

## Original Article

# Vitamin K nutritional status and undercarboxylated osteocalcin in postmenopausal osteoporotic women treated with bisphosphonates

Jun Iwamoto MD<sup>1</sup>, Tetsuya Takada MD<sup>2</sup>, Yoshihiro Sato MD<sup>3</sup>

<sup>1</sup>*Institute for Integrated Sports Medicine, Keio University School of Medicine, Tokyo, Japan*

<sup>2</sup>*Department of Internal Medicine, Hiyoshi Medical Clinic, Kanagawa, Japan*

<sup>3</sup>*Department of Neurology, Mitate Hospital, Fukuoka, Japan*

Serum undercarboxylated osteocalcin (ucOC) is an index of vitamin K nutritional status in treatment-naive postmenopausal osteoporotic women. The purpose of the present study was to reveal the association between vitamin K nutritional status and serum ucOC concentrations in postmenopausal osteoporotic women taking bisphosphonates. Eighty-six postmenopausal women with osteoporosis (age range: 47-90 years) initiated bisphosphonate treatment. Vitamin K nutritional status was evaluated using a simple vitamin K-intake questionnaire and serum ucOC concentrations were measured after 6 months of treatment. The patients were divided into two groups according to the simple vitamin K-intake questionnaire score: a low vitamin K-intake (score <40) group (n=67) and a normal vitamin K-intake (score ≥40) group (n=19). There were no significant differences between the groups in baseline parameters including age, height, body weight, body mass index, serum alkaline phosphatase (ALP), urinary cross-linked N-terminal telopeptides of type I collagen (NTX), and changes in serum ALP and urinary NTX concentrations during the 6-month treatment period. However, the mean serum ucOC concentration after 6 months of treatment was significantly higher in the low vitamin K-intake group (2.79 ng/mL) than in the normal vitamin K-intake group (2.20 ng/mL). These results suggest that 78% of postmenopausal osteoporotic women treated with bisphosphonates may have vitamin K deficiency as indicated by low vitamin K-intake and high serum ucOC concentrations, despite having a similar reduction in bone turnover to women who have normal vitamin K-intake.

**Key Words:** vitamin K, postmenopausal women, osteoporosis, undercarboxylated osteocalcin, nutrition

## INTRODUCTION

Vitamin K is a cofactor of  $\gamma$ -carboxylase, which converts three glutamic acid (Glu) residues in osteocalcin (OC) to  $\gamma$ -carboxyglutamic acid (Gla), and is thus essential for  $\gamma$ -carboxylation of OC.<sup>1-4</sup> Without this modification, OC becomes undercarboxylated OC (ucOC), which lacks structural integrity and the ability to bind to the mineral hydroxyapatite. The carboxylation reaction is completed as an intracellular posttranslational event, and secreted OC can no longer be carboxylated. Vitamin K deficiency impairs  $\gamma$ -carboxylation of OC, resulting in high serum concentrations of ucOC.

Although OC is a calcium binding protein that is strongly expressed in bone, its effects on bone remain obscure. However, a biomechanical analysis of the quality of the femur bones in OC-deficient mice showed that the yield energy (ductility of bone specimens), failure load (strength of the bone), and stiffness (elasticity of the bone) decreased dramatically after ovariectomy,<sup>5</sup> suggesting that OC may play an important role in bone biology, and that OC deficiency may be associated with bone fragility.

Vitamin K deficiency leads to OC deficiency. Associations between vitamin K deficiency, increased risk of hip

fracture, and bone mineral density (BMD) have been reported in elderly people.<sup>6-12</sup> High serum concentrations of ucOC are a risk factor for hip fractures in elderly women, independent of BMD.<sup>10</sup> One study showed that vitamin K deficiency was associated with a high prevalence of vertebral fractures in Japanese women.<sup>13</sup> Vitamin K deficiency, indicated by low serum phylloquinone concentrations, high serum ucOC concentrations, or possibly a low serum carboxylated OC to serum total OC ratio, may contribute to the increased risk of fractures in postmenopausal women and elderly people, and possibly to BMD loss in selected cohorts of elderly people.<sup>6-13</sup> In Japan, the cut-off value for increased fracture risk associated with vitamin K deficiency is set at 4.5 ng/mL in treatment-naive postmenopausal women.<sup>14</sup> However, in osteoporotic patients treated with amino-bisphosphonates, the cut-off

**Corresponding Author:** Dr Jun Iwamoto, Institute for Integrated Sports Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.

Tel: +81-3-3353-1211; Fax: +81-3-3352-9467

Email: jiwamoto@a8.keio.jp

Manuscript received 30 August 2013. Initial review completed 22 September 2013. Revision accepted 28 December 2013.

doi: 10.6133/apjcn.2014.23.2.15

value is reduced to 2.6 ng/mL,<sup>15</sup> because serum ucOC concentrations are correlated with bone turnover markers and bisphosphonates reduce bone turnover and subsequent serum ucOC concentrations in postmenopausal women.<sup>16,17</sup>

However, few studies have investigated the influence of vitamin K nutritional intake on serum ucOC concentrations in patients treated with bisphosphonate, and the association between vitamin K nutritional status and high ucOC remains to be established in postmenopausal women receiving bisphosphonates. The purpose of the present study was to evaluate the relationship between vitamin K nutritional status and high serum ucOC concentrations in postmenopausal osteoporotic women during bisphosphonate treatment.

## SUBJECTS AND METHODS

### *Study subjects*

Ninety-eight postmenopausal women with osteoporosis (age range: 47-90 years) who were receiving amino-bisphosphonates (alendronate or risedronate) were enrolled from our outpatient clinic (Hiyoshi Medical Clinic, Japan) during the 6 months period between October 1, 2012 and March 30, 2013. The inclusion criteria were spontaneous menopause, a fully-ambulatory status, a diagnosis of postmenopausal osteoporosis according to the Japanese diagnostic criteria,<sup>18,19</sup> and an osteoporosis-treatment-naïve status before bisphosphonate treatment. The exclusion criteria included glucocorticoid use, warfarin use, rheumatoid arthritis, gastrectomy, renal failure, or bone diseases including cancer-induced bone loss because of aromatase inhibitors, primary hyperparathyroidism, hyperthyroidism, Cushing syndrome, multiple myeloma, Paget's disease of the bone, osteogenesis imperfecta, or type 2 diabetes mellitus.

Age, height, body weight, body mass index, history of non-vertebral fractures after menopause, prevalence of vertebral fractures, bone mass, and biochemical markers were assessed in our outpatient clinic prior to osteoporosis treatment. Non-vertebral fractures included fractures of the hip, distal radius, proximal humerus, rib, pelvis, clavicle, femur, and lower leg. Thoracic and lumbar spine radiographs were taken to detect vertebral fractures. Blood and urine samples were obtained to assess serum calcium, phosphorus, alkaline phosphatase (ALP), and urinary cross-linked N-terminal telopeptides of type I collagen (NTX). After the initiation of bisphosphonates, urinary NTX concentrations were measured at 3 months, and serum calcium, phosphorus, and ALP concentrations were measured at 6 months. Vitamin K nutritional status was evaluated and serum ucOC concentrations were measured after 6 months of treatment using the simple vitamin K-intake questionnaire developed by Uenishi.<sup>20</sup> This questionnaire evaluates vitamin K nutritional status using scores based on natto (fermented soybean) and green vegetable intakes (Table 1).<sup>20</sup> Because multiple simultaneous measurements of bone markers were not permitted for health insurance reasons, serum ucOC concentrations were evaluated after 6 months of bisphosphonate treatment.

Twelve patients discontinued bisphosphonate treatment during the 6-month treatment period because of gastric

pain (n=1), tooth extraction (n=2), and noncompliance (n=9). Data for the 86 patients who continued bisphosphonate treatment for 6 months were analyzed. These patients were divided into two groups according to the simple vitamin K-intake questionnaire score: a low vitamin K-intake (score <40) group (n=67) and a normal vitamin K-intake (score ≥40) group (n=19). The cut-off value for vitamin K deficiency was score 40 as assessed by the simple vitamin K-intake questionnaire.<sup>20</sup> In the low vitamin K-intake group, 31 women were treated with alendronate (35 mg, weekly) and 36 with risedronate (17.5 mg, weekly). In the normal vitamin K-intake group, 7 women received alendronate (35 mg, weekly) and 12 received risedronate (17.5 mg, weekly). The doses of bisphosphonates used in the present study were recognized as effective doses in postmenopausal Japanese women with osteoporosis.<sup>21,22</sup> Baseline parameters, changes in biochemical markers and bone mass during the 6-month treatment period, and serum ucOC and vitamin K nutritional status after 6 months of treatment were compared between the two groups. The present study was approved by the Ethics Committee of Hiyoshi Medical Clinic.

### *Japanese diagnostic criteria for postmenopausal osteoporosis*

Postmenopausal osteoporosis was defined according to the Japanese diagnostic criteria<sup>18,19</sup> as (1) BMD <70% of the young adult mean (YAM) or the "presence" of osteopenia on X-ray images of the spine, and (2) BMD of 70-80% of the YAM or "possible" osteopenia on X-ray images of the spine, together with a history of osteoporotic fractures. Dual energy X-ray absorptiometry (DXA) of the spine is a useful technique for monitoring osteoporosis in Japanese women. Although quantitative ultrasound (QUS) parameters can predict the risk of hip, wrist, and total non-vertebral fractures for up to 10 years,<sup>23</sup> the reliability of speed of sound (SOS) in the calcaneus has not been fully established. The diagnosis of osteoporosis was therefore made using both SOS (<70% of the YAM or 70-80% of the YAM together with a history of osteoporotic fractures) and X-ray findings of the spine (i.e., presence of osteopenia or possible osteopenia, along with a history of osteoporotic fractures).

### *Assessment of morphometric vertebral fractures*

Plain lateral X-ray films of the thoracic and lumbar spine were obtained to detect evidence of prevalent morphometric vertebral fractures. Based on the Japanese criteria, a vertebral fracture was defined according to the vertebral height on lateral X-ray films.<sup>18,19</sup> Briefly, the vertebral height was measured at the anterior (A), central (C), and posterior (P) parts of the vertebral body, and the presence of a vertebral fracture was confirmed when (1) a reduction in the vertebral height of >20% (A, C, and P) compared with the height of the adjacent vertebrae was observed, (2) the C/A or C/P was <0.8, or (3) the A/P was <0.75. Assessment for vertebral fractures was performed at the T4-L4 level.

### *Measurement of bone mass*

SOS of the left calcaneus was measured using a QUS device (CM-200; Elk Corp., Osaka, Japan). The coeffi-

coefficients of variation ( $100 \times$  standard deviation (SD)/mean) of five measurements, with repositioning within 24 hours each time, was 0.17-0.43% among three healthy adults and 0.25-0.35% among three osteoporotic patients.

#### **Blood and urinary biochemical tests**

Serum and urine samples were obtained from each patient and subjected to biochemical analyses. Serum calcium, phosphorus, and ALP concentrations were measured using standard laboratory techniques. Urinary NTX concentrations were measured using an enzyme immunoassay.

#### **Statistical analysis**

Data were expressed as the mean $\pm$ SD. Unpaired t-test was used to compare the baseline characteristics, percent changes in biochemical markers and SOS, urinary NTX concentrations after 3 months of treatment, and serum ALP, ucOC concentrations and vitamin K-intake scores after 6 months of treatment between the two groups. The Fisher's exact test was used to compare the numbers of subjects with prevalent vertebral fractures and a history of non-vertebral fractures between the two groups. All statistical analyses were performed using the Stat View J-5.0 program (SAS Institute, Cary, NC, USA). The significance level was set at  $p < 0.05$  for all the comparisons.

## **RESULTS**

#### **Baseline characteristics of study subjects**

Table 2 shows the baseline characteristics of the study subjects. The mean age of the participants was 70.4 years in the low vitamin K-intake group ( $n=67$ , 78%) and 69.7 years in the normal vitamin K-intake group ( $n=19$ , 22%). The mean vitamin K-intake questionnaire score was significantly lower in the low vitamin K-intake group than in the normal vitamin K-intake group (26.4 vs. 48.7, respectively,  $p < 0.001$ ). However, there were no significant differences in age, height, body weight, body mass index, SOS, number of subjects with prevalent vertebral fractures, number of subjects with a history of non-vertebral fractures, and serum calcium, phosphorus, ALP, or urinary NTX concentrations between the two groups. Seven women in the low vitamin K-intake group (10.4%) had prevalent vertebral fractures and four (6.0%) had a history of non-vertebral fracture (all wrist fractures). Three subjects in the normal vitamin K-intake group (15.8%) had prevalent vertebral fractures and none (0%) had histories

of non-vertebral fractures. All non-vertebral fractures had completely healed, as assessed by plain radiographs.

In particular, the median (interquartile ranges) of serum ALP was 249 IU/L (212-293 IU/L) in the low vitamin K-intake group and 245 IU/L (203-289 IU/L) in the normal vitamin K-intake group. The respective median (interquartile ranges) of urinary NTX was 54.1 nmol BCE/mmol Cr (40.1-70.0 nmol BCE/mmol Cr) and 44.5 (28.5-68.1 nmol BCE/mmol Cr).

#### **Percentage changes in SOS and biochemical markers after bisphosphonate treatment**

Table 3 shows that urinary NTX concentrations decreased after 3 months of treatment and SOS increased and serum ALP concentrations decreased after 6 months of treatment in both groups. The mean rate of urinary NTX decrease was 38.5% in the low vitamin K-intake group and 36.6% in the normal vitamin K-intake group. The respective mean rate of serum ALP decrease was 17.5% and 22.4%, respectively. The respective mean rate of SOS increase was 0.36% and 0.62%, respectively. However, there were no significant differences in the percentage changes in urinary NTX, serum calcium, phosphorus, ALP concentrations or SOS between the two groups.

The mean urinary NTX concentration after 3 months of treatment was 32.5 nmol BCE/mmol Cr in the low vitamin K-intake group and 26.6 nmol BCE/mmol Cr in the normal vitamin K-intake group with no significant difference between the two groups. The respective mean serum ALP concentration after 6 months of treatment was 209 IU/L and 192 IU/L with no significant difference between the two groups.

#### **Vitamin K nutritional status and serum ucOC after 6 months of treatment**

Figure 1 shows serum ucOC concentrations after 6 months of treatment in the low and normal vitamin K-intake groups. The mean serum ucOC concentration was significantly higher in the low vitamin K-intake group than in the normal vitamin K-intake group (2.79 ng/mL and 2.20 ng/mL, respectively).

#### **Adverse events**

Twelve women dropped out of the study during the 6-month treatment period. However, no severe adverse event was observed in any participants.

**Table 1.** Simple vitamin K-intake questionnaire

Simplified vitamin K-intake questionnaire (Eat normally = a handful or small bowlful)				
Fermented soybeans (1 pack 50 g)	1. Almost none	2. One to three times/ week	3. Four to five times/ week	4. One or more times/day
Vegetables (per meal)	1. Almost none	2. Eat a few	3. Eat normally	4. Eat plenty
Points table for the simplified vitamin K-intake questionnaire (Total score <40 pts = insufficient vitamin K intake expected and measurement of serum undercarboxylated osteocalcin recommended)				
Fermented soybeans	1. 0 pts	2. 10 pts	3. 25 pts	4. 40 pts
Vegetables	1. 0 pts	2. 10 pts	3. 15 pts	4. 25 pts

**Table 2.** Baseline characteristics of study subjects

	Low vitamin K-intake group (n=67)	Normal vitamin K-intake group (n=19)	<i>p</i> -value
Vitamin K-intake questionnaire score	26.4±8.5	48.7±8.6	<0.001
Age (years)	70.4±9.5	69.7±9.9	NS
Height (m)	1.54±0.06	1.54±0.07	NS
Body weight (kg)	51.6±7.0	49.9±5.9	NS
Body mass index (kg/m <sup>2</sup> )	21.7±3.1	21.1±1.2	NS
Prevalence of vertebral fracture (%)	10.4	15.8	NS
History of non-vertebral fracture (%)	6	0	NS
SOS (m/s)	1472±17	1468±11	NS
YAM of SOS (%)	67.3±7.8	65.2±4.8	NS
Serum calcium (mg/dL)	9.1±0.3	9.2±0.4	NS
Serum phosphorus (mg/dL)	3.5±0.5	3.5±0.3	NS
Serum ALP (IU/L)	258±69	247±67	NS
Urinary NTX (nmol BCE/mmol Cr)	56.8±22.3	52.7±30.3	NS

Data are expressed as mean±SD. Unpaired *t*-tests were used to compare baseline characteristics of study subjects between groups. Fisher's exact tests were used to compare percentages of patients with prevalent vertebral fractures and history of non-vertebral fractures between groups.

SOS: speed of sound; YAM: young adult mean; ALP: alkaline phosphatase; NTX: cross-linked N-terminal telopeptides of type I collagen; BCE: bone collagen equivalent; Cr: creatinine; NS: not significant.

**Table 3.** Percentage changes in SOS and biochemical markers after bisphosphonate treatment

	Low vitamin K-intake group (n=67)	Normal vitamin K-intake group (n=19)	<i>p</i> -value
SOS (m/s)	0.36±0.97	0.62±0.84	NS
Serum calcium (mg/dL)	-0.10±4.33	0.65±3.79	NS
Serum phosphorus (mg/dL)	-2.3±14.3	-4.0±15.5	NS
Serum ALP (IU/L)	-17.5±17.1	-22.4±15.1	NS
Urinary NTX (nmol BCE/mmol Cr)	-38.5±24.4	-36.6±33.8	NS

Data are expressed as mean±SD. Unpaired *t*-tests were used to compare the changes in SOS and biochemical markers after bisphosphonate treatment between groups. The percentage changes in SOS, serum calcium, phosphorus, and ALP after 6 months of treatment and the percentage change in urinary NTX after 3 months of treatment were evaluated.

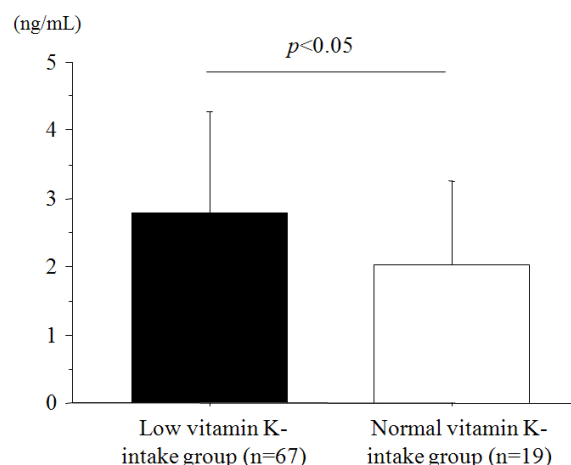
SOS: speed of sound; ALP: alkaline phosphatase; NTX: cross-linked N-terminal telopeptides of type I collagen; BCE: bone collagen equivalent; Cr: creatinine; NS: not significant.

## DISCUSSION

Serum ucOC is an index of vitamin K nutritional status in treatment-naïve postmenopausal osteoporotic women. However, the association between vitamin K nutritional status and high ucOC remains to be established in postmenopausal women receiving bisphosphonates. The present study confirmed that 78% of postmenopausal osteoporotic women treated with bisphosphonates might have had vitamin K deficiency as indicated by low vitamin K-intake and high serum ucOC concentrations, despite having a similar reduction in bone turnover to women who had normal vitamin K-intake.

Our previous studies showed that alendronate (35 mg weekly) and risedronate (17.5 mg weekly) reduced serum ALP and urinary NTX concentrations by 34.7-44.9% (at 3 months) and 17.2-19.9% (at 6 months), respectively, from baseline values, and increased SOS by 0.60-0.68% (at 6 months) from baseline in postmenopausal women with osteoporosis.<sup>24,25</sup> In the present study, urinary NTX concentrations were reduced by 36.6-38.5% at 3 months and serum ALP concentrations by 17.5-22.4% at 6 months. SOS was increased by 0.36-0.62%. The changes in bone turnover markers and SOS in the present study were compatible with those in previous studies.<sup>24,25</sup>

There are two types of naturally-occurring forms of vitamin K, phyloquinone (vitamin K1) and menaquinones



**Figure 1.** Serum ucOC levels after 6 months of bisphosphonate treatment. ucOC: undercarboxylated osteocalcin.

(vitamin K2). All forms of vitamin K contain a 2-methyl-1,4-naphthoquinone ring as part of their structure, and individual forms differ in the length and degree of saturation of a variable aliphatic side chain attached at the 3-position. Vitamin K1 is rich in green vegetables, while vitamin K2 is rich in natto (fermented soybean).<sup>26</sup> Thus, the simple vitamin K-intake questionnaire developed by Uenishi<sup>20</sup> was considered suitable for evaluating vitamin

K nutritional intake. A significant positive correlation between vitamin K nutritional intake according to this simple vitamin K-intake questionnaire and the food frequency questionnaire for dietary intake of calcium and other nutrients has been demonstrated in terms of bone health in adult Japanese women (Spearman's rank correlation coefficient,  $r^2=0.899$ ,  $p<0.0001$ ).<sup>20,27</sup> Furthermore, there was a significant negative correlation between the simple vitamin K-intake questionnaire scores and serum concentrations of ucOC in treatment-naïve postmenopausal women (mean age: 64.9 years) (Spearman's rank correlation coefficient,  $r^2=-0.404$ ,  $p<0.0001$ ).<sup>20</sup> These results demonstrate an association between simple vitamin K-intake questionnaire scores and serum ucOC concentrations in postmenopausal women with osteoporosis.

Alendronate (5 mg daily) reduced serum OC concentrations by approximately 45% from the baseline value in postmenopausal Japanese women with osteoporosis.<sup>21</sup> No data are available regarding risedronate.<sup>22</sup> Serum ucOC concentrations are decreased in postmenopausal women with osteoporosis according to the reduction in bone turnover markers following bisphosphonate treatment.<sup>16</sup> The reduction in serum OC concentrations levelled off after 6 months of bisphosphonate treatment, so evaluation of serum ucOC concentrations after 6 months may be considered reasonable. However, the association between vitamin K nutritional status and high ucOC in postmenopausal osteoporotic patients treated with bisphosphonates still remains to be confirmed. In the present study, 78% of postmenopausal osteoporotic patients treated with bisphosphonates were considered to have vitamin K deficiency as indicated by low vitamin K-intake and high serum ucOC concentrations (mean: 2.79 ng/mL), even though bone turnover markers were reduced similarly to those in patients who had normal vitamin K-intake and low serum ucOC concentrations (mean: 2.20 ng/mL). Because high concentrations of ucOC concentrations (>2.6 ng/mL) are thought to be an independent risk factor for incident fractures in osteoporotic patients treated with bisphosphonates,<sup>15</sup> nutritional management of vitamin K appears to be essential to reduce the occurrence of fractures in postmenopausal osteoporotic women treated with bisphosphonate who have vitamin K deficiency.

The vitamin K-dependent  $\gamma$ -glutamyl carboxylase (GGCX) carboxylates vitamin K-dependent proteins, including bone Gla protein (osteocalcin) and matrix Gla protein. Differences in GGCX activity have been demonstrated between the common genotypes associated with BMD in elderly Japanese women.<sup>28</sup> Another study showed an influence of GGCX gene polymorphisms on the response of serum ucOC after treatment with menatetrenone (vitamin K<sub>2</sub>, menaquinone-4) among Thai women.<sup>29</sup> The GGCX polymorphism may therefore affect the carboxylase activity of vitamin K and contribute to individual differences in susceptibility to osteoporosis and response to vitamin K. There seem to be genetic contributions to the actions of vitamin K in human populations, and the influence of GGCX gene polymorphisms on the relationship between vitamin K nutritional status and serum ucOC concentrations in individual patients thus needs to be considered. Further studies are needed to address this issue.

This study had some limitations. First, DXA is the gold standard in the diagnosis of osteoporosis, but we used SOS as measured by QUS to evaluate bone mass. There might therefore have been a bias in terms of patient inclusion and exclusion. Second, despite no significant differences between the two groups, both urinary NTX level after 3 months of treatment and serum ALP concentration after 6 months of treatment tended to be higher in the low vitamin K-intake group than in the normal vitamin K-intake group, which could have affected serum ucOC concentrations after 6 months of treatment, because serum ucOC concentrations might have been influenced by bone turnover status.<sup>17</sup> Considering the modest number of the study subjects, there remains the possibility that the lack of significance was due to the type-II error. Third, we did not measure serum 25-hydroxyvitamin D concentrations to evaluate vitamin D insufficiency/deficiency. Vitamin D supplementation is uncommon in Japan, and the measurement of serum 25-hydroxyvitamin D concentrations is not covered by health insurance. Because low serum concentrations of 25-hydroxyvitamin D are associated with a poorer response to bisphosphonate treatment,<sup>30</sup> vitamin D nutritional status could have had an influence on the results of the present study.

In conclusion, the results of this study suggest that 78% of postmenopausal osteoporotic patients treated with bisphosphonates may have vitamin K deficiency as indicated by low vitamin K-intake and high serum ucOC concentrations, despite similar reductions in bone turnover to women who have normal vitamin K-intake. Because high concentrations of ucOC concentrations are thought to be an independent risk factor for incident fractures in osteoporotic patients treated with bisphosphonates, nutritional management of vitamin K appears to be essential to reduce the occurrence of fractures in postmenopausal osteoporotic women treated with bisphosphonate who have vitamin K deficiency.

#### AUTHOR DISCLOSURES

The authors report no funding sources and no conflicts of interest in this paper. All authors contributed this manuscript; Dr Jun Iwamoto and Dr Tetsuya Takada treated patients with bisphosphonates. Dr Yoshihiro Sato organized the study and edited the manuscript.

#### REFERENCES

1. Hauschka PV, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiol Rev.* 1989;69:990-1047.
2. Koshihara Y, Hoshi K. Vitamin K<sub>2</sub> enhances osteocalcin accumulation in the extracellular matrix of human osteoblasts in vitro. *J Bone Miner Res.* 1997;12:431-8. doi: 10.1359/jbmr.1997.12.3.431.
3. Shearer MJ. Vitamin K. *Lancet.* 1995;345:229-34. doi: 10.1016/S0140-6736(95)90227-9.
4. Vermeer C, Jie KS, Knapen MH. Role of vitamin K in bone metabolism. *Annu Rev Nutr.* 1995;15:1-22. doi: 10.1146/annurev.nutr.15.1.1.
5. Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C et al. Increased bone formation in osteocalcin-deficient mice. *Nature.* 1996;382:448-52.
6. Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in

- elderly men and women. *Am J Clin Nutr.* 2000;71:1201-8.
7. Kaneki M, Hodges SJ, Hosoi T, Fujiwara S, Lyons A, Crean SJ et al. Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. *Nutrition.* 2001;17:315-21. doi: 10.1016/S0899-9007(00)00554-2.
  8. Szulc P, Chapuy MC, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. *J Clin Invest.* 1993;91:1769-74. doi: 10.1172/JCI116387.
  9. Szulc P, Chapuy MC, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture: a three year follow-up study. *Bone.* 1996;18:487-8. doi: 10.1016/8756-3282(96)00037-3.
  10. Feskanich D, Weber P, Willett WC, Rockett H, Booth SL, Colditz GA. Vitamin K intake and hip fractures in women: a prospective study. *Am J Clin Nutr.* 1999;69:74-9.
  11. Luukinen H, Käkönen SM, Pettersson K, Koski K, Laippala P, Lövgren T, Kivelä SL, Väänänen HK. Strong prediction of fractures among older adults by the ratio of carboxylated to total serum osteocalcin. *J Bone Miner Res.* 2000;15:2473-8. doi: 10.1359/jbmr.2000.15.12.2473.
  12. Vergnaud P, Garnero P, Meunier PJ, Bréart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. *J Clin Endocrinol Metab.* 1997;82:719-24. doi: 10.1210/jc.82.3.719.
  13. Tsugawa N, Shiraki M, Suhara Y, Kamao M, Ozaki R, Tanaka K, Okano T. Low plasma phylloquinone concentration is associated with high incidence of vertebral fracture in Japanese women. *J Bone Miner Metab.* 2008;26:79-85. doi: 10.1007/s00774-007-0790-8.
  14. Tsugawa N, Shiraki M, Kamao M, Kamao M, Ozaki R, Tanaka K, Okano T. Usefulness of serum undercarboxylated osteocalcin measurement as a predictor for clinical fractures. *Osteoporosis Japan.* 2010;18:254-6. (in Japanese)
  15. Shiraki M, Yamazaki Y, Shiraki Y, Hosoi T, Tsugawa N, Okano T. High level of serum undercarboxylated osteocalcin in patients with incident fractures during bisphosphonate treatment. *J Bone Miner Metab.* 2010;28:578-84. doi: 10.1007/s00774-010-0167-2.
  16. Hirao M, Hashimoto J, Ando W, Ono T, Yoshikawa H. Response of serum carboxylated and undercarboxylated osteocalcin to alendronate monotherapy and combined therapy with vitamin K2 in postmenopausal women. *J Bone Miner Metab.* 2008;26:260-4. doi: 10.1007/s00774-007-0823-3.
  17. Yamauchi M, Yamaguchi T, Nawata K, Takaoka S, Sugimoto T. Relationships between undercarboxylated osteocalcin and vitamin K intakes, bone turnover, and bone mineral density in healthy women. *Clin Nutr.* 2010;29:761-5. doi: 10.1016/j.clnu.2010.02.010.
  18. Orimo H, Sugioka Y, Fukunaga M, Muto Y, Hotokebuchi T, Gorai I, Nakamura T, Kushida K, Tanaka H, Ikai T, Ohhashi Y. Diagnostic criteria of primary osteoporosis. *J Bone Miner Metab.* 1998;16:139-50. doi: 10.1007/s007740050038.
  19. Orimo H, Hayashi Y, Fukunaga M, Sone T, Fujiwara S, Shiraki M et al. Diagnostic criteria for primary osteoporosis: year 2000 revision. *J Bone Miner Metab.* 2001;19:331-7. doi: 10.1007/s007740170001.
  20. Uenishi K. Development and evaluation of simple vitamin K intake questionnaire. *Osteoporosis Japan.* 2011;19:515-8. (in Japanese)
  21. Uchida S, Taniguchi T, Shimizu T, Kakikawa T, Okuyama K, Okaniwa M et al. Therapeutic effects of alendronate 35 mg once weekly and 5 mg once daily in Japanese patients with osteoporosis: a double-blind, randomized study. *J Bone Miner Metab.* 2005;23:382-8. doi: 10.1007/s00774-005-0616-5.
  22. Kishimoto H, Fukunaga M, Kushida K, Shiraki M, Itabashi A, Nawata H et al. Efficacy and tolerability of once-weekly administration of 17.5 mg risedronate in Japanese patients with involutional osteoporosis: a comparison with 2.5-mg once-daily dosage regimen. *J Bone Miner Metab.* 2006;24:405-13. doi: 10.1007/s00774-006-0706-z.
  23. Fujiwara S, Sone T, Yamazaki K, Yoshimura N, Nakatsuka K, Masunari N, Fujita S, Kushida K, Fukunaga M. Heel bone ultrasound predicts non-spine fracture in Japanese men and women. *Osteoporos Int.* 2005;16:2107-12. doi: 10.1007/s00198-005-2008-z.
  24. Iwamoto J, Takada T, Sato Y, Matsumoto H. Influence of treatment with alendronate on the speed of sound, an ultrasound parameter, of the calcaneus in postmenopausal Japanese women with osteoporosis: a clinical practice-based observational study. *Ther Clin Risk Manag.* 2012;8:287-93. doi: 10.2147/TCRM.S32794.
  25. Iwamoto J, Takada T, Sato Y, Matsumoto H. Effect of risedronate on speed of sound in postmenopausal women with osteoporosis. *World J Orthop.* 2013;4:316-22. doi: 10.5312/wjo.v4.i4.316. eCollection 2013.
  26. Iwamoto J, Sato Y, Takeda T, Matsumoto H. High-dose vitamin K supplementation reduces fracture incidence in postmenopausal women: a review of the literature. *Nutr Res.* 2009;29:221-8. doi: 10.1016/j.nutres.2009.03.012.
  27. Uenishi K, Ishida H, Nakamura K. Development of a simple food frequency questionnaire to estimate intakes of calcium and other nutrients for the prevention and management of osteoporosis. *J Nutr Sci Vitaminol (Tokyo).* 2008;54:25-9. doi: 10.3177/jnsv.54.25.
  28. Kinoshita H, Nakagawa K, Narusawa K, Goseki-Sone M, Fukushi-Irie M, Mizoi L et al. A functional single nucleotide polymorphism in the vitamin-K-dependent gamma-glutamyl carboxylase gene (Arg325Gln) is associated with bone mineral density in elderly Japanese women. *Bone.* 2007;40:451-6. doi: 10.1016/j.bone.2006.08.007.
  29. Songpatanasilp T, Chailurkit LO, Chantprasertyothin S, Ongphiphadhanakul B, Taechakraichana N. Effect of GGCC gene polymorphism on the responses of serum undercarboxylated osteocalcin and bone turnover markers after treatment with vitamin K2 (menatetrenone) among postmenopausal Thai women. *J Bone Miner Metab.* 2011;29:606-14. doi: 10.1007/s00774-011-0263-y.
  30. Ishijima M, Sakamoto Y, Yamanaka M, Tokita A, Kitahara K, Kaneko H, Kurosawa H. Minimum required vitamin D level for optimal increase in bone mineral density with alendronate. *Calcif Tissue Int.* 2009;85:398-404. doi: 10.1007/s00223-009-9295-x.

## Original Article

## Vitamin K nutritional status and undercarboxylated osteocalcin in postmenopausal osteoporotic women treated with bisphosphonates

Jun Iwamoto MD<sup>1</sup>, Tetsuya Takada MD<sup>2</sup>, Yoshihiro Sato MD<sup>3</sup>

<sup>1</sup>Institute for Integrated Sports Medicine, Keio University School of Medicine, Tokyo, Japan

<sup>2</sup>Department of Internal Medicine, Hiyoshi Medical Clinic, Kanagawa, Japan

<sup>3</sup>Department of Neurology, Mitate Hospital, Fukuoka, Japan

### 用双磷酸盐治疗的绝经后骨质疏松妇女的维生素 K 的营养状况和未羧化的骨钙素浓度

血清未羧化的骨钙素(ucOC)是治疗初始的绝经后骨质疏松妇女的维生素 K 营养状况的指标。本研究的目的是揭示服用双磷酸盐的绝经后骨质疏松妇女维生素 K 的营养状况和血清 ucOC 的浓度之间的关联。86 位绝经后骨质疏松妇女开始双磷酸盐治疗(年龄范围 47-90 岁)。采用简单的维生素 K 摄入量调查问卷评估维生素 K 的营养状况,治疗 6 个月后测定血清中 ucOC 的浓度。根据简单的维生素 K 摄入量调查问卷评分将患者分为两组:低维生素 K 摄入组(得分 <40, n=60)和正常维生素 K 摄入组(得分 ≥40, n=19)。两组患者的基线参数包括年龄、身高、体重、体质指数、血清碱性磷酸酶(ALP)和尿 N-末端肽交联的 I 型胶原蛋白(NTX),以及 6 个月治疗期间血清 ALP 和尿 NTX 浓度的变化之间没有显著差异。然而治疗 6 个月之后,低维生素 K 摄入组的平均血清 ucOC 浓度(2.79 ng/mL)显著高于正常维生素 K 摄入组(2.20 ng/mL)。这些结果表明,尽管 78%用双磷酸盐治疗的绝经后骨质疏松妇女与正常维生素 K 摄入量的妇女有相似的骨转化降低,但从低维生素 K 摄入量和高血清 ucOC 浓度看出他们可能缺乏维生素 K。

**关键词:** 维生素 K、绝经后妇女、骨质疏松症、未羧化的骨钙素、营养