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*Corresponding author : Motoko Taguchi, Ph. D.

Professor, Faculty of Sport Sciences, Waseda University, 2-579-15 Mikajima, Tokorozawa, Saitama, 359-1192, Japan.

Tel: +81-4-2947-6778

E-mail: mtaguchi@waseda.jp

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

Tatsuya Ishizu[1] / **Suguru Torii**[1,2] / **Motoko Taguchi**[1,2*]

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

[Purpose] The current study aimed to determine the association between serum uncarboxylated osteocalcin (ucOC) levels and dietary vitamin K intake in female Japanese athletes.

[Methods] The nutritional profile and food group intake of 52 Japanese female athletes were investigated using a digital photographic method with data obtained from 3-day dietary records. The food groups were categorized into 18 groups in line with the standard tables of food composition in Japan. Fasting blood samples were collected for serum ucOC levels, and dual-energy X-ray absorptiometry (DXA) evaluated body composition and bone parameters.

[Results] The results showed that dietary vitamin K intake level was 235 ± 148 μg/day, and approximately 70% (n = 36) of participants consumed more than the adequate intake (AI) level, based on the Dietary Reference Intakes for Japanese females aged 18–29. Serum ucOC levels were negatively associated with daily vitamin K intake ($r = −0.388$, $P = 0.004$) and calcium ($r = −0.596$, $P = 0.004$) after adjusting for energy intake.

[Conclusion] Our study revealed that serum ucOC levels were negatively associated with dietary vitamin K intake in female Japanese athletes. Serum ucOC levels reflected dietary vitamin K intake in female athletes. In summary, female athletes consume more vitamin K than the general population to maintain bone health. Furthermore, our results indicated that serum ucOC levels might be linked to dietary calcium intake.

[Keywords] female athletes, bone quality, uncarboxylated osteocalcin, dietary habits, vitamin K, bone health

INTRODUCTION

Several studies involving athletes have revealed that bone mineral density (BMD) is a critical factor contributing to bone health[1-4]. Bone strength is defined by two main factors: BMD and bone quality[5]. Bone quality is evaluated based on bone microstructure, metabolism, microfractures, and calcification[5]. Few investigations have studied the relationship between bone quality and nutritional status in Japanese athletes[6-8], 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[9,10].

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[11]. Osteocalcin (OC) is secreted by osteoblasts that contain three glutamate residues[12]. These 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[13]. In addition, serum ucOC is not incorporated into the bone matrix, and increased serum ucOC levels indicate vitamin K deficiencies[13]. 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[14]. Furthermore, higher bone metabolism leads to increased the 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[15], lower lumbar spine BMD[9], and a greater risk of hip fractures[16,17] 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[8]. 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 following parameters for each participant: energy, nutrition, and food group intake. Food groups were classified into 18 categories identified by the Ministry of Education, Culture, Sports, Science, and Technology[18]. The nutritional calculation software (Wellness 21 version.2.85, Top Business System, Okayama, Japan) following the Standard Tables of Food Composition in Japan - 2015 (Seventh Revised Version)[18] was 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 D_3 [25(OH)D_3], 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)D_3 levels. Blood samples were collected from all participants, irrespective of their menstrual phase. The blood analyses were outsourced to LSI Medience 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[19]. 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 dietary vitamin K intake and food group consumption. Spearman correlation coefficients (r) were categorized based on Cohen's score[20], 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/m^2). Moreover, the mean whole-body BMD

Table 1. Demographic characteristics of the participants.

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/m^2)	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)D_3 (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 ± 250
Whole-body BMD (g/cm^2)	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.

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.

Table 2. Daily nutritional and food group intakes in the participants.

	Total (n = 52)	Dietary recommendations[a, †]
Nutritional variables		
Total energy (kcal)	1,929 ± 393	-
Protein (g/ kg BW)	1.4 ± 0.5	1.2 – 2.0[†]
Fat (% of total energy)	32.4 ± 6.2	20 – 30[*]
Carbohydrate (g/ kg BW)	4.7 ± 1.1	6.0 – 10.0[†]
Calcium (mg)	508 ± 231	650[**]
Vitamin K (µg)	235 ± 148	150[***]
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; *** adequate intake; † 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)D_3 (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/cm^2)	-0.039	0.784
Whole-body Z-score	0.106	0.455

Data are presented as r (Spearman's correlation coefficient).

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 K_1 (phylloquinone), primarily in green and yellow vegetables, and vitamin K_2 (menaquinone), which is contained in fermented foods such as natto. In our study, the total amounts of vitamins K_1 and K_2 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[21,22]. 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 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[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[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[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[26]. As an additional factor in carboxylase activity, vitamin K can support bone formation and OC carboxylation[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 postmenopausal Japanese women, and a cutoff value of 4.5 ng/dL of serum ucOC levels can be used for such prediction[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)[15] 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 1 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.

Our study had two limitations. First, a causal relationship was unidentified due to the cross-sectional nature of the study. Longitudinal follow-up is necessary to determine the effects of high serum ucOC levels on BMD and stress fracture occurrence in athletes. Second, the sample size was small. More studies are required to assess the relationship between serum ucOC levels and vitamin K intake in larger samples of female and male athletes.

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.

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