Association between serum uncarboxylated osteocalcin levels and nutritional intake in Japanese female athletes

Tatsuya Ishizu¹ / Suguru Torii¹,² / Motoko Taguchi¹,²

¹. Waseda Institute of Sports Nutrition, Saitama, Japan
². Faculty of Sport Sciences, Waseda University, Saitama, Japan

INTRODUCTION

Several studies involving athletes have revealed that bone mineral density (BMD) is a critical factor contributing to bone health. Bone strength is defined by two main factors: BMD and bone quality. Bone quality is evaluated based on bone microstructure, metabolism, microfractures, and calcification. Few investigations have studied the relationship between bone quality and nutritional status in Japanese athletes, and further research is needed. The serum uncarboxylated osteocalcin (ucOC) levels are an indicator of bone quality and may be associated with nutritional intake. Previous studies conducted in elderly populations have revealed that dietary vitamin K intake is related to serum ucOC levels.

Vitamin K has multiple roles such as blood coagulation, arterial calcification, and bone metabolism in the human body. Vitamin K has been recognized as a cofactor in the action of vitamin K-dependent carboxylase, which converts glutamic acid to γ-carboxyglutamyl acid residues. Osteocalcin (OC) is secreted by osteoblasts that contain three glutamate residues are γ-carboxylated independent of vitamin K. Carboxylated OC binds to calcium hydroxide phosphate. When dietary vitamin K intake is deficient, OC is not fully carboxylated and circulates as ucOC into the blood. In addition, serum ucOC is not incorporated into the bone matrix, and increased serum ucOC levels indicate vitamin K deficiencies. Therefore, vitamin K directly supports bone metabolism.

Exercise enhances an osteogenic effect, and bone metabolism in athletes is higher than that in more sedentary populations. Furthermore, higher bone metabolism leads to increased production of OC precursors. Increased vitamin K levels are required for normal bone formation in athletes. Vitamin K deficiency in athletes may increase their serum ucOC levels. High serum ucOC levels have been linked with uncoupled bone biomarkers, lower lumbar spine BMD, and a greater risk of hip fractures in the non-athletic population. Consequently, consuming more dietary vitamin K should be considered to avoid bone health concerns. However, the relationship between serum ucOC levels and nutritional intake among female athletes remains unclear. The current study
aimed to determine the relationship between serum ucOC levels and vitamin K intake in female Japanese athletes.

**METHODS**

**Design**

This study conducted a secondary cross-sectional analysis of female athletes (n = 52). Our previous study explained the study design and methods in detail. Data were collected during regular training periods. The inclusion criteria for the study were as follows: a) no medication use that might influence metabolism or hormones, b) no current injury, c) non-smoker, d) no history of fractures in the last six months; e) non-pregnant, f) self-reported regular menstrual cycle (25-38 days), and g) not using oral contraceptives. The current study was approved by the Human Research Ethics Committee of Waseda University (2017-096 and 2020-141) in accordance with the ethical principles of the Declaration of Helsinki.

**Participants**

Female athletes (n = 52) aged 18–25 years participated in this study (rhythmic gymnastics, n = 13; softball, n = 10; field hockey, n = 9; middle- and long-distance running, n = 8; soccer, n = 7; high jump, n = 2; figure skating, n = 2; and race walking, n = 1). Each participant was provided with details regarding the study’s purpose, procedures, and potential risks before providing informed consent.

**Assessment of dietary intake**

This study used 3-day weighed food records using a digital photographic method to analyze the parameters for each participant: energy, nutrition, and food group intake. Food groups were classified into 18 categories (Wellness 21 version.2.85, Top Business System, Okayama, Japan) following the Standard Tables of Food Composition in Japan - 2015 (Seventh Revised Version) were used to analyze the daily dietary intake.

**Blood sampling**

Fasting blood samples were collected to analyze serum ucOC, tartrate-resistant acid phosphatase 5b (TRACP-5b), amino-terminal propeptide of type 1 procollagen (P1NP), insulin-like growth factor (IGF-1), 25-hydroxyvitamin D3, 25(OH)D3, and growth hormone (GH) levels. An electrochemiluminescent immunoassay method was used to evaluate serum ucOC, P1NP, IGF-1, and GH levels. TRACP-5b levels were assessed using an enzyme immunoassay. In addition, liquid chromatography-tandem mass spectrometry was used to analyze 25(OH)D3 levels. Blood samples were collected from all participants, irrespective of their menstrual phase. The blood analyses were outsourced to LSI Medicine Corporation (Tokyo, Japan).

**BMD measurement**

All participants underwent dual-energy X-ray absorptiometry (DXA; Hologic QDR-4500, Hologic Inc., MA, USA) to assess bone mineral content (BMC), BMD, and body Z-score. The Z-score indicates a deviation from the normal age- and sex-matched means beyond the standard deviation (SD). According to the manufacturer, the mean coefficient of variance of the measurements was < 1%.

**Statistical analysis**

IBM SPSS Statistics for Windows v.28 (IBM Japan, Ltd., Tokyo, Japan) was used for statistical analysis. All data were tested for normality using the Shapiro–Wilk test and expressed as mean ± SD. Differences were considered statistically significant at P < 0.05. Dietary vitamin K and vitamin K-related food group intake data were computed using the residual energy-adjusted method. The relationship between serum ucOC levels and several variables (nutritional profile and bone parameters) was explored using Spearman rank correlation analysis. We analyzed the relationship between vitamin K intake and food group consumption. Spearman correlation coefficients (r) were categorized based on Cohen’s score, and values between 0.10 and 0.29 were considered small, between 0.30 and 0.49 as a medium, and between 0.50 and 1.0 as a large association.

**RESULTS**

**Characteristics of participants**

Descriptive characteristics of participants are shown in Table 1. The participants exhibited a healthy BMI range (20.5 ± 1.9 kg/m²). Moreover, the mean whole-body BMD

| Variables                      | Total (n = 52)   |
|--------------------------------|-----------------|
| Age (y)                        | 20.0 ± 1.2      |
| Height (cm)                    | 161.2 ± 5.3     |
| Body weight (kg)               | 53.4 ± 6.2      |
| BMI (kg/m²)                    | 20.5 ± 1.9      |
| Body fat (%)                   | 18.2 ± 3.7      |
| FM (kg)                        | 9.8 ± 2.8       |
| FFM (kg)                       | 43.6 ± 4.1      |
| TRACP-5b (mU/dL)               | 384 ± 138       |
| P1NP (μg/L)                    | 82.8 ± 33.2     |
| ucOC (ng/mL)                   | 5.83 ± 3.28     |
| 25(OH)D3 (ng/mL)               | 21.9 ± 9.1      |
| IGF-1 (ng/mL)                  | 239 ± 56        |
| GH (ng/mL)                     | 7.0 ± 6.1       |
| Whole-body BMC (g)             | 2.167 ± 0.25    |
| Whole-body BMD (g/cm²)         | 1.15 ± 0.08     |
| Whole-body Z-score             | 1.4 ± 1.4       |

*Data are presented as mean ± SD; BMI: body mass index; FM: fat mass; FFM: fat-free mass; BMC: bone mineral content; BMD: bone mineral density.*

**Table 1. Demographic characteristics of the participants.**
Z-scores were 1.4 ± 1.4, whereas three participants had values < -1.0.

Characteristics of dietary intake
The daily nutritional and food intake groups are presented in Table 2. Vitamin K intake was 235 ± 148 µg/day, and approximately 70% (n = 36) of the participants consumed more than the adequate intake (AI) level for vitamin K based on the Dietary Reference Intakes (DRIs) for Japanese 2020.

Correlation analysis
The relationship between serum ucOC levels and nutritional intake is shown in Table 3. Correlation analysis showed a significant negative association between serum ucOC levels and daily intake of calcium (r = -0.596, P < 0.001) and vitamin K (r = -0.388, P = 0.004) after adjusting for energy intake.

We also analyzed the association between serum ucOC levels and food intake. Serum ucOC levels were negatively correlated with the intake of soybean products (r = -0.326, P = 0.018) and vegetables (r = -0.405, P = 0.003) after adjusting for energy intake. We also explored the association between dietary vitamin K intake and food consumption. This analysis showed a significant negative association between dietary vitamin K intake and daily consumption of soybean products (r = 0.543, P < 0.001) and vegetables (r = 0.705, P < 0.001) after adjusting for energy intake. The associations between serum ucOC levels and bone parameters are shown in Table 4. Serum ucOC levels were positively correlated with serum P1NP levels as a bone formation marker (r = 0.512, P < 0.001). No significant medium or large correlations were identified, except for serum PINP levels.

DISCUSSION
This study analyzed the relationship between serum ucOC levels and dietary vitamin K intake in female Japanese athletes. Serum ucOC levels are associated with dietary vitamin K intake, and vegetables and soybean products are good sources of vitamin K in the Japanese diet. This study is the first to determine the relationship between serum ucOC levels and nutritional intake in female athletes.

In this study, serum ucOC levels were significantly correlated with the daily intake of vitamin K (r = -0.388, P = 0.004), soybean products (r = -0.326, P = 0.018), and vegetables (r = -0.405, P = 0.003) after adjusting for energy intake. The natural forms of vitamin K are vitamin K1 (phylloquinone), primarily in green and yellow vegetables, and vitamin K2 (menaquinone), which is contained in fermented foods such as natto. In our study, the total amounts of vitamins K1 and K2 were considered vitamin K intake. The strong positive correlation between vitamin K intake and consumption of soybean products and vegetables observed in this study indicated that these food groups represent the primary sources of vitamin K. The AI level for vitamin K specified in the Japanese DRIs 2020 is the amount required for the carboxylation of glutamate residues, which are blood coagulation factors in the liver. The AI level for vitamin K is set at 150 µg/day for the general Japanese female population aged 18-29 years; however, this value does not consider whether this amount is sufficient to maintain bone health. High serum ucOC indicates insufficient dietary vitamin K intake in bone and risk of osteoporotic fracture independent of BMD. In addition, exposure of bone to high serum ucOC levels may lead to impaired BMD in the future, regardless of sporting activity. Therefore, this study recruited female

| Table 2. Daily nutritional and food group intakes in the participants. |
|-----------------------------|-----------------------------|
| Nutritional variables       | Dietary recommendations* †  |
| Total (n = 52)              |                             |
| Total energy (kcal)         | 1,929 ± 303                 |
| Protein (g/ kg BW)          | 1.4 ± 0.5                   |
| Fat (% of total energy)     | 32.4 ± 6.2                  |
| Carbohydrate (g/ kg BW)     | 4.7 ± 1.1                   |
| Calcium (mg)                | 508 ± 231                   |
| Vitamin K (µg)              | 235 ± 148                   |
| Food group variables        |                             |
| Soybean products (g)        | 45.8 ± 55.6                 |
| Vegetables (g)              | 198.3 ± 115.2               |
| Data are presented as mean ± SD; BW: body weight; a DRIs 2020 for Japanese females aged 18 – 29 years; * tentative dietary goal for preventing lifestyle-related diseases; † recommended dietary allowance; 1 adequate intake; 2 dietary guideline from the American College of Sports Medicine for nutrition and athletic performance. |

| Table 3. Correlation between serum ucOC levels and energy-adjusted nutritional intake. |
|-----------------------------------------------|-----------------------------------------------|
| Serum ucOC (ng/mL)                          | r  | P-value |
| Protein (g/ kg BW)                          | -0.401 | 0.003 |
| Carbohydrate (g/ kg BW)                     | -0.254 | 0.069 |
| Calcium (mg)                                | -0.596 | < 0.001 |
| Vitamin K (µg)                              | -0.388 | 0.004 |
| Data are presented as r (Spearman’s correlation coefficient); BW: body weight. |

| Table 4. Correlation between serum ucOC levels and bone parameters. |
|-----------------------------|-----------------------------|
| ucOC (ng/mL)                | r  | P-value |
| P1NP (µg/L)                 | 0.512 | < 0.001 |
| TRACP5b (mU/dL)             | 0.126 | 0.374 |
| 25(OH)D3 (ng/mL)            | -0.230 | 0.102 |
| IGF-1 (ng/mL)               | 0.231 | 0.099 |
| GH (ng/mL)                  | 0.297 | 0.032 |
| Whole-body BMC (g)          | 0.210 | 0.135 |
| Whole-body BMD (g/cm²)      | -0.039 | 0.784 |
| Whole-body Z-score          | 0.106 | 0.455 |
| Data are presented as r (Spearman’s correlation coefficient). |
athletes who engaged in a variety of sports. Moreover, previous studies have displayed that > 500 µg/day of vitamin K is required for coagulation factor activation in the liver to lower serum ucOC levels in the non-athletic population\textsuperscript{23,24}. Our participants’ average dietary vitamin K intake was 235 ± 148 µg/day, with most participants consuming more vitamin K than the AI level. Nevertheless, since athletes have increased bone metabolic turnover due to daily training\textsuperscript{14}, it can be inferred that the dietary vitamin K intake needed to maintain bone health is higher than that of the Japanese guideline. Therefore, it is necessary to recommend a vitamin K intake that takes into account vitamin K deficiency in the bone. Serum ucOC levels also displayed a significant negative correlation with dietary calcium intake (r = −0.596, P < 0.001). A meta-analysis indicated that the combined effects of vitamin K and calcium intake reduced serum ucOC levels more effectively than vitamin K alone\textsuperscript{25}. Calcium hydroxyapatite crystals are deposited in the type I collagen matrix of bone and enhance bone strength. Calcium utilization in the body is partially regulated by vitamin K, which promotes calcium-bone binding\textsuperscript{26}. As an additional factor in carboxylase activity, vitamin K can support bone formation and OC carboxylation\textsuperscript{27}. These findings indicated that dietary vitamin K and calcium represent essential factors for lowering serum ucOC levels. Consequently, appropriate daily vitamin K intake is essential for maintaining bone health in athletes. Japanese foodstuffs, including soybean products such as natto, tofu, and vegetables such as Japanese mustard spinach and spinach, are relatively rich in dietary vitamin K and calcium. Dietary vitamin K and calcium deficiency may unfavorably influence bone metabolism, resulting in stress fractures and future osteoporosis in athletes. Therefore, it is possible to consume the necessary vitamins and minerals by consuming a variety of foods. Nutritional education is necessary to improve dietary vitamin K and calcium intake in young female athletes to maintain bone health.

Serum ucOC levels predict clinical fractures in post-menopausal Japanese women, and a cutoff value of 4.5 ng/dL of serum ucOC levels can be used for such prediction\textsuperscript{28}. However, no research has been conducted to determine whether a serum ucOC of 4.5 ng/dL should be set as a cutoff value for young female athletes. Therefore, we explored the correlation between serum ucOC levels and bone parameters using exploratory analysis. Among the bone parameters, serum ucOC levels were strongly positively correlated with serum P1NP levels as bone formation markers (r = 0.512, P < 0.001). Kalkwarf et al. (2004)\textsuperscript{19} demonstrated a significant positive association between serum ucOC levels and the bone biomarker levels (bone formation marker BAP and the bone resorption marker cross-linked N-telopeptide of type I collagen (NTx)). Their results indicated increased vitamin K levels are linked to lowered bone biomarker levels (bone resorption and bone formation). Therefore, our participants may have had insufficient dietary vitamin K intake to support bone formation. In contrast, this study found no relationship between serum ucOC levels and the bone resorption marker, serum TRACP-5b levels. This discrepancy may be due to differences in the bone resorption markers used or differences in our sample size. Our study revealed that serum ucOC levels were linked with dietary vitamin K intake in Japanese female athletes. Female athletes may require more vitamin K intake than the general population to maintain bone health, although most participants in this study consumed more vitamin K than the AI level.

In conclusion, our study revealed that serum ucOC levels are linked with dietary vitamin K intake in female Japanese athletes. Female athletes may consume more vitamin K than the general population to maintain bone health, although most participants in this study consumed more vitamin K than the AI level. In addition to vitamin K, calcium intake may be related to serum ucOC levels.

ACKNOWLEDGEMENTS

We wish to thank all participants in this study.

REFERENCES

1. Gibbs JC, Nativ A, Barrack MT, Williams NI, Rauh MJ, Nichols JF, De Souza MJ. Low bone density risk is higher in exercising women with multiple triad risk factors. Med Sci Sports Exerc. 2014;46:167-76.

2. Nativ A, Kennedy G, Barrack MT, Abdelkerim A, Goolsby MA, Arends JC, Seeger LL. Correlation of MRI grading of bone stress injuries with clinical risk factors and return to play: a 5-year prospective study in collegiate track and field athletes. Am J Sports Med. 2013;41:1930-41.

3. Tenforde AS, Parziale AL, Popp KL, Ackerman KE. Low bone mineral density in male athletes is associated with bone stress injuries at anatomic sites with greater trabecular composition. Am J Sports Med. 2018;46:30-6.

4. Nose-Ogura S, Yoshino O, Dohi M, Kigawa M, Harada M, Kawahara T, Osuga Y, Saito S. Low bone mineral density in elite female athletes with a history of secondary amenorrhea in their teens. Clin J Sport Med. 2020;30:245-50.

5. NIH consensus development panel on osteoporosis prevention, diagnosis, and therapy. JAMA. 2001;285:785-95.

6. Taguchi M, Moto K, Lee S, Torii S, Hongu N. Energy intake deficiency promotes bone resorption and energy metabolism suppression in Japanese male endurance runners: a pilot study. Am J Mens Health. 2020;14:155798320905251.

7. Miyamoto T, Oguma Y, Sato Y, Kobayashi T, Ito E, Tani M, Miyamoto K, Nishiwaki Y, Ishida H, Otani T, Matsumoto H, Matsumoto M, Nakamura M. Elevated creatine kinase and lactic acid dehydrogenase and decreased osteocalcin and uncarboxylated osteocalcin are associated with bone stress injuries in young female athletes.
ucOC and nutritional intakes in female athletes

8. Ishizu T, Tori S, Takai E, Miura N, Taguchi M. Japanese female athletes with low energy availability exhibit low multiple food group intake and increased tartrate-resistant acid phosphatase 5b levels: a cross-sectional study. *J Phys Fitness Sports Med.* 2022;11:107-16.

9. 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.

10. Binkley NC, Krueger DC, Engelke JA, Foley AL, Suttle JW. Vitamin K supplementation reduces serum concentrations of under-gamma-carboxylated osteocalcin in healthy young and elderly adults. *Am J Clin Nutr.* 2000;72:1523-8.

11. 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.

12. Price PA, Baukol SA. 1,25-dihydroxyvitamin D3 increases serum levels of the vitamin K-dependent bone protein. *Biochem Biophys Res Commun.* 1981;99:928-35.

13. Sokoll LJ, Sadowski JA. Comparison of biochemical indexes for assessing vitamin K nutritional status in a healthy adult population. *Am J Clin Nutr.* 1996;63:566-73.

14. Banfi G, Lombardi G, Colombini A, Lippi G. Bone metabolism markers in sports medicine. *Sports Med.* 2010;40:697-714.

15. Kalkwarf HJ, Khoury JC, Bean J, Elliot JG. Vitamin K, bone turnover, and bone mass in girls. *Am J Clin Nutr.* 2004;80:1075-80.

16. 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.

17. 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.

18. Standard tables of food composition in Japan - 2015 - (seventh revised version). *Ministry of Education, Culture, Sports, Science and Technology.* 2015.

19. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr.* 1997;65:1220-8.

20. Cohen J. Statistical power analysis for the behavioral sciences. *Elsevier Science & Technology;* 1977, 109-43.

21. Tsugawa N, Uenishi K, Ishida H, Minekami T, Doi A, Koike S, Takase T, Kamao M, Mimura Y, Okano T. A novel method based on curvature analysis for estimating the dietary vitamin K requirement in adolescents. *Clin Nutr.* 2012;31:255-60.

22. Iwamoto J, Takeda T, Uenishi K, Ishida H, Sato Y, Matsumoto H. Urinary levels of cross-linked N-terminal telopeptide of type I collagen and nutritional status in Japanese professional baseball players. *J Bone Miner Metab.* 2010;28:540-6.

23. Binkley NC, Krueger DC, Kawahara TN, Engelke JA, Chappell RJ, Suttle JW. A high phylloquinone intake is required to achieve maximal osteocalcin gamma-carboxylation. *Am J Clin Nutr.* 2002;76:1055-60.

24. Bügel S. Vitamin K and bone health in adult humans. *Vitam Horm.* 2008;78:393-416.

25. Hu L, Ji J, Li D, Meng J, Yu B. The combined effect of vitamin K and calcium on bone mineral density in humans: a meta-analysis of randomized controlled trials. *J Orthop Surg Res.* 2021;16:592.

26. Hamidi MS, Gajic-Veljanoski O, Cheung AM. Vitamin K and bone health. *J Clin Densitom.* 2013;16:409-13.

27. Furie B, Bouchard BA, Furie BC. Vitamin K-dependent biosynthesis of gamma-carboxyglutamic acid. *Blood.* 1999;93:1798-808.

28. Nishizawa Y, Miura M, Ichimura S, Inaba M, Imanishi Y, Shiraki M, Takada J, Chaki O, Hagino H, Fukunaga M, Fujiiwa S, Miki T, Yoshimura N, Ohta H. Executive summary of the Japan osteoporosis society guide for the use of bone turnover markers in the diagnosis and treatment of osteoporosis (2018 edition). *Clin Chim Acta.* 2019;498:101-7.