subjects at high 25(OH)D levels. Further studies should address this issue.

The present study showed that the serum 25(OH)D concentration of 50 nmol/L or lower was associated with low BMD (t score ≤ -2.5 SD) of both the lumbar spine and femoral neck (no significant increase in the prevalence of low BMD was observed in the <30 nmol/L group due to limited sample size). Study findings also suggest that vitamin D insufficiency is more strongly associated with low BMD in the femoral neck than in the lumbar spine.

Despite the significant associations observed between serum 25(OH)D concentration and BMD, the low R^2 values associated with vitamin D status in multivariate analysis indicate that it accounted for only a small proportion of the variance in BMD in the study population. Results of the present study are in line with the findings of two recent population-based investigations targeting postmenopausal women. The Rancho Bernardo Study [16] showed a slight but significant association between serum 25(OH)D and femoral BMD, and the OFELY Study [17] showed serum 25(OH)D not to be a significant determinant of bone loss. On the other hand, there have been two clinic-based studies in which the serum 25(OH)D concentration was correlated moderately with both spinal and femoral BMDs in postmenopausal women [18,19]. As such, the strength of the association between vitamin D status and BMD seems to depend on which population is targeted.

Numerous studies have shown an inverse association between the serum 25(OH)D and intact PTH serum concentrations [20–22]. The present study confirmed such an association with a threshold of 50 nmol/L of the serum 25(OH)D concentration for elevated serum intact PTH concentrations. This finding suggest that maintenance of serum 25(OH)D concentrations of at least 50 nmol/L is essential for maintaining bone health in postmenopausal Japanese women.

This study failed to confirm an association between serum 25(OH)D concentration and markers of bone turnover. Gallagher et al. [23] also reported no or only a slight association between the serum 25(OH)D concentration and markers on bone turnover in a healthy elderly population. On the other hand, Jesudanson et al. [24] showed a negative association between serum 25(OH)D concentration and serum bone resorption markers and alkaline phosphatase levels in postmenopausal women attending an osteoporosis clinic. Furthermore, an inverse relationship between serum 25(OH)D and markers of bone turnover was found in postmenopausal women with established osteoporosis [25]. Taken together, these studies suggest an association between the serum 25(OH)D and markers of bone turnover may be observed in frail populations, such as osteoporotic women, but not in the general population of postmenopausal women.

Our study also demonstrated that serum intact PTH is associated with BMD of the femoral neck, but not with BMD of the lumbar spine. The lack of the association with the lumbar spine may be due to the fact that PTH affects cortical bone mass [26] to a greater extent than spongy bone mass or because PTH does not have as great of an effect on bone mass in elderly Asian

populations compared to their European counterparts [20]. Moreover, BMD of the femoral neck was independently associated with serum PTH and 25(OH)D, which suggests that each plays an independent role in bone metabolism and bone mass. PTH may affect BMD partly via increased bone turnover because high serum PTH was associated with both serum OC and NTX in this study. On the other hand, serum 25(OH)D may affect BMD not via increased bone turnover, as serum 25(OH)D did not link to bone turnover markers in this study but probably via increased calcium absorption in the intestine. The cross-sectional nature of this study has limitations in its ability to make causal relationships, and this hypothesis should be confirmed by a longitudinal study.

Low levels of vitamin D have been reported to be associated with impaired physical functions [27,28]. To the contrary, the present study failed to demonstrate such an association between vitamin D status and muscle strength or the TUG test. The lack of the associations in this study may be due to relatively good vitamin D status (mean serum 25[OH]D concentration, 55.6 nmol/L), the study population being relatively young (mean age, 64.5 years), or ethnicity [29].

The elderly Japanese population has some characteristics in terms of diet and bone health that make them different from other general populations. They have lower calcium intake and higher vitamin D intake than elderly whites [12]. In the present population, 95% of the subjects had total calcium intake of less than 800 mg/day, a daily calcium requirement in Japan [30]. Their low calcium intake (527 mg/day) might diminish an effect of vitamin D on bone, and increase of calcium intake is hypothesized to alter strength of the association between vitamin D status and bone mass.

This study had some limitations. This study employed a cross-sectional design, which is limited in its ability to detect causal relationships. An intervention trial is needed in order to establish causality. In addition, subjects' participation rate of this study was approximately 50%, and thus selection bias may have occurred. For example, it is likely that healthier or more active women tended to participate in this study. Generalizations of our results to other populations should thus be made with caution.

In summary, the present study was the largest study to date to examine the relationship between vitamin D levels and bone health among Asian postmenopausal women. Our results suggest that higher serum 25(OH)D concentrations are associated with increased BMD of the femoral neck, and that a serum 25(OH)D concentration of at least 70 nmol/L is needed to obtain high BMD of the femoral neck, and that of at least 50 nmol/L is needed to achieve normal PTH levels and prevent low BMD. While significant associations were observed between vitamin D status and BMD of the femoral neck, the contribution of vitamin D status to BMD is relatively small, suggesting a role for other factors in low bone mass.

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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.

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ノート

腸内細菌のパントテン酸前駆体の投与がパントテン酸欠乏幼若ラットの成長とパントテン酸の尿中排泄量におよぼす影響

[1] 滋賀県立大学人間文化学部生活栄養学科，[2] 第一ファインケミカル株式会社

福渡　努[1]，三木　啓子[1]，東野　勲[2]，柴田　克己[1]

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Effects of Feeding Pantothenic Acid Precursors on Microflora, Growth and Urinary Excretion of Pantothenic Acid in Young Pantothenic Acid-deficient Rats

Tsutomu Fukuwatari[1], Keiko Miki[1], Isao Azumano[2] and Katsumi Shibata[1]

[1]Department of Food Science and Nutrition, School of Human Cultures, The University of Shiga Prefecture
[2]Daiichi Fine Chemical Co., Ltd.

Pantothenic acid is very unstable under acid or alkaline conditions to be hydrolyzed to pantolactone and β-alanine. There is no information of the pantothenate deficiency which might be caused by hydrolysis with gastric acid. It may be possible that pantothenate is synthesized again from pantolactone and β-alanine by enterobacteria. Accordinngly when pantolactone and β-alanine was administered to pantothenate-deficient infant rats, the growth and pantotheate content in liver and urine were compared with normal rats. Consequently pantolactone and β-alanine has not supported the growth similar to pantothenate-deficiency.
Key words: pantothenic acid, pantolactone, β-alanine, urine, rat

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緒　言

パントテン酸は酸やアルカリに不安定で，パントテン酸の前駆体でもあるパントラクトンとβ-アラニンに加水分解される．このことから，胃酸によってパントテン酸が加水分解される可能性があるが，パントテン酸欠乏の発生は全く報告されていない．その理由の一つに，腸内細菌によってパントラクトンとβ-アラニンからパントテン酸が再合成され，そのパントテン酸を利用している可能性が挙げられている．さらに，ヒトを含むほ乳動物の大腸に生息する腸内細菌はパントテン酸の *de novo* 生合成経路も有しており，合成された一部を利用していると言われている．また，肉を中心とした食事よりも野菜の多い食事を摂ったときや，ヒトにセルロースを摂取させたときに，糞中のパントテン酸含量が高くなるという報告がある[1]．しかし，糞中のパントテン酸含量が高くなっても，尿中に排泄されるパントテン酸量には明らかな増大は認められていないか，あるいは若干増大している．一方，ヒトにパントテン酸を含まない食事を10週間にわたって投与すると，尿中のパントテン酸排泄量は速やかに検出限界以下に減少したが，実験最終日においても血液中のパントテン酸含量は低下せず，欠乏と思われる症状は認められなかった，という報告がある[2]．この欠乏実験の結果は，腸内細菌によって合成されたパントテン酸の利用が示唆されるが，腸内細菌合成のパントテン酸の体内利用性に関しては未だに不明である．

[1] 〒522-8533　滋賀県彦根市八坂町2500

そこで，我々は，栄養学の常法を使用して，幼若ラットの飼料摂取量と成長を指標として，パントテン酸の *de novo* 生合成経路の直前の前駆体であるパントラクトンと β-アラニンを投与することで，腸内細菌の合成したパントテン酸の体内利用性を検討したので報告する．その結果，少なくとも幼若ラットにおいては，パントラクトンと β-アラニンの同時投与によるパントテン酸効果はないと判断された．

実験方法

1．動物飼育

本実験は滋賀県立大学動物実験委員会の承認を受けた．飼育室の温度は 22℃前後，湿度は 50%前後に維持し，明暗サイクルは，午前 6 時～午後 6 時を明，午後 6 時～午前 6 時を暗とした．

3 週齢の Wistar 系雄ラット 25 匹を日本クレア株式会社より購入した．直ちに，平均体重がほぼ均等になるように 5 匹ずつ 5 群に分け，ラット用代謝ケージに入れた．その日から，Table 1 に示した飼料を与えた．AIN-93-VX 配合に従った 20%カゼイン食投与群（パントテン酸含量は 0.00147%）を正対照群とし，パントテン酸を含まない 20%カゼイン食投与群を負対照群とした．試験食群として，①正対照群に与えた 20%カゼイン食に含まれるパントテン酸と等モルのパントラクトンと β-アラニンを含む飼料を投与した群，②正対照群に与えた 20%カゼイン食に含まれるパントテン酸と等モルのパントラクトンのみを含む飼料を投与した群，③正対照群に与えた 20%カゼイン食に含まれるパントテン酸と等モルの β-アラニンのみを含む飼料を投与した群を作製した．飼育期間は 28 日間である．飼料と水は自由摂取とし，1 日ないし 2 日おきに新しいものに交換した．ラットの世話は午前 8 時～10 時の間に行い，体重と飼料摂取量を測定した．実験開始日を Day 0 として，飼育最終日の Day 28 の 1 日尿（Day 28 の午前 9 時～ Day 29 午前 9 時：24 時間）を集めた．尿は塩酸酸性下で集め，採尿後，分析に供するまで－20℃で保存した．

採尿終了後の Day 29 の午前 9 時～ 10 時に断頭にて屠殺した．また，尿中の B 群ビタミン量を測定した．

2．化学薬品

ビタミンフリーミルクカゼイン，ショ糖，L-メチオニンは和光純薬工業株式会社（大阪）より購入した．コーンオイルは味の素株式会社（東京）より購入した．α-コーンスターチ，ミネラル混合（AIN-93-G-MX）[3]，ビタミン混合（AIN-93-VX）[3] はオリエンタル酵母株式会社より購入した．チアミン塩酸塩（$C_{12}H_{17}ClN_4OS$-HCl = 337.27），リボフラビン（$C_{17}H_{20}N_4O_6$ = 376.37），ニコチンアミド（$C_6H_6N_2O$ = 122.13），パントテン酸カルシウム（$C_{18}H_{32}N_2O_{10}$-Ca = 476.54）および β-アラニン（$C_3H_7NO_2$ = 89.09）は，和光純薬工業株式会社より購入した．パントラクトン（$C_6H_{10}O_3$ = 130.14）は Sigma（ミズーリ，米国）より購入した．4-ピリドキシン酸（4-PIC，$C_8H_9NO_4$ = 183.16）は ICN Pharmaceuticals（カリフォルニア，米国）が製造したものを，和光純薬工業株式会社を通して得た．N^1-Methylnicotinamide（MNA）塩化物（$C_7H_9N_2O$-HCl = 159.61）は東京化成工業株式会社（東京）より得た．N^1-メチル-2-ピリドン-5-カルボキサミド（2-Py，$C_7H_8N_2O_2$ = 152.15）と N^1-メチル-4-ピリドン-3-カルボキサミド（4-Py，$C_7H_8N_2O_2$ = 152.15）は，Pullman と Colowick[4]，および Shibata ら[5] の方法で合成した．他の化学薬品は市販品の中で最高純度のもの使用した．

Table 1. Composition of the diets.

	Positive control	Negative control	Pantolactone	β-Alanine	Pantolactone + β-Alanine
Vitamin-free milk casein	20	20	20	20	20
L-Methionine	0.2	0.2	0.2	0.2	0.2
Gelatinized cornstarch	50.2	50.2	50.2	50.2	50.2
Sucrose	25.1	25.1	25.1	25.1	25.1
Mineral mixture (AIN-93-G-MX)	3.5	3.5	3.5	3.5	3.5
Vitamin mixture (AIN-93-VX, PaA free)	1.0	1.0	1.0	1.0	1.0
Calcium pantothenate	0.0016	0	0	0	0
Pantolactone	0	0	0.00087	0	0.00087
β-Alanine	0	0	0	0.0006	0.0006

Values are expressed g per 100 g of diet.

3. 分　析

3-1. チアミンの測定

尿を0.45μmフィルターで濾過し，その濾液20μLを直接HPLCに注入した．定量方法は文献6に示したポストカラム-HPLC法にしたがった．分析条件は，カラム：Shodex Rs-pak NN-614（φ 6.0 x 150 mm），移動相および流速：0.2 mol/L NaH_2PO_4，1.0 mL/min，反応液1：0.01％ $K_3Fe(CN)_6$，0.15 mL/min，反応液2：15％NaOH，0.15 mL/min，カラム温度：40℃，検出器：蛍光光度計，励起波長365 nm，蛍光波長435 nmとした．

3-2. リボフラビンの測定

尿を0.45μmフィルターで濾過し，その濾液20μLを直接HPLCに注入した．定量方法は文献7に示したHPLC法にしたがった．分析条件は，カラム：Tosoh ODS-80Ts（φ 4.6 x 250 mm），移動相：10 mmol/L NaH_2PO_4（pH 5.5）：メタノール（70：30, v/v），流速：0.8 mL/min，カラム温度：40℃，検出器：蛍光光度計，励起波長445 nm，蛍光波長530 nmとした．

3-3. ピリドキサルの異化代謝産物の4-ピリドキシン酸の測定

尿を0.45μmフィルターで濾過し，その濾液20μLを直接HPLCに注入した．定量方法は文献8に示したHPLC法にしたがった．分析条件は，カラム：TSKgel ODS-120A（φ 4.6 x 250 mm），移動相：2.2％リン酸（pH 2.2）：メタノール（90：10, v/v），流速：1.0 mL/min，カラム温度：40℃，検出器：蛍光光度計，励起波長355 nm，蛍光波長436 nmとした．

3-4. シアノコバラミンの測定方法

尿を常法に従い，KCN溶液を用いて尿中のビタミン B_{12} をシアノコバラミンに変化させ，安定化処理を行った[9]．その処理尿を適当量添加したビタミン B_{12} 定量用基礎培地（日水製薬株式会社）2 mLに*Lactobacillus leichmanii*, ATCC 7830を接種した．16時間培養後，比色計を用いて660 nmにおける濁度を測定した．

3-5. ニコチンアミドの異化代謝産物のMNA，2-Py，4-Pyの測定方法

尿中のニコチンアミドおよびその異化代謝産物であるMNA，2-Py，4-Pyを測定し，この合計を総ニコチンアミド代謝産物とした．尿中のニコチンアミド，2-Py，4-Pyは以下の方法で同時定量した[5]．尿1 mLに内部標準として1 mg/mLのイソニコチンアミドを10μL加えた．炭酸カリウム1.2 gを添加した後，ジエチルエーテル5 mLを加えて，5分間室温でよく混合し，エーテル層を取り出した．エーテルによる抽出操作を2回繰り返し，取り出したエーテルを蒸発乾固させた．この乾固物を水0.5 mLに溶解し，溶解液を0.45μmフィルターで濾過し，濾液20μLをHPLCに注入した．分析条件は，カラム：Chemcosorb 7-ODS-L（φ 4.6 x 250 mm），移動相：10 mmol/L KH_2PO_4（pH 3.0）：アセトニトリル（96：4, v/v），流速：1.0 mL/min，カラム温度：25℃，検出器：紫外分光光度計260 nmとした．内部標準であるイソニコチンアミドのピーク面積から回収率を求め，ニコチンアミド，2-Py，4-Py量を算出した．

尿中MNA含量を測定するために[10]，尿0.1 mL，水0.7 mL，1 mol/Lイソニコチンアミド0.2 mL，0.1 mol/Lアセトフェノン溶液0.5 mLを混合した後，6 mol/L NaOH溶液1mLを加えて10分間氷冷した．99％ギ酸0.5 mLを加えて15分間氷中で放置した後，沸騰水浴中で5分間放置し，十分に氷冷してから遠心上清を0.45μmフィルターで濾過し，その濾液20μLをHPLCに注入した．分析条件は，カラム：Tosoh ODS 80Ts（φ 4.6 x 250 mm），移動相：1 g/L 1-ヘプタスルホン酸ナトリウムおよび1 mmol/L EDTA-2Naを含む20 mmol/L KH_2PO_4（pH 3.0）：アセトニトリル（97:3, v/v），流速：1.0 mL/min，カラム温度：40℃，検出器：蛍光光度計，励起波長382 nm，蛍光波長440 nmとした．

3-6. パントテン酸の測定

尿を適当量添加したパントテン酸定量用基礎培地（日水製薬株式会社）2 mLに*Lactobacillus plantarum* ATCC 8014を接種した．16時間培養後，比色計を用いて660 nmにおける濁度を測定した[11]．

3-7. 葉酸の測定

尿を適当量添加した葉酸定量用基礎培地（DIFCO）2 mLに*Lactobacillus casei* ATCC 2733を接種した．22時間培養後，比色計を用いて660 nmにおける濁度を測定した[12]．

3-8. ビオチンの測定方法

尿を適当量添加したビオチン定量用基礎培地（日水製薬株式会社）2 mLに*Lactobacillus plantarum* ATCC 8014を接種した．22時間培養後，比色計を用いて660 nmにおける濁度を測定した[13]．

4. 有意差検定

すべてのデータは平均値±SEMで示した．有意差検定は，まず一元配置の分散分析を行い，有意差が認められた時にはStudent-Newman-Keulsの多重比較テストを行った．$p < 0.05$ を有意差ありと判定した．検定には統計ソフトGraphPad Prism version 4.03（GraphPad Software, San Diego, CA, USA）を用いた．

結果と考察

1. 体重増加量と飼料摂取量におよぼす影響

離乳したての3週齢のラットを被検動物とした．正対照群には1％AIN-93-VX配合を含む20％カゼイン食（パントテン酸含量は0.00147％）を，負対照群にはパントテン酸を含まない1％AIN-93-VX配合を含む20％カゼイン食を投与した．試験食群には，①正対照群と等モルのパントラクトンとβ-アラニンを含む飼料を，②正対照群と等モルのパントラクトンのみを含む飼料を，③正対照群

と等モルの β-アラニンのみを含む飼料をそれぞれ投与した．投与期間は 28 日間とした．Fig. 1 に示したように，試験群の三つの群すべてにおいて体重の増加量と飼料摂取量は負対照群と同じであり，パントラクトンと β-アラニンはラットの成長に全く寄与していないことが明らかとなった．

2. 臓器重量におよぼす影響

Table 1 に示した正・負対照群，三つの試験飼料を幼若ラットに 28 日間投与した後の，脳，肺，心臓，腎臓，肝臓，脾臓，胃，精巣の各重量を Table 2 に示した．Fig. 1 に示した飼料摂取量および体重増加量と同様に，負対照群と三つの試験群の臓器重量は正対照群に比して低値を示した．

3. 尿中，糞中，血液中，肝臓中のパントテン酸含量

正・負対照群，三つの試験群の尿中，糞中，血液中，肝臓中のパントテン酸含量を Table 3 に示した．正対照群のパントテン酸含量がいわゆる正常値である．負対照群では明らかに尿中への排泄量が低下し，肝臓中の含量も低い値を示した．血液中の値は，我々の用いた方法では，すべての群において検出限界以下であった．パントテン酸は *L. plamtarum* を用いる微生物定量方法を用いているが，感度が悪いため，血液中の値の測定は常に検出限界程度の値となり，きわめて精度の低い値となる．これは，血液中の結合型のパントテン酸を *L. plamtarum* が利用できる遊離型にするための処理により希釈度が高くなることに起因する．現在，適正な処理方法を検討中である．

糞中のパントテン酸の値も，血液と肝臓中の値と同じく，正対照群が負対照群よりも高い値を示した．また，大腸に生息する細菌のパントテン酸前駆体であるパントラクトン，あるいは β-アラニンを添加した飼料を投与しても，糞中のパントテン酸量は負対照群とほぼ同じであった．一方，パントラクトンと β-アラニン同時投与群の糞中パントテン酸含量は，正対照群との間に有意の差異は認められなかった．したがって，パントテン酸の前駆体の投与は，腸内細菌のパントテン酸生合成を促進しているものと考えられた．しかしながら，肝臓中と尿中のパントテン酸量は，負対照群と全く同じであったことから，大腸内で合成されたパントテン酸をラットは利用しにくいことが明らかとなった．このことは，肉を中心とした食事よりも野菜の多い食事を摂ったとき，また，ヒトにセルロースを摂取させたとき，糞中のパントテン酸含量が高くなるが，糞中のパントテン酸含量が高くなっても尿中に排泄されるパントテン酸量には明らかな増加は認められていないか，あるいは若干増加しているという報告[1]と一致するものであった．

緒言で述べたように，摂取したパントテン酸が，胃内でパントラクトンと β-アラニンに加水分解され，そして，腸内細菌によってパントラクトンと β-アラニンからパン

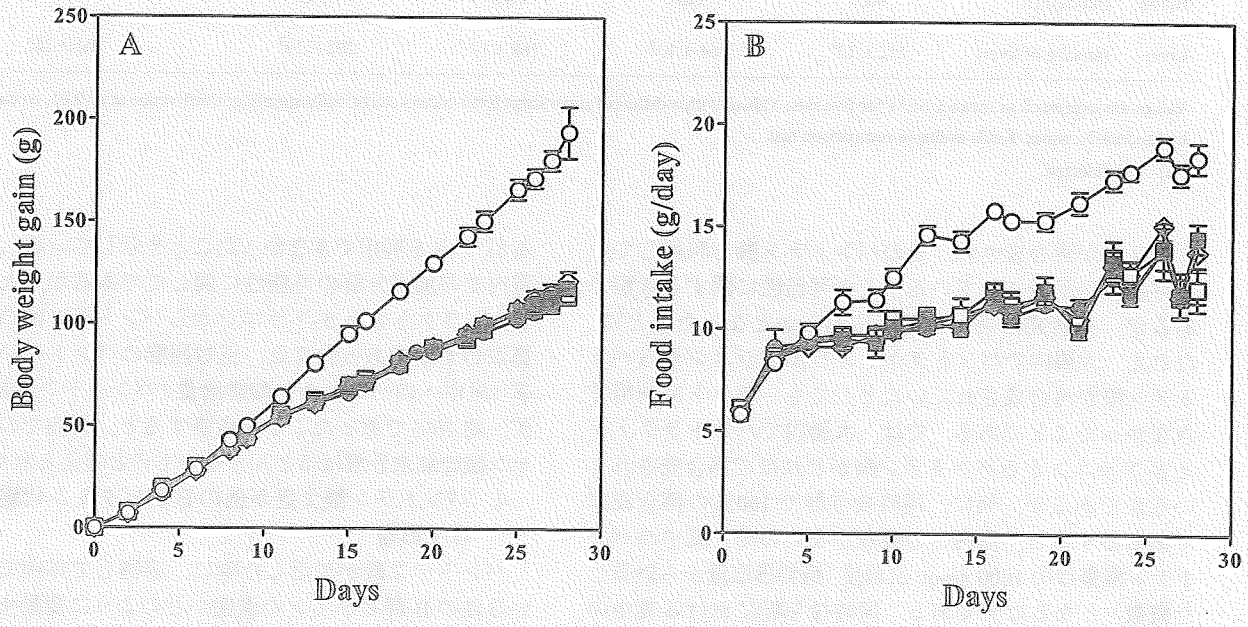

Fig. 1. Effects of dietary pantolactone, β-alanine, and simultaneous administration of pantolactone and β-alanine on the body weight gain (A) and food intake (B).

Weaning rats of the Wistar strain were fed with a pantothenic acid-containing diet (positive control, ○), and pantothenic acid-free diet (negative control, ◎). As the test groups, the rats were fed with a PaA-free diet containing pantolactone (▨), β-alanine (□), or pantolactone and β-alanine (◇) for 28 days. Values are means ± SEM for five rats.

Table 2. Effects of dietary pantolactone, β-alanine, and simultaneous administration of pantolactone and β-alanine on the weights of various organs and tissues

	Positive control	Negative control	Pantolactone	β-Alanine	Pantolactone + β-Alanine
Brain (g)	1.26 ± 0.02[a]	1.16 ± 0.02[b]	1.17 ± 0.02[b]	1.16 ± 0.01[b]	1.18 ± 0.01[b]
Lung (g)	1.42 ± 0.05[a]	0.88 ± 0.06[b]	0.97 ± 0.06[b]	1.06 ± 0.08[b]	0.91 ± 0.05[b]
Heart (g)	0.87 ± 0.02[a]	0.62 ± 0.01[b]	0.63 ± 0.01[b]	0.62 ± 0.03[b]	0.64 ± 0.02[b]
Kidney (g)	1.81 ± 0.02[a]	1.58 ± 0.03[b]	1.53 ± 0.03[b]	1.47 ± 0.05[b]	1.47 ± 0.05[b]
Liver (g)	10.33 ± 0.27[a]	8.00 ± 0.43[b]	8.39 ± 0.30[b]	8.75 ± 0.62[a,b]	8.53 ± 0.40[a,b]
Spleen (g)	0.66 ± 0.01[a]	0.43 ± 0.02[b]	0.46 ±0.03[b]	0.46 ± 0.03[b]	0.43 ±0.03[b]
Stomach (g)	1.13 ± 0.03[a]	0.84 ± 0.04[b]	0.91 ± 0.03[b]	0.87 ± 0.05[b]	0.87 ±0.03[b]
Testis (g)	2.32 ± 0.08[a]	2.06 ± 0.06[b]	2.03 ± 0.06[b]	2.05 ± 0.02[b]	2.03 ± 0.06[b]

Values are expressed as mean ± SEM for five rats; Values with a different superscript letter means statistically significant difference at $p<0.05$, as calculated by Student-Newman-Keuls multiple comparison test.

Table 3. Effects of dietary pantolactone, β-alanine, and simultaneous administration of pantolactone and β-alanine on the concentrations of pantothenic acid in urine, feces, blood, and liver.

		Positive control	Negative control	Pantolactone	β-Alanine	Pantolactone + β-Alanine
Urine	(nmol/day)	814 ± 69[a]	11 ± 2[b]	11 ± 3[b]	16 ± 2[b]	10 ± 3[b]
Feces	(nmol/day)	152 ± 16[a]	47 ± 9[b]	63 ± 14[b]	71 ± 13[b]	97 ± 7[a,b]
Blood	(nmol/mL)	ND	ND	ND	ND	ND
Liver	(nmol/g of liver)	512 ± 12[a]	164 ± 40[b]	168 ± 17[b]	202 ± 19[b]	204 ± 24[b]

Values are expressed as mean ± SEM for five rats; Values with a different superscript letter means statistically significant difference at $p<0.05$, as calculated by Student-Newman-Keuls multiple comparison test.
ND : not detected.

トテン酸が再合成され，そのパントテン酸を利用している可能性が示唆されている．この可能性に関する考察であるが，本研究結果から確実に明らかとなったことは，たとえ，大腸内でパントラクトンとβ-アラニンからパントテン酸が再合成されていたとしても，ラットは利用できなかったことである．では，大腸内でパントラクトンとβ-アラニンからパントテン酸がどれほど再合成されているかであるが，仮に，同時投与群で100％の再合成率であるとすると，体内への吸収がないので，糞中のパントテン酸量は約1000 nmol/日程度（飼料摂取量から計算した概数）となるはずである．同時投与群における糞中のパントテン酸排泄量は約100 nmol/日（Table 3），負対照群が50 nmol/日程度（Table 3）であった．これらの結果は，摂取したパントラクトンとβ-アラニンから再合成されたパントテン酸量は非常にわずかであったこと，および負対照群群でもパントテン酸がある程度 *de novo* 合成しているが，それを利用できなかったことを示している．さらに，胃内での加水分解の可能性に関してであるが，大腸内でパントラクトンとβ-アラニンからパントテン酸の再合成量がわずかであったこと，正対照群のパントテン酸排泄率（尿中へのパントテン酸排泄量/パントテン酸摂取量）が，約70％であったことを考慮すると，胃内でのパントテン酸の加水分解はほとんどないものと考えられた．

4. パントテン酸欠乏が他のB群ビタミン代謝におよぼす影響

パントテン酸が欠乏した時に，協調して体内で働いている他のB群ビタミンの濃度がどのように変動するのかを調べた．Table 4に尿中の排泄量におよぼす影響を示した．値は1日尿中に排泄された量で示した．1日当たりのチアミン排泄量および総ニコチンアミド異化代謝産物排泄量は，正対照群が他の群に比して有意に高い値を示した．一方，他のビタミンにおいては，正の対照群が他

Table 4. Effects of dietary pantolactone, β-alanine, and simultaneous administration of pantolactone and β-alanine on the concentrations of other B-group vitamins in urine.

	Positive control	Negative control	Pantolactone	β-Alanine	Pantolactone + β-Alanine
Thiamin (nmol/day)	50.8 ± 7.5[a]	17.0 ± 4.2[b]	21.0 ± 3.7b	22.9 ± 7.1[b]	17.4 ± 1.7[b]
Riboflavin (nmol/day)	120 ± 17	116 ± 107	108 ± 10	124 ± 10	103 ± 9
4-PIC (nmol/day)	231 ± 12[a]	182 ± 8[b]	175 ± 15[b]	151 ± 17[b]	145 ± 9[b]
Nicotinamide and its metabolites (μmol/day)	6.72 ± 0.87[a]	3.89 ± 0.20[b]	4.00 ± 0.25[b]	3.24 ± 0.49[b]	4.17 ± 0.37[b]
Folic acid (nmol/day)	6.7 ± 0.9	7.2 ± 0.7	7.0 ± 0.7	7.2 ± 0.8	8.6 ± 0.8
Biotin (nmol/day)	4.01 ± 0.49[a]	1.85 ± 0.14[b]	1.28 ± 0.11[b]	1.77 ± 0.12[b]	1.92 ± 0.23[b]
Cyanocobalamin (pmol/day)	20.2 ± 3.5[a]	12.6 ± 1.0[b]	12.7 ± 0.7[b]	12.2 ± 1.1[b]	11.2 ± 2.0[b]

Values are expressed as mean ± SEM for five rats; Values with a different superscript letter means statistically significant difference at $p<0.05$, as calculated by Student-Newman-Keuls multiple comparison test.

の群に対して，明確に高い値を示すことはなかった．尿中の排泄量は，摂取したビタミン量に比例する．飼料摂取量は，Fig. 1 に示したように，正の対照群が負の対照群および三つの試験群に比して有意に高く，試験期間中の総飼料摂取量は，正の対照群を 100％ (382 g) とすると，負の対照群は 75％ (288 g)，パントラクトン群が 77％ (293 g)，β-アラニン群が 77％ (296 g)，パントラクトン＋ β-アラニン群が 76％ (296 g) であった．差異が認められたチアミンとナイアシンとその異化代謝産物の総計量を飼料摂取量で補正しても，有意に高い値を示した．ビタミンの代謝は体内よりも，尿の方が影響が現れやすい．摂取量が等しい時には，尿中への排泄量の低下は体内での必要量の亢進を意味する．逆に尿中への排泄量の増大は必要量の低下を意味する．つまり，パントテン酸欠乏によって，尿中のチアミンとナイアシンの排泄量が増大したことは，パントテン酸欠乏時にはチアミンとナイアシンの必要量が低下していることを意味している．

パントテン酸欠乏あるいはパントテン酸誘導体の摂取が，血液中および肝臓中の他の B 群ビタミン濃度におよぼす影響を検討した．血液，肝臓のいずれにおいても，チアミン，リボフラビン，ビタミン B_6，ニコチンアミド，葉酸，ビオチン，シアノコバラミンの各濃度には影響をおよぼさなかった．つまり，パントテン酸欠乏は，血液と肝臓中の他の B 群ビタミン濃度には影響をおよぼしにくいことが，はじめて明らかとなった．

まとめ

腸内細菌はパントテン酸をパントラクトンと β-アラニンから生合成できると言われている．このことは事実であるが，腸内で生成したパントテン酸をラットは体内で利用できていないことが，はじめて明確に証明された．おそらく，パントテン酸が生成している大腸においては，パントテン酸を吸収する機構が存在していないためであろう．ヒトについては，今後，同様の実験を行って確認する必要があるが，腸内で合成されたパントテン酸を体内で利用している可能性はきわめて低いことが予想される．

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