Note

Nutritional Therapy with Vitamin K₁ Is Effective in the Improvement of Vitamin K Status and Bone Turnover Markers in Patients with Severe Motor and Intellectual Disabilities

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Summary We have previously reported that patients with severe motor and intellectual disabilities (SMID) have a high prevalence of vitamin K deficiency both in the liver and bone. Thus, vitamin K therapy for SMID patients should be considered. In the present study, we have studied the efficacy of nutritional therapy with vitamin K₁ for improving their vitamin K status and bone metabolism markers in patients with SMID. During the 3-mo period, 19 patients under enteral feeding received vitamin K₁ treatment, the dose of which was determined to meet each subject's energy requirement. Biomarkers of vitamin K insufficiency; protein induced by vitamin K absence or antagonist-II (PIVKA-II), undercarboxylated osteocalcin (ucOC), intact osteocalcin (intact OC) and bone turnover markers (tartrate-resistant acid phosphatase-5b: TRACP-5b and bone alkaline phosphatase: BAP) were measured at baseline and post treatment. The ucOC/OC ratio was calculated as a more sensitive index than ucOC for vitamin K status in the bone. After treatment, the median vitamin K intake increased from 66 to 183 µg/d, and serum levels of PIVKA-II and ucOC/OC ratio were significantly decreased. Decrements of serum ucOC level and ucOC/OC ratio were significantly associated with vitamin K intake, indicating that both markers well reflect the dose-dependent vitamin K effects. Serum levels of BAP and TRACP-5b were significantly increased after vitamin K₁ therapy. Nutritional therapy with vitamin K₁ effectively improved the markers for vitamin K status and bone turnover, and was considered to be a good candidate for treatment in SMID patients.

Key Words vitamin K, severe motor and intellectual disabilities, protein induced by vitamin K absence (PIVKA-II), undercarboxylated osteocalcin (ucOC), γ-carboxylation, bone turnover

Severe motor and intellectual disabilities (SMID) occurs due to various causes such as cerebral palsy, cerebral injury, chromosome aberration, and congenital enzyme deficiency. SMID patients have multiple risks for malnutrition such as gastroesophageal reflux disease, and malabsorption (1). Furthermore, due to neurological abnormalities, patients with SMID have dysphagia, which necessitates enteral feeding. Some previous reports have indicated that SMID patients are likely to be deficient in their micronutrient status (2, 3). Patients with SMID have several medical complications such as aspiration syndromes, respiratory infections, seizures, and contractures, and are at higher risk for atherosclerosis and dementia. Orthopedic complications also occur including muscle wasting, osteoarthritis, bone loss, and low-trauma fractures (1, 4). Various risk factors for

fracture have been reported including decreased weight bearing, inadequate calcium and vitamin D intake, and exposure to medications that adversely affect bone mineral density (BMD) such as anticonvulsants (5).

Vitamin K is an essential co-factor for enzymatic, vitamin K-dependent γ-carboxylation of glutamic acid residues. It plays a key role in converting glutamic acid residues in vitamin K-dependent proteins to γ-carboxyl glutamic acid (Gla) residues (6, 7). This conversion allows blood coagulation factors to acquire the ability to bind calcium ion and leads to their activation (8). Other extrahepatic proteins such as osteocalcin (bone Gla protein) and matrix Gla protein are also γ-carboxylated by gamma-glutamyl carboxylase (GGCX) (9, 10). Recent evidence suggests that vitamin K deficiency is associated with increased risk of fracture (11).

Reports on the vitamin K status in SMID patients is quite limited (12, 13). We have previously studied the

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vitamin K status in patients with SMID (13). A substantial percentage of patients with SMID must be kept under enteral nutrition because of their dysphagia. Enteral nutrition is a risk for vitamin K insufficiency, since vitamin K content is not abundant enough in the enteral nutrition formulas. Antibiotics use is another risk, since it can impair vitamin K production by the intestinal bacteria. Also long-term use of antibiotics is inevitable in some patients with SMID. Enteral nutrition and long-term use of antibiotics were the factors responsible for vitamin K insufficiency, and their co-existence further worsened the vitamin K status. Therefore, intervention is needed to improve the vitamin K status in SMID patients. In the previous intervention studies, only drug therapy with vitamin K₂ was used. Drug therapy, however, costs more compared with nutritional therapy. If nutritional therapy with vitamin K₁ is comparably efficacious for improving the vitamin K status, it would be more cost-effective. Based on the above considerations, we have studied the therapy efficacy of nutritional therapy with vitamin K₁ in patients with SMID.

Materials and Methods

Subjects. The study subjects were 24 SMID patients (13 males and 11 females) living in the residential hospital, the Biwako Gakuen Kusatsu Medical and Welfare Center for Children and Persons with SMID. Detailed information about this study was provided to the subjects or their proxy, and written consent was obtained for their participation in this study. The study protocol was approved by the ethics committee of the institution described above (Approval number: 2009101). The exclusion criteria were those under therapy with vitamin K, warfarin, or multivitamin supplementation. Patients with pre-existing liver or bone disease were also excluded. None of the subjects had experienced overt signs or symptoms attributable to vitamin K deficiency. Finally, 19 patients with full-set of data before and after treatment were subjected to analysis.

Study design. Vitamin K₁ was added in enteral nutrition as a form of Racol® (Otsuka Pharmaceutical Factory, Inc.). Racol® used in the present study contains 125 µg/200 mL of vitamin K₁. Dose of Racol® was determined to meet the each subject's energy requirement. Added vitamin K amount ranged from 55 to 234 µg/d (median: 116 µg/d). This therapy was conducted for 3 mo. Biomarkers of vitamin K insufficiency and bone turnover markers were measured at baseline and 3 mo after starting the therapy.

Biochemical measurements. Blood was obtained from each patient after an overnight fasting. After centrifugation, the serum was maintained at -30°C until the analysis. The serum levels of protein induced by vitamin K absence-II (PIVKA-II) and undercarboxylated osteocalcin (ucOC) were measured by an electrochemiluminescence immunoassay (ECLIA) (Sekisui Medical Co., Ltd., Tokyo, Japan), which are markers for vitamin K status in the liver and bone, respectively. In the current study, the reference range for PIVKA-II was defined to be less than 28 mAU/mL, which is 2SD above the

Table 1. Characteristics of subjects at baseline.

	All subjects (n=19)
M/F	9/10
Age (y)	36.2±12.8
Body mass index (kg/m ²)	15.1±1.5
Ongoing antibiotics (n)	11
Energy and nutrients intake at baseline	
Energy intake (kcal/d)	1,000 (847, 1,100)
Protein intake (g/d)	50.9±10.0
Fat intake (g/d)	24.5±5.5
Carbohydrates intake (g/d)	141.9±26.7

Mean±SD or median (Q1, Q3).

healthy subjects' mean value of 14.2 mAU/mL (14). The cut-off value for serum ucOC concentration was 4.5 ng/mL (15). Serum intact osteocalcin (intact OC) was measured by immuno-radiometric assay (IRMA) (LSI Medience Corp., Tokyo, Japan). The ucOC/OC ratio was calculated as a sensitive index for vitamin K status in the bone. Serum levels of tartrate-resistant acid phosphatase-5b (TRACP-5b) and bone alkaline phosphatase (BAP) were measured by enzyme immunoassay (EIA) (DS Pharma Biomedical, Osaka, Japan) and chemiluminescence enzyme immunoassay (CLEIA) (Beckman Coulter Inc., Tokyo, Japan), respectively. TRACP-5b and BAP are markers of bone resorption and bone formation, respectively. The reference range for serum TRACP-5b is 170–590 mU/dL and 120–420 mU/dL in men and women, respectively. The reference range for serum BAP is 3.7–20.9 µg/L and 3.0–22.6 µg/L in men and women, respectively. The minimum significant change of BAP and TRACP-5b are 23.1% and 16.2%, respectively (16).

Assessment of the nutrients intake. The 7-d nutrients intake at baseline was assessed by the food record method. The intake of vitamin K was calculated by multiplying the amount of vitamin K supplied from the institution by the average percentage intake. Based on these records, the intake of vitamin K by the patients was calculated using a software program (Excel Eiyo-kun version 4.5 (Kenpakusya Co., Ltd., Tokyo, Japan)). The vitamin K intake/kg body weight was also calculated.

Statistical analyses. Statistical analyses were performed using the SPSS 23.0 J software program for Windows (SPSS, Japan Inc., Tokyo, Japan). The comparison of data at baseline and after 3-mo intervention was done with Wilcoxon signed rank test. The dose-dependency of vitamin K₁ therapy in the changes of PIVKA-II, ucOC and ucOC/OC were analyzed by Spearman's rank correlation.

Results

As shown in Table 1, 11 patients were receiving antibiotics therapy. All subjects were receiving only enteral nutrition. Table 2 shows the results from 3 mo intervention. Median vitamin K intake was 66 µg/d at baseline and 183 µg/d at post-therapy, respectively. No adverse

Table 2. Change of vitamin K intake biomarkers after 3-mo intervention.

	Baseline	After 3 mo	<i>p</i> value
Vitamin K intake (μg/d)	66 (54, 81)	183 (169, 246)	<0.001
Calcium intake (mg/d)	675±132	618±131	<0.001
Vitamin D intake (μg/d)	4.3 (3.5, 5.1)	4.1 (3.3, 4.7)	0.002
PIVKA-II (mAU/mL)	82 (59, 143)	47 (36, 79)	0.008
ucOC (ng/mL)	8.30 (4.68, 12.3)	6.15 (4.03, 11.0)	0.159
intact OC (ng/mL)	4.0 (2.8, 5.4)	5.5 (3.5, 6.1)	0.136
ucOC/OC ratio	2.27 (1.43, 2.93)	1.34 (0.88, 2.60)	0.003
BAP (μg/L)	15.9 (11.6, 18.1)	16.1 (13.6, 21.7)	0.028
TRACP-5b (mU/dL)	268 (189, 388)	332 (241, 446)	0.021

Median (Q1, Q3). *p* value represents the comparison of data at baseline and after 3-mo intervention by Wilcoxon signed rank test.
PIVKA-II: protein induced by vitamin K absence or antagonist-II, ucOC: undercarboxylated osteocalcin, intact OC: intact osteocalcin, TRACP-5b: tartrate-resistant acid phosphatase-5b, BAP: bone alkaline phosphatase.

Fig. 1. The dose-dependency relationship of vitamin K₁ intake and circulating levels of vitamin K related biomarkers. The correlations between increment of vitamin K intake and changes of circulating levels of vitamin K deficiency related biomarkers were analyzed by Spearman's correlation. PIVKA-II: protein induced by vitamin K absence or antagonist-II, ucOC: undercarboxylated osteocalcin, intact OC: intact osteocalcin. ΔVit.K intake (μg/d), ΔucOC/OC ratio, ΔPIVKA-II (mAU/mL) and ΔucOC (ng/mL) were calculated by values of 3 mo after starting the therapy minus those of baseline.

effects were observed during the study. At baseline, serum PIVKA-II level was above the upper normal range of 28 mAU/mL in all subjects, and serum level of ucOC was above the cut-off value of 4.5 ng/mL in 78.9% of subjects. After 3 mo therapy, only serum PIVKA-II concentration and ucOC/OC ratio were significantly decreased. Both serum levels of BAP and TRACP-5b were significantly increased without clinical minimum significance. There was a significant dose-dependency of vitamin K₁ intake in the changes of ucOC and ucOC/OC

ratio, but not in those of PIVKA-II (Fig. 1).

Discussion

In this paper, we have studied the therapeutic efficacy of vitamin K₁ by measuring the markers for vitamin K status; PIVKA-II and ucOC as well as bone metabolic markers. Despite previous intervention studies with vitamin K in subjects with cerebral palsy (17, 18), some issues remain to be clarified. First, only drug therapy with vitamin K₂ was used in previous studies. Second, most studies focused only on bone turnover markers and BMD, and only a few studies have evaluated biomarkers for vitamin K status (12). Therefore, we have studied the efficacy of nutritional therapy with vitamin K₁ employing various parameters for vitamin K status as well as bone metabolic markers.

In the current study, circulating concentration of vitamin K concentration was not measured. Although circulating vitamin K concentration responds to the alteration of vitamin K intake (19, 20), its concentration peak 6–10 h post-prandially (21). Thus, its concentration reflects short-term vitamin K intake (22). Additionally, there is currently no established threshold of circulating concentration of vitamin K that indicates insufficiency or deficiency (23).

Prothrombin, factor II of blood coagulation factors, is synthesized in the liver. Its glutamic acid residues are γ -carboxylated to the active form with calcium binding capacity by the action of GGCX, with vitamin K as the co-enzyme. Serum concentration of prothrombin, not γ -carboxylated yet (PIVKA-II), is increased in vitamin K insufficiency, and can be a marker for vitamin K insufficiency in the liver. OC is a bone matrix protein produced by osteoblast, and also γ -carboxylated vitamin K-dependently. Then serum concentration of ucOC can serve as a marker for vitamin K insufficiency in the bone.

At baseline, serum concentrations of PIVKA-II and ucOC were above the reference range in all and 78.9% of the patients, respectively, confirming that SMID patients are vitamin K deficient both in the liver and bone.

After treatment, serum concentration of PIVKA-II was significantly decreased, but serum level of ucOC was not significantly changed. Such results could be explained by the difference of vitamin K requirement in the liver and the bone. Previous vitamin K₁ depletion-repletion studies reported that the γ -carboxylation of prothrombin was restored at 100–200 $\mu\text{g}/\text{d}$, whereas that of osteocalcin required at least 300–500 $\mu\text{g}/\text{d}$ (24–26). In the current study, median vitamin K intake was 183 $\mu\text{g}/\text{d}$ after nutritional therapy, which could be good enough for hepatic γ -carboxylation of prothrombin, but still lower than the dose required for the γ -carboxylation of osteocalcin. There was no dose-dependency between Δ vitamin K intake and Δ PIVKA-II while significant dose-dependency were found between Δ vitamin K intake and Δ ucOC and Δ ucOC/OC ratio. Since the supplemented vitamin K intake value in the present study seemed to be reached a plateau to fulfill the requirement for the liver function, there was no dose-dependency between serum PIVKA-II level and

vitamin K intake. The vitamin K intake requirement value for the liver is lower than those for the bone. This supplemented value range, however, was insufficient for bone, and the significant relationship was seen between serum ucOC level and vitamin K intake.

Caution is needed, however, in the interpretation of serum ucOC concentration. In a high-turnover bone, such as in hyperparathyroidism or hyperthyroidism, serum OC concentration is increased because of enhanced osteoblastic bone formation. Then, serum ucOC level can be increased even in the absence of vitamin K insufficiency. Conversely, in the low-turnover bone, serum ucOC concentration may not be increased even in vitamin K deficiency, and could be underestimated. Therefore, ucOC/OC ratio has been adopted as a more accurate indicator of vitamin K status than absolute ucOC level (27). In the current study, ucOC/OC ratio was significantly decreased, which is inconsistent with the previous reports that higher vitamin K₁ intake is needed for the restoration of γ -carboxylation in the bone (24–26). Such inconsistency may be due to the difference in the study subjects. Previous reports are from the healthy subjects who are unlikely to be vitamin K deficient, whereas SMID patients are quite likely to be severely vitamin K deficient for a long time. Generally, vitamin supplementation would be more efficacious to the subjects with severe deficiency than those without it. Since there was a significant dose-dependency of vitamin K₁ intake in the changes of ucOC and ucOC/OC ratio, treatment with vitamin K₁ yielded improvement of vitamin K status in the bone.

Bone turnover status can be evaluated by measuring the bone metabolic markers. In the current study, BAP and TRACP-5b were employed as the bone formation marker and bone resorption marker, respectively. In our study, these serum bone turnover markers were significantly increased. The reason for increased TRACP-5b may be related to calcium and vitamin D intake. Previous studies described that calcium supplementation decreased bone resorption markers (28–31). In fact, the mean calcium intake and the median vitamin D intake of our patient were significantly decreased from baseline, since these nutrients contained in Racol[®] were lower than those in other enteral nutrition used by our subjects at the baseline. Serum BAP level was also increased in our subjects, while one intervention study has reported that vitamin K₁ therapy did not alter BAP level in healthy postmenopausal women (32). Such discrepancy may be due to low turnover bone in SMID patients. SMID patients are under long-term immobilization and have an extremely low turnover bone (1, 33). Therefore, it is possible that vitamin K₁ did not stimulate bone formation in healthy postmenopausal women with normal bone turnover, but stimulated it in SMID patients with suppressed bone turnover. However, the above explanation remains a hypothesis, and further studies are required.

The present study has some limitations. First one is the limited number of the study subjects included and the lack of control group with no intervention. How-

ever, we believe that our study is not so small-sized considering the low prevalence of adult patients with SMID. Second, intervention duration was 3 mo, and longer intervention study is needed for the long-term efficacy of an intervention. Third, this study was performed within a context of routine medical care, and the amount of added vitamin K could not be fixed. Fourth, there were significant alterations of serum PIVKA-II and ucOC in the present study. However, it did not reach a clinically significant alteration level. Since the cut-off values of PIVKA-II and ucOC adopted in the present study were setting for healthy subjects, it was questionable to use for SMID subjects. Therefore, it is considered that the alterations without clinical significance have a certain worth for SMID subjects.

Our strength is that intervention efficacy of nutritional therapy in vitamin K₁ was confirmed. As far as we have searched, such study has not been previously reported in SMID patients. Additionally, our present results would be of clinical relevance. Racol[®] used in the present study containing 125 µg of vitamin K₁ in 200 mL was discontinued after our study was completed. Currently available one contains far less amount of vitamin K₁. As discussed above, much more vitamin K is required for reducing fracture risk than for avoiding blood coagulation abnormality. The importance of vitamin K in fracture prevention does not seem to be adequately acknowledged.

In conclusion, vitamin K₁ therapy was considered to be favorable for SMID patients in view of that it has effectively improved markers for vitamin K status as well as bone turnover markers.

Authorship

Research conception and design: A. Kuwabara; investigation: A. Nagae and M. Kitagawa; resource: K. Tozawa; statistical analysis of the data: A. Kuwabara; interpretation of the data: A. Kuwabara, K. Tanaka; writing of the manuscript: A. Kuwabara and K. Tanaka; supervision: M. Kumode.

Disclosure of state of COI

No conflicts of interest to be declared.

REFERENCES

- 1) Sheridan KJ. 2009. Osteoporosis in adults with cerebral palsy. *Dev Med Child Neurol* **51**(Suppl 4): 38–51.
- 2) Grammatikopoulou MG, Daskalou E, Tsigga M. 2009. Diet, feeding practices, and anthropometry of children and adolescents with cerebral palsy and their siblings. *Nutrition* **25**: 620–626.
- 3) Kilpinen-Loisa P, Pihko H, Vesander U, Paganus A, Ritanen U, Mäkitie O. 2009. Insufficient energy and nutrient intake in children with motor disability. *Acta Paediatr* **98**: 1329–1333.
- 4) Sugiyama T, Takaki T, Saito T, Taguchi T. 2007. Vitamin K therapy for cortical bone fragility caused by reduced mechanical loading in a child with hemiplegia. *J Musculoskelet Neuronal Interact* **7**: 219–223.
- 5) Ozel S, Switzer L, Macintosh A, Fehlings D. 2016. Informing evidence-based clinical practice guidelines for children with cerebral palsy at risk of osteoporosis: an update. *Dev Med Child Neurol* **58**: 918–923.
- 6) Suttie JW. 1990. Warfarin and vitamin K. *Clin Cardiol* **13** (4 Suppl 6): VI-16–18.
- 7) Hoang QQ, Sicheri F, Howard AJ, Yang DS. 2003. Bone recognition mechanism of porcine osteocalcin from crystal structure. *Nature* **425**: 977–980.
- 8) Vermeer C. 1990. Gamma-carboxyglutamate-containing proteins and the vitamin K-dependent carboxylase. *Biochem J* **266**: 625–636.
- 9) Booth SL. 1997. Skeletal functions of vitamin K-dependent proteins: not just for clotting anymore. *Nutr Rev* **55**: 282–284.
- 10) Vermeer C, Shearer MJ, Zittermann A, Bolton-Smith C, Szulc P, Hodges S, Walter P, Rambeck W, Stöcklin E, Weber P. 2004. Beyond deficiency: potential benefits of increased intakes of vitamin K for bone and vascular health. *Eur J Nutr* **43**: 325–335.
- 11) Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA, Wilson PW, Ordovas J, Schaefer EJ, Dawson-Hughes B, Kiel DP. 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.
- 12) Yoshikawa H, Yamazaki S, Watanabe T, Abe T. 2003. Vitamin K deficiency in severely disabled children. *J Child Neurol* **18**: 93–97.
- 13) Nagae A, Kuwabara A, Tozawa K, Kumode M, Takeuchi Y, Tanaka K. 2013. Enteral nutrition and antibiotic use increase the risk for vitamin K deficiency in patients with severe motor and intellectual disabilities. *e-SPEN J* **8**: 31–36.
- 14) Kozu K, Nakanishi T, Okuda H, Watanabe K, Saito S, Tanaka M, Akahane Y, Kawai T, Suzuki H. 1996. Development of PIVKA-II measuring reagent (EDO38) by ECL technology and its performance characteristics. *Rinsho to Kenkyu* **73**: 2656–2664 (in Japanese).
- 15) Nishimura J, Arai N, Fujimatsu J. 2007. Measurement of undercarboxylated osteocalcin by electrochemiluminescence immunoassay with the “Picolumi ucOC” kit. *Igaku to Yakugaku* **57**: 523–525 (in Japanese).
- 16) Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepan J; Committee of Scientific Advisors of the International Osteoporosis Foundation. 2000. The use of biochemical markers of bone turnover in osteoporosis. Committee of Scientific Advisors of the International Osteoporosis Foundation. *Osteoporos Int* **11**: S2–17.
- 17) Tanaka Y, Shibata R. 2000. Effects of vitamin K₂ administration in the patients with severely motor and intellectual disabilities: assessment of bone metabolic marker and bone mineral density. *No To Hattatsu* **32**: 491–496 (in Japanese).
- 18) Kodama Y, Okamoto Y, Kubota T, Hiroyama Y, Fukami H, Matsushita K, Kawano Y. 2017. Effectiveness of vitamin K₂ on osteoporosis in adults with cerebral palsy. *Brain Dev* **39**: 846–850.
- 19) Booth SL, Tucker KL, McKeown NM, Davidson KW, Dallal GE, Sadowski JA. 1997. Relationships between dietary intakes and fasting plasma concentrations of fat-soluble vitamins in humans. *J Nutr* **127**: 587–592.
- 20) Booth SL, O'Brien-Morse ME, Dallal GE, Davidson KW, Gundberg CM. 1999. Response of vitamin K status to different intakes and sources of phyloquinone-rich foods: Comparison of younger and older adults. *Am J Clin Nutr* **70**: 368–377.

- 21) Novotny JA, Kurilich AC, Britz SJ, Baer DJ, Clevidence BA. 2010. Vitamin K absorption and kinetics in human subjects after consumption of ¹³C-labelled phyloquinone from kale. *Br J Nutr* **104**: 858–862.
- 22) Sokoll LJ, Booth SL, O'Brien ME, Davidson KW, Tsaioun KI, Sadowski JA. 1997. Changes in serum osteocalcin, plasma phyloquinone, and urinary gamma-carboxyglutamic acid in response to altered intakes of dietary phyloquinone in human subjects. *Am J Clin Nutr* **65**: 779–784.
- 23) Shea MK, Booth SL. 2016. Concepts and controversies in evaluating vitamin K status in population-based studies. *Nutrients* **8**(1): 8.
- 24) Booth SL, Martini L, Peterson JW, Saltzman E, Dallal GE, Wood RJ. 2003. Dietary phyloquinone depletion and repletion in older women. *J Nutr* **133**: 2565–2569.
- 25) Schurgers LJ, Shearer MJ, Hamulyák K, Stöcklin E, Vermeer C. 2004. Effect of vitamin K intake on the stability of oral anticoagulant therapy: dose-response relationships in healthy subjects. *Blood* **104**: 2682–2689.
- 26) Binkley NC, Krueger DC, Kawahara TN, Engelke JA, Chappell RJ, Suttie JW. 2002. A high phyloquinone intake is required to achieve maximal osteocalcin gamma-carboxylation. *Am J Clin Nutr* **76**: 1055–1060.
- 27) Gundberg CM, Nieman SD, Abrams S, Rosen H. 1998. Vitamin K status and bone health: an analysis of methods for determination of undercarboxylated osteocalcin. *J Clin Endocrinol Metab* **83**: 3258–3266.
- 28) Palacios S, Castelo-Branco C, Cifuentes I, von Helde S, Baró L, Tapia-Ruano C, Menéndez C, Rueda C. 2005. Changes in bone turnover markers after calcium-enriched milk supplementation in healthy postmenopausal women: a randomized, double-blind, prospective clinical trial. *Menopause* **12**: 63–68.
- 29) Cleghorn DB, O'Loughlin PD, Schroeder BJ, Nordin BE. 2001. An open, crossover trial of calcium-fortified milk in prevention of early postmenopausal bone loss. *Med J Aust* **175**: 242–245.
- 30) Martini L, Wood RJ. 2002. Relative bioavailability of calcium-rich dietary sources in the elderly. *Am J Clin Nutr* **76**: 1345–1350.
- 31) Bonjour JP, Benoit V, Rousseau B, Souberbielle JC. 2012. Consumption of vitamin D- and calcium-fortified soft white cheese lowers the biochemical marker of bone resorption TRAP 5b in postmenopausal women at moderate risk of osteoporosis fracture. *J Nutr* **142**: 698–703.
- 32) Binkley N, Harke J, Krueger D, Engelke J, Vallarta-Ast N, Gemar D, Checovich M, Chappell R, Suttie J. 2009. Vitamin K therapy reduces undercarboxylated osteocalcin but does not alter bone turnover, density, or geometry in healthy postmenopausal North American women. *J Bone Miner Res* **24**: 983–991.
- 33) Höglér W, Ward L. 2015. Osteoporosis in children with chronic disease. In: *Calcium and Bone Disorders in Children and Adolescents*, 2nd, revised edition (Allgrove J, Shaw NJ, eds), p 176–195. Karger Publishers, Basel.