

The Affect of Lifestyle on Bone Mineral Density and Bone Turnover in Young Women

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Received 6 September 2019/ Accepted 11 November 2019

Keywords: Bone mineral density, bone turnover, vitamin D, vitamin K, young women

Introduction: This research aimed to evaluate the effect of lifestyle factors such as nutrient intake and physical activity on bone mineral density (BMD) and bone turnover in young women. **Materials and Methods:** BMD was assessed using Quantitative Ultrasound; lifestyle-related factors such as dietary habits, and physical activity were examined using questionnaires in 194 female college students. The biochemical markers of bone turnover were measured in the Osteopenia (BMD below the Young Adult Mean [YAM] -1.0SD, 16 subjects) and Normal (above the YAM-1.0SD, 31 subjects) groups. **Results:** The percentage of osteopenia was 11.9%. Calcium and magnesium intake ($p<0.05$), and physical activity ($p<0.1$) were found to be factors influencing BMD. The level of osteocalcin and type 1 procollagen N-terminal propeptide (P1NP) were higher in the Osteopenia group than in the Normal group ($p<0.05$). There was tendency that showed relationship between the level of undercarboxylated osteocalcin (ucOC) and BMD ($p<0.1$). The level of bone-specific alkaline phosphatase was significantly higher in the 25OH vitamin D insufficiency group compared to sufficiency group ($p<0.05$). The levels of OC, tartrate-resistant acid phosphatase-5b and P1NP were lower in the ucOC <4.5 ng/ml group compared to ≥ 4.5 ng/ml group ($p<0.01$, $p<0.05$, $p<0.1$), respectively. **Conclusion:** This study showed that BMD in young women is affected by calcium and magnesium intake, physical activity, and vitamin D and K levels. It was suggested that the insufficiency of vitamin D and K might be contributable to low BMD through the change of bone turnover.

INTRODUCTION

It is known that bone mass in women reaches its peak in the latter half of the teens and then maintains a steady level between the twenties and thirties, declining thereafter [4,15,22]. While osteoporosis develops during and after menopause due to the sudden decrease in estrogen levels [6], maximizing and preserving bone mineral density (BMD) are highly important for primary prevention of osteoporosis.

Young generation might undergo drastic changes in terms of lifestyle and social environment. Several recent studies have showed that BMD is lower in young women who follow extreme dietary practices, consume an unbalanced diet, lack regular exercise, and exhibit abnormal menstruation patterns. Tonnesen et al. indicated that exercise levels in the previous 7 days were positively associated with the BMD [24]. Considering the nutrition aspects, vitamin D insufficiency remain a worldwide issue. 25OHvitamin D (25OHVD) is the major circulating form of vitamin D present in the blood and has been shown to be linked to measures of bone health [16,28].

However, to the best of our knowledge, no study has evaluated the nutritional status by studying intake and blood concentration, measuring bone turnover, and combining analysis of both lifestyle and bone turnover in otherwise healthy young women with low BMD. Moreover, very few studies have analyzed the effect of physical activity (encompassing overall daily activity) on BMD and bone turnover in young women.

Therefore, in this study, we comprehensively and objectively examined lifestyle-related factors such as nutrient intake and physical activity in healthy young women, and investigated which among these factors influenced BMD and bone turnover.

MATERIALS AND METHODS

Subjects

A total of 199 female college students from K University (faculty of health science) and S University (school of nursing, cultures, and environmental science) were enrolled in this study. We explained the purpose and methods of the study to the subjects and obtained informed consent from them before the study started. Five

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subjects who had pre-existing disease or were taking medications (steroid and hormone agents) that could affect BMD were excluded from this study. The remaining 194 subjects were included in the analysis.

Methods

1. Questionnaires

The self-administered questionnaires comprised questions concerning demographic characteristics such as body type (age, height, weight, body mass index [BMI], menstrual cycle, history of fracture, exercise habits during junior and senior high school) and lifestyle-related factors such as history of losing weight, changes in the menstrual cycle during losing weight, eating habits (frequency of breakfast, lunch, and dinner, regularity of diet, and nutrient intake), physical activity, and period of sunlight exposure (total time spent outdoors). A regular menstrual cycle was defined as being between 25 to 38 days, with a variability <7 days. The frequency of every meal (breakfast, lunch, dinner) over a week were evaluated at four levels (eat almost every day/four or five times a week/two or three times a week/not eating). The regularity of diet was defined as consuming a meal in the same time zone every day.

The nutrient intake (vitamin D, vitamin K, calcium [Ca], magnesium [Mg], zinc [Zn], and phosphorus [P]) was measured using the Brief-type self-administered Diet History Questionnaire (BDHQ) [13]. The BDHQ is a questionnaire that asks about the consumption frequency of selected food to estimate the dietary intake of 58 food and beverage items during the preceding month. The insufficiency of nutrient intake was diagnosed based on the criteria in Japan [18].

The physical activity was measured using the International Physical Activity Questionnaire (IPAQ) short version [3]. Insufficient physical activity was defined as <3Mets×60minutes per day [11].

2. BMD measurement

The BMD of the right calcaneus of all subjects was measured using Quantitative Ultrasound (QUS) (Hitachi, Japan, AOS-100SA).

The evaluation of BMD was based on the criteria provided by the World Health Organization [26]. Based on the results of BMD measurements, the subjects were divided into 2 groups: the Osteopenia group, which included those with a BMD below the Young Adult Mean (YAM) -1.0 SD, T-score, $x < 89$, and the Normal group, which included those with a BMD above the YAM-1.0 SD, T-score, $x \geq 89$.

3. Measurement of biochemical markers of bone turnover

For bone formation markers, osteocalcin (OC) and type 1 procollagen N-terminal propeptide (P1NP) in plasma were measured using electro chemiluminescence immunoassay (ECLIA) and bone specific alkaline phosphatase (BAP) was measured using chemiluminescent enzyme immunoassay. Regarding absorption markers, tartrate-resistant acid phosphatase-5b (TRACP-5b) was measured using enzyme immunoassay. About determinant hormone and bone turnover nutrient-related factors, parathyroid hormone (PTH) and undercarboxylated osteocalcin (ucOC) were measured using ECLIA, and 25OHVD was measured using radioimmunoassay. Ca and P levels were measured using the Arsenazo III and Molybdate direct method, respectively. Based on the results of BMD, the biochemical markers of bone turnover were measured in the Osteopenia group (16 subjects who approved blood test out of all subjects with low BMD) and the Normal group (31 subjects whose BMI matched with those of 16 subjects). We further divided the subjects into groups based on the 25OHVD (<20 ng/ml group vs. ≥20 ng/ml), and ucOC (<4.5 ng/ml vs. ≥4.5 ng/ml) levels.

The BMD and bone turnover were compared between groups based on background and lifestyle habits (BMI; underweight/normal weight/overweight, menstrual cycle; regular/abnormal, history of fracture; yes/no, past exercise habits; junior high school; yes/no, past exercise habits; senior high school; yes/no, history of losing weight; yes/no, changes in the menstrual cycle during losing weight; yes/no, the frequency of each meal intake; eat almost every day/four or five times a week/two or three times a week/not eating, regularity of diet; good/bad, intake of each nutrient; sufficiency/insufficiency, physical activity; sufficiency/insufficiency, period of sunlight exposure; ≥15 minutes/<15 minutes). The bone turnover was compared between the Osteopenia and Normal BMD groups. Nutrient intakes were compared among each group based on the lifestyle habits.

Statistical analyses

All statistical analyses were carried out using the analysis software IBM SPSS Statistics 18 for Windows. In the descriptive analysis of participant characteristics, numerical data were expressed as median. Differences in the distribution of baseline characteristics were tested using the Mann-Whitney U test (compares 2 groups) or Kruskal-Wallis test (compares 3 groups or more). Significance was defined as a *p* value <0.05. Tendency was defined as 0.05 < *p* value <0.1.

Ethical Considerations

The study protocol was approved by the ethics committees of Kobe University Graduate School of Health Sciences and University of Shiga Prefecture (No. 477).

RESULTS

Questionnaire results

1. Characteristics of subjects (Table I)

Among the subjects, 64.4% (125/194) and 35.6% (69/194) were studying nursing and other subjects. The median of age was 20 (from 18 to 25) years. The median of BMI was 20.3 (from 15.1 to 28.5) kg/m². A total of 39.7% (77/194) of the subjects had abnormal menstrual cycle. Regarding exercise habits, 66.0% (128/194) of the subjects reported regular exercise habits in junior high school, while 55.2% (87/194) reported the regular exercise habits in during senior high school.

		n	%	Median(Minimum-Maximum)
Age (years)				20.0 (18.0-25.0)
Height (cm)				158.0 (145.8-171.7)
Weight (kg)				51.3 (35.5-68.0)
BMI (m/kg ²)	Underweight	25	12.9	} 20.3 (15.1-28.5)
	Normal weight	164	84.5	
	Overweight	5	2.6	
Menstrual cycle	Normal	111	57.2	
	Abnormal	77	39.7	
History of fracture	Yes	44	22.7	
	No	127	65.5	
Past exercise habits ; Junior high school	Yes	128	66.0	
	No	66	34.0	
Past exercise habits ; High school	Yes	87	55.2	
	No	107	44.8	

2. Lifestyle habits (Table II)

The percentage of subjects with a history of weight loss was 24.2% (47/194); among these, 27.7% (13/47) reported menstrual cycle changes during losing weight. The percentages of subjects who ate breakfast almost every day, four or five times a week, two or three times a week, and not at all were 72.7% (141/194), 13.4% (26/194), 8.8% (17/194), and 5.2% (10/194), respectively. Among the subjects, 54.1% (105/194) had regular eating habits. Vitamin D, vitamin K, Ca, and Mg intake per day were 6.8 (from 0.2 to 25.0) µg, 185.1 (from 26.4 to 789.9) µg, 380.3 (from 102.4 to 1193.5) mg, and 163.7 (from 59.6 to 446.2) mg respectively. The physical activity was 137.1 (from 0.0 to 3039.4) Mets·min. The period of sunlight exposure per day was 60.0 (from 0.0 to 480.0) min.

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Table II. Lifestyle habits		n=194	
		n	%
		Median (Minimum-Maximum)	
Dietary intake			
History of losing weight	Yes	47	24.2
	No	147	75.8
Changes in the menstrual cycle during losing weight	Yes	13	6.7
	No	32	16.5
Breakfast intake	Eat almost every day	141	72.7
	Eat 4 or 5 times a week	26	13.4
	Eat 2 or 3 times a week	17	8.8
	Not eating	10	5.2
Lunch intake	Eat almost every day	189	97.4
	Eat 4 or 5 times a week	4	2.1
	Eat 2 or 3 times a week	1	0.5
	Not eating	0	0.0
Dinner intake	Eat almost every day	175	90.2
	Eat 4 or 5 times a week	16	8.2
	Eat 2 or 3 times a week	3	1.5
	Not eating	0	0.0
Regularity of diet	Good	105	54.1
	Bad	87	44.8
Nutrients intake	Vitamin D (µg/day)	6.8	(0.2-25.0)
	Vitamin K (µg/day)	185.1	(26.4-789.9)
	Ca (mg/day)	380.3	(102.4-1193.5)
	Mg (mg/day)	163.7	(59.6-446.2)
	P (mg/day)	772.6	(253.2-1738.5)
	Zn (mg/day)	6.3	(1.3-16.0)
Physical activity (Mets·min/day)		137.1	(0.0-3039.4)
Period of sunlight exposure (min/day)		60.0	(0.0-480.0)

BMD and biochemical markers of bone turnover (Table III)

The median of BMD was 103 (from 78 to 184) %. There were 23 subjects with BMD <89% (11.9%).

The levels of OC, P1NP, BAP and TRACP-5b were 17.3 (from 4.0 to 45.1) ng/ml, 54.1 (from 19.9 to 180.0) ng/ml, 12.7 (from 7.5 to 40.0) µg/l, and 203.5 (from 14.6 to 433.0) mU/dl, respectively. The PTH level was 39.0 (from 15.0 to 116.0) pg/ml. The levels of 25OHVD, ucOC, Ca, and P were 19.0 (from 13.0 to 25.0) ng/ml, 4.1 (from 0.4 to 16.7) ng/ml, 9.3 (from 8.6 to 10.3) mg/dl and 4.0 (from 3.0 to 4.7) mg/dl, respectively. The proportion of subjects whose 25OHVD level was <20 ng/ml was 57.4% (27/47), while that of subjects whose ucOC level was ≥4.5 ng/ml was 38.3% (18/47).

Table III. Bone mineral density, biochemical markers of bone turnover

		Median (Minimum-Maximum)	
Bone mineral density (n=194)			
BMD (%)	103.0	(78.0-184.0)	
	Normal	171 (88.1%)	
	Osteopenia	23 (11.9%)	
Biochemical markers of bone turnover (n=47 ※n=46)			
OC (ng/ml)	17.3	(4.0-45.1)	
P1NP (ng/ml)	54.1	(19.9-180.0)	
BAP (µg/l) ※	12.7	(7.5-40.0)	
TRACP-5b (mU/dl) ※	203.5	(14.6-433.0)	
PTH (pg/ml)	39.0	(15.0-116.0)	
25OHVD (ng/ml)	19.0	(13.0-25.0)	
ucOC (ng/ml)	4.1	(0.38-16.7)	
Ca (mg/dl) ※	9.3	(8.6-10.3)	
P (mg/dl)	4.0	(3.0-4.7)	

Factors affecting BMD (Table IV)

There were significant differences in BMD among the three BMI groups ($p < 0.05$). The BMD of the underweight group was the lowest, and that of the overweight group was the highest. The subjects in the group with regular exercise habits in the past had a significantly higher BMD compared to the group without regular exercise ($p < 0.01$). Significant differences in BMD were seen between the sufficient and the insufficient groups regarding the following nutrients; Ca (108.0% vs. 102.0%, $p < 0.05$) and Mg (108.0% vs. 102.0%, $p < 0.05$). The BMD tended to be higher in the sufficient Zn intake group than in the insufficient Zn intake group (106.0% vs. 101.0%, $p < 0.1$). The BMD tended to be higher in the group with sufficient physical activity than in the insufficient physical activity group (106.0% vs. 102.0%, $p < 0.1$).

There were no significant relationships between BMD and menstrual cycle, history of fracture, history of losing weight, the change of menstrual cycle during losing weight, frequency of each meal intake, regularity of diet, and periods of sunlight exposure.

Table IV. Factors affecting BMD

Characteristics of subject	n	BMD	
		Median (Minimum-Maximum)	p score
BMI	Underweight	25	96.0 (78.0-117.0)
	Normal weight	164	103.0 (84.0-184.0)
	Overweight	5	107.0 (104.0-173.0)
Past exercise habits ; Junior high school	Yes	128	106.0 (78.0-173.0)
	No	66	97.0 (84.0-184.0)
Past exercise habits ; High school	Yes	87	107.0 (86.0-138.0)
	No	107	99.0 (78.0-184.0)
Lifestyle habits			
Nutrients intake			
Ca	Sufficiency	23	108.0 (86.0-184.0)
	Insufficiency	171	102.0 (78.0-173.0)
Mg	Sufficiency	24	108.0 (86.0-184.0)
	Insufficiency	170	102.0 (78.0-173.0)
Zn	Sufficiency	55	106.0 (86.0-184.0)
	Insufficiency	139	101.0 (78.0-134.0)
Physical activity	Sufficiency	81	106.0 (86.0-138.0)
	Insufficiency	113	102.0 (78.0-184.0)

* $p < 0.05$ ** $p < 0.01$

Factors affecting the bone turnover

The serum level of BAP was significantly higher in the group that showed menstrual cycle changes during losing weight, than in the group without changes ($p < 0.05$). The P1NP level tended to be lower in the group that showed menstrual cycle changes during losing weight, than in the group without changes ($p < 0.1$). The P1NP level was significantly lower in the group with regular exercise habits in the past than in the group without the same ($p < 0.05$). The OC and TRACP-5b levels were significantly lower in the group with sufficient physical activity than in the group without ($p < 0.05$).

The 25OHVD level was significantly lower in the group that showed menstrual cycle changes during losing weight, than in the group without changes ($p < 0.05$). There were no significant relationships between the 25OHVD level and the intake of vitamin D and periods of sunlight exposure.

Correlations between BMD and biochemical markers of bone turnover

Table V shows the correlations between BMD and biochemical markers of bone turnover. The OC and P1NP levels were higher in the osteopenia group than in the normal BMD group ($p < 0.05$). The serum level of ucOC tended to be higher in the osteopenia group than in the normal BMD group (4.5 ng/ml vs. 3.7 ng/ml, $p < 0.1$).

Table V. Correlation between BMD and biochemical markers of bone turnover

BMD	n	OC		BAP		P1NP		TRACP-5b		25OH vitaminD		ucOC	
		Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score
Normal	31	16.4 (4.0-31.9)	.02*	12.7 (7.5-17.9)	.95	52.3 (19.9-89.0)	.02*	204.0 (14.6-433.0)	.75	19.0 (13.0-25.0)	.11	3.7 (0.4-9.6)	.06
Osteopenia	16	19.2 (13.9-45.1)		12.7 (7.7-40.0)		60.8 (41.3-180.0)		200.0 (116.0-387.0)		17.5 (14.0-22.0)		4.5 (3.3-16.7)	

* $p < 0.05$

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Correlations between bone turnover and nutrient-related factors

Table VI shows the correlations between biochemical markers of bone turnover and 25OHVD and ucOC levels. The level of BAP was significantly higher in the group with 25OHVD <20 ng/ml than in the group with ≥20 ng/ml (13.7 μg/l vs. 12.1 μg/l, $p<0.05$). The levels of OC and TRACP-5b were significantly lower in the group with ucOC <4.5 ng/ml to ≥4.5 ng/ml (14.2 ng/ml vs. 21.2 ng/ml, $p<0.01$, 177.0 mU/dl vs. 222.0 mU/dl, $p<0.05$, respectively). The level of P1NP tended to be lower in the group with ucOC <4.5 ng/ml than in the group with ≥4.5 ng/ml (51.0 ng/ml vs. 58.9 ng/ml, $p<0.1$).

Table VI. Correlations between bone turnover and nutrient-related factors

	n	OC		BAP		P1NP		TRACP-5b	
		Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score
25OHVD	less than 20ng/ml	27	16.9 (4.0-45.1)	13.7 (7.7-40.0)	55.2 (19.9-180.0)	206.5 (14.6-433.0)			
	20ng/ml or more	20	18.7 (11.4-24.0)	12.1 (7.5-17.6)	12.1 (7.5-17.6)	203.5 (134.0-336.0)	.13	.15	.53
ucOC	less than 4.5ng/ml	29	14.2 (4.0-19.7)	12.8 (7.5-17.9)	51.0 (19.9- 95.2)	177.0 (16.6-280.0)			
	4.5ng/ml or more	18	21.2 (16.4-45.1)	12.5 (9.9-40.0)	58.9 (39.1-180.0)	222.0 (134.0-433.0)	.00**	.08	.01*

* $p<0.05$ ** $p<0.01$

Eating habits and nutrient intake (Table VII)

A higher frequency of breakfast was seen to result in higher intake of vitamin K, Ca, Mg, P, and Zn ($p<0.01$, $p<0.01$, $p<0.05$, $p<0.05$, $p<0.05$, respectively). The subjects in the group with regular eating habits showed a significantly higher intake of vitamin D, P, and Zn than the subjects in the group without regular eating habits ($p<0.05$, $p<0.05$, $p<0.05$, respectively). The Ca, Mg, and vitamin K intake tended to be higher in the group with regular eating habits than in the group without regular eating habits ($p<0.1$).

Table VII. Eating habits and nutrients intake

	n	VitaminD intake		VitaminK intake		Ca intake		Mg intake		P intake		Zn intake	
		Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score	Median (Minimum-Maximum)	p score
Breakfast intake													
Eat almost every day	141	6.7 (0.3-25.0)		210.7 (29.8-789.9)		407.3 (102.4-1193.5)		173.3 (69.8-446.2)		795.6 (276.5-1738.5)		6.6 (2.3-16.0)	
Eat 4 or 5 times a week	26	9.3 (0.2-19.5)	.58	155.4 (45.4-626.8)		351.0 (119.8-1143.9)		167.5 (84.7-357.3)		775.0 (421.7-1660.4)		6.2 (3.0-12.6)	
Eat 2 or 3 times a week	17	7.2 (1.2-14.2)		126.1 (31.3-125.7)	.01**	258.9 (141.1-622.6)	.00**	141.5 (80.4-310.8)	.03*	626.6 (360.6-1473.6)	.02*	5.2 (2.9-13.9)	.02*
Not eating	10	4.8 (0.3-24.7)		125.7 (26.4-355.3)		236.2 (106.8-537.4)		123.2 (59.6-301.4)		567.1 (253.2-1147.4)		5.3 (1.3-8.7)	
Regularity of diet													
Good	105	7.2 (0.2-25.0)	.02*	210.7 (29.8-725.6)	.06	402.4 (119.8-1193.5)	.08	173.4 (75.3-446.2)	.07	823.4 (276.5-1738.5)	.03*	6.6 (2.3-12.7)	.03*
Bad	87	5.8 (0.3-24.7)		173.3 (26.4-789.9)		339.4 (106.8-1143.9)		158.3 (59.6-386.9)		700.4 (253.2-1736.7)		5.8 (1.3-16.0)	

* $p<0.05$ ** $p<0.01$

DISCUSSION

We demonstrated in this study that BMI, past exercise habits (when subject were in junior or senior high school), nutrition intake (Ca and Mg), and physical activity affect BMD, and the prevalence of osteopenia in our subjects was 11.9%. Furthermore, it was suggested that the insufficiency of vitamin D and K might be contributable to low BMD through the change of bone turnover in young women. The new finding is the insufficiency of both of vitamin D and vitamin K related to a high bone turnover rate in young women.

The rate of osteopenia in university women students is 11.9% in this study, which may be a little bit higher than that seen in a previous study [27]. There is a possibility that recent remarkable change of lifestyle may be one of factors reducing the BMD among young women. The fact that about 12% of young women already have low BMD and that their bone mass decreases by roughly 20% after menopause [23] may result in high prevalence of osteoporosis in future. Therefore, it is an urgent health concern to examine whether recent changes in lifestyle affect BMD or bone turnover in young women.

This study demonstrated that BMI and regular exercise habits in the past significantly affected BMD in young women and there was a tendency that showed a relationship between physical activity and BMD. Physical activity levels in this study refer not only to intentional exercise, but also to the total amount of movement activity in one's daily life. Miyahara et al. indicated that current total energy to be quantified in term of METs·h/day expenditure significantly contributed to the BMD [20]. Callegari et al. reported that moderate to high intensity physical activity is effective in increasing BMD [1]. Moderate intensity physical activity includes familiar daily life movements, such as normal walking for shopping or pleasure, cleaning, descending stairs, and riding a bicycle. In Japan, only 11.6% young women had regular exercise habits [17]. We believe that even a moderate increase in regular movement in daily life might help increase the BMD. It has been reported that young women whose current activity levels were high enjoyed physical education in school more than those with low activity levels [2]. It is essential that health personnel and educators should promote the creation of physical activity encompassing overall daily life in elementary, junior, and senior high school students.

Significant correlations were observed between the intake of Ca and Mg and BMD. Although correlation of Ca and Mg intake with BMD has been reported [5,10,12,28], there are few reports of the correlation of Mg intake with BMD in young women. It was indicated that the intakes of these nutrients were below the normal range in young women [17]. Insufficient intake of Ca and Mg contributes to low BMD among young women.

The association between nutrient intake and BMD has been shown in many studies, however, the correlations among the intake and blood concentrations of various nutrients, and their effects on BMD and bone turnover are less well known, especially regarding vitamin D and vitamin K. We showed that the serum concentrations of 25OHVD and ucOC were related to bone turnover and BMD. The deficiency of both of vitamin D and vitamin K was seen to lead to a high bone turnover rate in this study. Furthermore, ucOC level tended to affect BMD. The level of 25OHVD didn't relate to BMD directly. However, 25OHVD related to bone turnover. Moreover, bone turnover related to BMD. The continuous acceleration of bone turnover may result in the decrease of BMD subsequently. Therefore, we considered 25OHVD related to BMD indirectly. Vitamin D is not only supplied from dietary intake, but also from exposing the skin to the sun. Approximately 90 % of the vitamin D in the body is produced in the skin [14], and it has been calculated that even in summer, about 15 minutes of exposure to the sun is necessary[25]. Low serum 25OHVD concentration leads to a decrease in calcium absorption and increased PTH secretion, which may result in accelerated bone formation and absorption [9]. Ohta *et al.* demonstrated a significant positive correlation between vitamin D intake and the level of 25OHVD; the vitamin D intake threshold for maintaining 25OHVD levels at 20 ng/mL or higher was 11.6 µg/day or greater [21]. The median of level of 25OHVD in this study was 19.0 ng/ml, which is less than 20 ng/ml. The median of vitamin D intake from food products in this study was 6.8 µg per day, which was higher than the intake described in the Japanese Standards (5.5 µg per day) [19]. These results imply that we need to reconsider the intake standards for bone health.

There was a correlation between ucOC levels and BMD. Vitamin K is the cofactor for the enzyme that converts ucOC to OC. OC is synthesized by osteoblasts during the mineralization phase of bone formation [8]. In this study, subjects with high ucOC seem to be high OC. It is possible that vitamin K insufficiency invited high bone absorption, further high bone absorption invited high bone formation.

We found correlations between the intake levels of vitamin D and K, Ca, and Mg, and breakfast and eating habit rhythms. Gibney *et al.* reported that breakfast contributes 16 to 20 % of the daily energy, 1.0 to 55.8 % of vitamin D, 22.1 to 35.7 % of Ca, and 15.0 to 35.8 % of Mg intake [7]. It is very important that health professionals and educators teach young women the healthy habits such as eating breakfast and regular meals.

In conclusion, this research showed that BMD in young women was affected by Ca and Mg intake, physical activity, and the levels of vitamin D and K. Furthermore, it was suggested that insufficiency of vitamin D and K might be contribute to low BMD by affecting bone turnover. It is essential that health professionals and educators teach young women how to prevent osteopenia and promote bone health: eating breakfast, regular dietary habits and physical activity (encompassing overall daily activity).

ACKNOWLEDGMENTS

This work was supported in part by Grant-in-Aid for Scientific Research 16K20797 from the Japanese Ministry of Education, Science, and Culture.

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