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# Bone Mineral Density and Bone Turnover among Young Women in Chiang Mai, Thailand

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**The present study was carried out to investigate the influence of lifestyle on bone mineral density (BMD) and bone turnover among young women in Chiang Mai, Thailand. A total of 177 young women affiliated with Chiang Mai University hospital were enrolled. Firstly, Questionnaires about their lifestyle and the Osteoporosis Knowledge Test (OKT) were examined. The measurement of BMD was assessed by Quantitative Ultrasound (QUS). Secondly, Based on the measurement of BMD, the subjects were divided into 2 groups, a Low BMD group (L group: less than YAM-1.0SD) and a Normal BMD group（N group: more than YAM-1.0SD）. L group ( n= 23) and N group ( n= 23) were examined using Osteocalcine (OC), type 1 collagen cross-linked N-telopeptide (NTx) and undercarboxylated osteocalcin (ucOC) as bone turnover markers, and serum Ca, 1,25-$(OH)_2$VitaminD, VitaminK1 and Vitamin K2(MK-4) as bone turnover related factors. Based on the results, the percentage of Low BMD group was 23.2%. Concerning lifestyle and BMD, the BMD of the low cheese intake group was 99.7±17.0 and the BMD of the high cheese intake one was 110.0±23.3 ($p < 0.05$). The BMD of the fracture experience group was 82.5±11.6 and the BMD of no-fracture group was 103.3±19.6 ($p < 0.05$). There were significant differences in ucOC and 1,25-$(OH)_2$VitaminD between L and N groups ($p < 0.05$). It was suggested that BMI, food and fracture experience might affect BMD level and suppression of bone formation might have contributed to the low BMD group among young women in Chiang Mai, Thailand.**

## INTRODUCTION

It is known that bone mass in women reaches its peak in the latter half of their teens and thereafter maintains a steady level between their twenties and thirties (5,21,31). It has been postulated that the future risk of osteoporosis declines as maximum peak bone mass increases (2,12,23). Recent studies have shown that bone mineral density (BMD) is lower in young women who follow extreme dietary practices, consume an unbalanced diet, lack regular exercise and exhibit abnormal menstruation patterns (4). Maximizing the attainment of optimal BMD is a potential primary prevention strategy for osteoporosis (20, 21).

Hip fracture is a major public health problem in Asia. It has been projected that by next century, 50% of all hip fractures in the world will occur in Asia (11,13,19,33). Recently, the prevalence of osteoporosis has been increasing in Thailand (1,24). In a nation-wide survey taken during 2000-2001, the age–adjusted prevalence of osteoporosis in Thailand ranging in age from 40-80 years was 13.6% and 19.8% for femoral neck and lumbar spine, respectively (28). The average daily calcium intake was demonstrated to be 265mg/day (29,30). Nippawan studied the self-efficacy of education on the primary prevention of osteoporosis, which involved education on both exercise and calcium intake among Thai nursing students (26, 27), and found that self-efficacy was important for education.

It is therefore believed that a healthy lifestyle contributes to maximum BMD. No studies, however, have been conducted on the relationship between lifestyle and bone turnover in otherwise healthy young women with low BMD. We therefore analyzed a number of lifestyle–related factors among healthy young women in Chiang Mai, northern Thailand, in order to determine which factors influenced BMD, and measured the blood levels of bone turnover-related factors to determine the characteristics of bone turnover in young women presenting with low BMD.

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## SUBJECTS AND METHODS

### 1. Subjects

A total of 177 healthy young women between the ages of 20 and 30 who provided informed consent participated in the study. All subjects were affiliated with Chiang Mai University hospital, in northern Thailand, and worked as nurses and administrative employees, or were nursing and medical students undergoing practical training. Subjects who presented with disease and or were taking medications that could affect BMD were excluded from the study. All enrolled subjects had their BMD measured for the first time in this study.

### 2. Methods

#### 1) Questionnaires

The self-administered questionnaires consisted of questions concerning demographic characteristics such as body type (age, height, weight, BMI) and occupation (University student, medical worker), lifestyle-related factors such as eating habits (milk, cheese, yogurt, fish, meat, tofu, beans, vegetable, calcium supplements), diet history (age of initiation, frequency, weight loss), exercise experience (regularity, duration, weight-bearing exercise while engaged in walking), menstruation (age of menarche, menstruation cycle), disease (past medical history, past history of bone fracture), and other items (family history of osteoporosis, history of pregnancy, lactation, smoking, alcohol, coffee). The questionnaires were written in both Japanese and English, and the English version was translated into the Thai language by a Thai/English bilingual expert. Subjects were free to refuse participation in the study.

#### 2) Osteoporosis knowledge test (OKT)

Osteoporosis knowledge was measured by the modified version of the Osteoporosis Knowledge Test (OKT). The OKT (Kim, Horan, Gebder,& Pastel,1991) is a 24-item questionnaire that measures knowledge of risk factors and strategies for osteoporosis prevention. The OKT has three subscales: risk factors of osteoporosis, calcium intake and exercise. The OKT score was calculated as the sum of these scores, ranging between 0 (all incorrect) and 26 (all correct).

#### 3) BMD measurement

The BMD of calcaneus of all subjects was measured using Quantitative Ultrasound (QUS) (GE-HEALTHCARE, USA) and Osteoreport version 5.12, Data Record Program.

Based on the results of BMD measurements, the subjects were divided into 2 groups: Low BMD group, defined as below YAM-1.0SD T-score, $x < 89$ (YAM; Young Adult Mean), and a Normal BMD group, defined as above YAM-1.0SD, $x \geqq 89$. The Low BMD group was further divided into 2 groups: Low BMD 1 ($x < 80$) and Low BMD 2 ($x \geqq 80$).

#### 4) Measurement of bone turnover markers and related factors

For bone formation markers, Osteocalcine (OC) in plasma was measured using RIA; for bone absorption markers, type 1 collagen cross-linked N-telopeptide (NTx) was measured using ELISA; and for bone turnover-related factors, undercarboxylated osteocalcin (ucOC) was measured also using ELISA. Ca serum level was measured using ArnenazoⅢ, 1,25-$(OH)_2$Vitamin D using RIA, and Vitamin K1 and Vitamin K2 (MK-4) using HPLC.

Bone turnover markers and bone turnover-related factors were compared among three groups of subjects, Low BMD 1 (n=8) and Low BMD 2 (n=15), respectively, were assigned and matched with the control group (n=23) ranging selected subjects whose BMD was 88% or more of the YAM (Normal BMD group).

#### 5) Statistical Analysis

Subject demographic information, results of questionnaires, results of the OKT test, BMD, bone turnover markers and related factors were analyzed by SPSS version 20. The data was expressed as mean ± SD. Differences in the distribution of baseline characteristics were tested using the Chi-square test, student t-test or Kruskal Wallis test. Significance was defined by a P value $<0.05$ (two-tailed).

#### 6) Ethical committee

The study protocol was approved by the ethics committees of both Kobe University, Faculty of Health Sciences and Chiang Mai University, Faculty of Medicine.

## RESULTS

A total of 177 subjects were enrolled in the study. The characteristics of the subjects are displayed in Table I. The average age (year), height (cm), weight (kg), BMI ($kg/m^2$), and menarche (year) were 23.4±2.5 (min. 20.0, max.29.0), 159.5±5.2 (145.0, 175.0), 52.1±9.0 (38.0, 93.0), 20.5±3.1 (15.6, 34.2), and 12.7±1.6 (9.0, 19.0), respectively.

Table I. Subjects characteristics (n=177)

| Items (unit) | mean ± SD | mini. | max. |
|---|---|---|---|
| Age (year) | 23.4 ± 2.5 | 20.0 | 29.0 |
| Height (cm) | 159.5 ± 5.2 | 145.0 | 175.0 |
| Weight (kg) | 52.1 ± 9.0 | 38.0 | 93.0 |
| BMI (kg/m$^2$) | 20.5 ± 3.1 | 15.6 | 34.2 |
| Menarche (year) | 12.7 ± 1.6 | 9.0 | 19.0 |

Table II shows the results of BMD measurement as assessed by QUS. The mean BMD of all subjects was 102.8±19.8（min.72.0, max.162.0）. BMD in the Normal BMD group (n=136) and Low BMD group (n=41) was 109.0±18.1 and 82.2±4.9, respectively. Subjects in the Low BMD groups, which accounted for approximately 23.2% of the total number of subjects, presented with a mean BMD and percentage of Low BMD 1 (n=13) and Low BMD 2 (n=28) of 75.9±3.0 and 85.1±2.2, and 7.3% and 15.8%, respectively.

Table II. Measurement of BMD among Normal, Low 1 and Low 2 groups assessed by QUS (n=177)

| Group | | BMD mean ± SD | mini. | max. | n | % |
|---|---|---|---|---|---|---|
| Total BMD | 70≦x | 102.8 ± 19.7 | 72.0 | 162.0 | 177 | 100 |
| Normal BMD | 89≦x | 109.0 ± 18.1 | | | 136 | 76.8 |
| Low BMD ( 1+2) | 70≦x <89 | 82.2 ± 4.9 | | | 41 | 23.2 |
| Low BMD 1 | 70≦x <80 | 75.9 ± 3.0 | | | 13 | 7.3 |
| Low BMD 2 | 80≦x <89 | 85.1 ± 2.2 | | | 28 | 15.8 |

Table III shows BMD according to subject characteristics. There were no significant differences in age, height, or menarche between the Low BMD and Normal BMD group. A positive relationship was observed between weight and BMD, however no significant difference between height and BMD was seen among three groups (Low 1, Low 2 and Normal). A positive tendency was observed between BMD and BMI among the three groups (p=0.096).

Table III. Comparison of subjects characteristics among Low 1, Low 2 and Normal (n=177)

| | Low BMD 1 70≦x <80 n= 13 | Low BMD 2 80≦x <89 n= 28 | Normal 89≦x n = 136 | |
|---|---|---|---|---|
| Age (year) | 23.2 ± 2.4 | 24.1 ± 2.4 | 23.3 ± 2.5 | |
| Height (cm) | 159.6 ± 4.2 | 159.6 ± 6.2 | 159.4 ± 5.1 | |
| Weight (kg) | 48.9 ± 5.2 | 51.3 ± 10.2 | 52.6 ± 9.0 | ** |
| BMI (kg/m$^2$) | 19.2 ± 1.9 | 20.0 ± 3.1 | 20.7 ± 3.1 | |
| Menarche (year) | 12.7 ± 1.3 | 12.9 ± 1.8 | 12.6 ± 1.6 | |

** P < 0.01

Table IV shows BMD according to items in the lifestyle related questionnaires. Subjects in the regular cheese intake 'yes' group (110.0±23.3) had a significantly higher BMD compared with the 'no' group (99.7±17.0). In contrast, subjects in the fracture experience 'yes' group (82.5±11.6) had a significantly lower BMD compared with the 'no' group (103.3±19.6). There were no significant differences in food regularity, milk intake and fish intake between the Low BMD and Normal BMD group. There were no significant differences in diet and exercise experience or in family history between the two groups.

Table IV. Correlation between lifestyle and BMD (n=177)

| | n | BMD mean ± SD | P-value |
|---|---|---|---|
| Meal regularity (3 times a day) | | | |
| yes. | 145 | 103.2 ± 20.4 | |
| no. | 32 | 101.3 ± 16.2 | |
| Milk intake (current regularity) | | | |
| yes. | 70 | 103.5 ± 21.7 | |
| no. | 107 | 102.4 ± 18.3 | |
| Cheese intake (current regularity) | | | |
| yes. | 54 | 110.0 ± 23.3 | * |
| no. | 123 | 99.7 ± 17.0 | |
| Fish intake(current regularity) | | | |
| yes. | 70 | 102.5 ± 19.0 | |
| no. | 107 | 103 ± 20.2 | |
| Vegetable intake(current regularity) | | | |
| yes. | 160 | 102.0 ± 19.8 | |
| no. | 17 | 110.4 ± 17.3 | |
| Diet experience | | | |
| yes. | 87 | 104.5 ± 19.4 | |
| no. | 87 | 101.0 ± 20.2 | |
| Current exercise (current regularity) | | | |
| yes. | 107 | 104.5 ± 20.2 | |
| no. | 70 | 100.3 ± 18.7 | |
| Fracture history | | | |
| yes. | 4 | 82.5 ± 11.6 | * |
| no. | 173 | 103.3 ± 19.6 | |
| Family history | | | |
| yes. | 41 | 101.5 ± 20.7 | |
| no. | 136 | 103.2 ± 19.4 | |

* $P < 0.05$ VS Normal

Table V shows the results of knowledge assessed by OKT. The average total scores (Q1-Q26) of all subjects (n=177) was 60.0%, with a mean of Low BMD 1 (n=13), Low BMD 2 (n=23), Normal BMD (n=136) of 15.1±4.5, 15.5±5.7, and 16.0±3.7, respectively. The percentage of correct answers for each type of knowledge regarding osteoporosis, e.g., risk factor knowledge, exercise knowledge, calcium knowledge and Vitamin D knowledge, were 55.6%, 64.3%, 60.6%, and 80.0%, respectively. Although comparison of mean scores among each group indicated no significant differences, the Normal BMD group had higher total scores than Low BMD groups.

TableV. Correlation between knowledge assessed by OKT and BMD among Low 1, Low 2 and Normal (n=177)

| Group | Low BMD | | Normal BMD | P-value |
|---|---|---|---|---|
| | Low BMD 1 | Low BMD 2 | Normal | Low and N |
| Item | 70≦x <80 n=13 | 80≦x <89 n=28 | 89≦x n=136 | |
| Risk factors of Osteoporosis (Q1-Q9) | 5.5 ± 1.3 | 4.7 ± 2.5 | 5.0 ± 1.9 | 0.476 |
| Exercise Knowledge(Q10-Q16) | 3.9 ± 2.3 | 4.5 ± 1.7 | 4.5 ± 1.5 | 0.459 |
| Calcium Knowledge(Q17-Q24) | 4.1 ± 1.6 | 4.8 ± 2.1 | 4.9 ± 1.6 | 0.344 |
| Vitamin D Knowledge(Q25-Q26) | 1.5 ± 0.8 | 1.6 ± 0.7 | 1.7 ± 0.7 | 0.419 |
| Total Scores(Q1-Q26) | 15.1 ± 4.5 | 15.5 ± 5.7 | 16.0 ± 3.7 | 0.373 |

Table VI shows a comparison between Low BMD and Normal BMD groups regarding measurements of bone turnover markers and related factors. Significant differences in 1,25-$(OH)_2$ Vitamin D and ucOC were observed between the Low and Normal BMD group.

Table VI. Comparison of bone turnover and bone turnover related factors between Low BMD and Normal

| | unit | Low BMD n=23 mean±SD | Normal BMD n=23 mean±SD | P-value n=46 | |
|---|---|---|---|---|---|
| Ca | mg/dL | 9.40±0.50 | 9.58±0.34 | 0.152 | |
| 1,25-(OH)2VitaminD | pg/mL | 66.70±22.53 | 85.74±24.48 | 0.012 | * |
| VitaminK1 | ng/mL | 0.37±0.18 | 0.51±0.45 | 0.180 | |
| VitaminK2(MK-4) | ng/mL | 0.10±0.17 | 0.10±0.07 | 0.834 | |
| OC | ng/mL | 8.44±2.52 | 7.38±1.54 | 0.091 | |
| NTx | nmolBCE/L | 13.34±2.93 | 13.86±4.80 | 0.662 | |
| ucOC | ng/mL | 5.09±2.67 | 3.58±1.01 | 0.017 | * |

* P < 0.05 VS Normal

## DISCUSSION

The prevalence of Low BMD(x＜YAM-1.0SD) as assessed by QUS in young women in Thailand was 23.2%. Furthermore, it was suggested that weight, BMI, food, fracture history and knowledge level regarding osteoporosis all may affect BMD, with bone turnover in young women with Low BMD characterized by decreased bone formation. These results indicate the possibility that unhealthy lifestyle habits in youth can result in lower BMD through decreased bone formation.

Base on the results, the percentages of Low BMD, Low BMD 1 and Low BMD 2 groups were 23.2%, 7.3%, 15.8%, respectively. The percentage of Low BMD was higher than expected before we started the study. Chijiwa et al demonstrated that the percentage of Low BMD (x＜YAM-1.0SD) in Japanese healthy young women amounted to 4.7% (4). The percentage of Low BMD in young women in Chiang Mai, Thailand was higher than seen in Japan.

There are few reports of the BMD in young women in Thailand. All subjects in this study had their BMD examined for the first time and were not previously aware of their bone health. All subjects physically looked healthy despite some of them being in the Low BMD group.

The future risk of fracture decreases as maximum peak bone mass increases. Females acquire about 1000g of bone mineral or 40%-50% of their adult skeletal mass and achieve peak hip bone mineral density during adolescence, so it is important to understand the contributions of modifiable lifestyle determinates particularly exercise fitness, body composition, and calcium intake on bone development (20). In recent years, younger generations have undergone drastic changes in terms of lifestyle and social environment, and number of studies have showed low BMD in young women who have abnormal menstruation patterns, follow extreme dieting practices, consume unbalanced diets and lack regular exercise (7,8).

The questionnaires concerning lifestyle related factors employed in this study also showed that weight, calcium intake and fracture history significantly affect BMD in young women. Examination of subject BMI revealed, a tendency for those with higher BMI to also have a higher BMD. A positive correlation was seen between weight and BMD, although there was no significant difference in height and BMD among three groups (Low BMD 1, Low BMD 2 and Normal BMD groups). It is therefore important to measure and manage BMD in young women with weight loss, low calcium intake and presence of fracture history in terms of primary prevention of osteoporosis (3,7,12,21,23). The subjects in the regular cheese intake 'yes' group had a significantly higher BMD compared with the 'no' group. Pngchaiyakul (2008) reported that milk drinking is uncommon in northern Thailand. Moreover, the amount of milk consumption was minimal as reflected by the average daily calcium intake being far less than the recommended value. The mean daily calcium intake is 265 mg (29). Although recently people in the urban area of Chiang Mai have accepted Western food, those in rural areas consume traditional foods that are low in calcium content. We found that subjects who had rich calcium intake often ate pizza or spaghetti with cheese.

Subjects in the fracture history 'yes' group had a significantly lower BMD compared with the 'no' group. It is well known that the risk of future fractures is low in those with a high BMD.

The mean score of OKT in this study was 60.0%,which is lower than expected based on the occupations of the subjects. Osteoporosis has frequently been called a "silent disease" due to the fact that it is asymptomatic until a fracture occurs (32). Identification of factors contribute to the achievement of optimal or peak bone mass during this augmentation period may therefore be very important determinants of bone mass later in life and hence may reduce the risk of or delay the occurrence of future fractures (22). Results of this study showed a tendency in which the Normal group had a higher BMD than the Low BMD 1 and Low BMD 2 groups in osteoporosis knowledge regarding exercise, calcium and Vitamin D. The study focused on young women's education regarding primary prevention of osteoporosis since subjects with higher BMD appeared to have higher knowledge scores.

Next, we measured bone turnover markers to analyze bone turnover in young women with Low BMD, and we demonstrated no significant difference in the serum level of OC and NTx between the Low BMD and Normal BMD groups. This observation differs from results of past studies, which describe a decrease in bone turnover conditions related to the BMD decrease seen from menopause to old age (4). In other words, estrogen deficiency promotes bone desorption in menopause, and as a result, bone formation activity increases in response to this change. Therefore, in menopause, both bone desorption and formation rise, leading to a high bone turnover rate. However, bone metabolism in old age is frequently characterized by a low turnover rate when both desorption and formation decrease. In young women with low BMD, bone turnover did not change, indicating that this phenomenon cannot be classified as either high or low turnover (6,9). It was suggested that the suppression of bone formation as a result of insufficient uptake of Vitamin D or K might be contributory to the Low BMD seen in young women in Chiang Mai, Thailand. Traditional north Thai- food, which has low calcium intake might contribute to low bone mass in this instance. Further studies in other communities should yield additional information leading to improvement in the primary prevention of osteoporosis.

Recently, diet and nutrition deficiencies have become a worldwide problem. In November 2010, the Institute of Medicine (ICM) in the USA stated that a serum 25(OH) Vitamin D level of 20 ng/mL is sufficient maintain good health in 97.5% of the general population, and recommended a Vitamin D intake that was much lower than was previously recommended by many experts, although Vitamin D sufficiency is defined by a serum 25(OH) Vitamin D level of more than 30 ng/mL. Vitamin D deficiency in young women in Thailand has become a significant problem. We must improve their uptake of Vitamin D through education, particularly regarding Vitamins D and K.

Limitations of this study included the following. First, although subjects in the study should have been enrolled from the general community, they instead consisted of medical workers and students affiliated with Chiang Mai University Hospital. The second limitation of this study consisted of errors that are inherent when using QUS to measure BMD. Currently, DXA is the accepted standard for conducting BMD measurements.

However, QUS was found to be useful for the primary prevention of osteoporosis among young healthy women due to its portability and lack of X-ray risk. It has been reported that BMD measurements are not widely available in some communities due to high cost and lack of equipment (10,15-17,25,34). Somsak (2007) reported that osteoporosis self-assessment tools used for the Asian(OSTA) index score had a low sensitivity (36-48%) but high specificity (71-75%) for identifying osteopenic menopausal women (3). QUS is widely required at the community level among young women in Asia. Further studies at the community level should clarify additional issues that will improve the education program regarding osteoporosis in Thailand.

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