The Association of Dietary Calcium, Bone Mineral Density and Biochemical Bone Turnover Markers in Rural Thai Women

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Objective: *To investigate the relative contribution of dietary calcium intake on bone mineral density (BMD) and biochemical bone turnover markers in rural Thai women.*
Material and Method: *A cross-sectional investigation was designed in 255 rural Thai women. Usual dietary calcium intake was determined by 3-day food records and quantitative food-frequency questionnaire. BMD was measured by DXA. The three markers for bone turnover event: serum total alkaline phosphatase, serum N-mid osteocalcin and type I collagen C-telopeptide, including serum calcium and were determined in 125 women in the present study.*
Results: *An average daily calcium intake in the present study was 265 mg/day. Two hundred and thirty three out of 255 women (87%) consumed dietary calcium less than half of the recommended value and only 3% of women (n = 7) had calcium intake > 800 mg/day. After controlling certain parameters: age and body mass index, women who consumed higher amount of dietary calcium had significantly higher BMD at all sites. Moreover, highly increased bone turnover markers were observed in those with lowest quartile calcium intake. Women with osteopenia and osteoporosis were older, lower BMI, consumed less calcium and had significantly higher values of all biochemical bone turnover markers than those who had normal BMD.*
Conclusion: *The present study showed that a habitual diet of the rural Thai population might not provide enough calcium as needed for bone retention and for prevention of bone loss in the following years. Modification of eating pattern by promotion of increased consumption of locally available calcium rich food may be beneficial for prevention of osteoporosis among this population.*

Keywords: *Calcium, Dietary, Bone marker, Bone mineral density, Epidemiology*

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Calcium intake among women has drawn more attention in the recent years since several studies showed a significant relationship between low calcium intake and risk of osteoporosis as well as the relationship between increased calcium intake and maximizing peak bone mass during adolescent years[1-5]. The average calcium intake has been observed to be in a low level among Asian people, who have an eating habit of low dairy products. The mean dietary calcium intakes among Hong Kong and Chinese population were between 350-450 mg/day[6,7]. These values of calcium intake were about half of that found in a Western population. In Thailand, the average calcium intake of

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adults who lived in urban area has also been reported to be low (~361 mg/day)[8]. The majority of these subjects consumed calcium in the amount less than half of the recommended value and only 2% of the study subjects could have dietary calcium at the recommended level (800 mg/day).

Meta-analysis from the study in young and middle age men and women[9], and also in postmenopausal women[10] showed that a high dietary calcium intake has a positive effect on bone mineral density (BMD) and calcium supplementation (~1,000 mg/day) could prevent bone loss in premenopausal women[9]. However, previous studies reported that the association between the calcium supplementation and osteoporotic fracture were not consistent[11-13]. A systematic review of the literature to assess the effectiveness of calcium supplementation and/or dietary calcium for the prevention of osteoporotic fractures in postmenopausal women, showed that calcium supplementation (~1,050 mg/day) could reduce the risk of osteoporotic fracture from 2-70%[11]. Some studies reported that calcium supplementation alone[12] or calcium plus vitamin D supplementation[13] did not significantly reduce osteoporotic fractures, but it was effective in those patients with good long-term compliance[12]. Although the cause of osteoporosis is multi-factorial, these findings reflect an important and essential role of dietary calcium on attainment of peak bone mass and prevention of bone loss and also osteoporotic fractures in the following years[14].

Therefore, the initial purpose of the present study was to determine the calcium intake and its major food sources among adult Thai women living in a rural area. The authors' second attempt was to correlate the dietary calcium intake with BMD as well as biochemical bone turnover markers and including characterization of those who are at risk of osteoporosis based on nutritional factor and biochemical bone turnover markers.

Material and Method

Study subjects

The present study was a cross-sectional study, conducted in the rural area of Khon Kaen province, Northeast of Thailand. The study participants were recruited from two sub-districts (Nongtoom and Koksri) of Muang district, Khon Kaen province. Initially, the potential participants were randomly selected from a list of members of a local population and were invited to participate in the present study. The participants were recruited if they were 20-85 years old and in good general health as judged by physical examination. The exclusion criteria included any chronic diseases, any diseases, conditions or medications known to affect bone or calcium metabolism, a history of calcium supplementation, use of corticosteroids, hormone replacement therapy, or a history of bone fracture. The present study was approved by the ethical committee of the Faculty of Medicine, Khon Kaen University, and informed consent was obtained from all participants.

Estimation of calcium intake

All interviews were done at the participants' homes by a skilled field worker. Where possible, they underwent a second interview at the study center (Department of Medicine, Faculty of Medicine, Khon Kaen University) to assist in verifying and validating the integrity of the responses. The usual calcium intake was assessed by using a 3-day food record and an interviewer-administered quantitative food frequency questionnaire which contained 73 food items that covered all calcium rich food as well as locally available food items, commonly consumed in this area. Frequency of consumption was reported in 8 categories: never, once per month, 2-3 times/month, 1-2 times/week, 3-4 times/week, 5-6 times/week, once per day, and 2-3 times/day. The participants were asked to identify the consumed food items, frequency of consumption during the past month and indicating the amount of each food consumed. The real food models, weighing scale and standard measuring cups and spoons were used for portion size estimation during an interview. The daily calcium intake was calculated by converting the amount of food consumed in household unit to the amount consumed in grams then multiplied by the calcium content obtained from the Thai food composition table. In addition to the dietary intake questionnaire, the participants were asked about their history of milk consumption during childhood, alcohol consumption together with current and past smoking habits.

Measurements

Anthropometry

Body weight and height were measured on the day of BMD measurement. Body weight (including light indoor clothing) was measured using an electronic balance (accuracy 0.1 kg) and standing height (without shoes) with a stadiometer (nearest 0.1 cm). Body mass index (BMI) was then calculated as the ratio of weight (in kg) over height (in m^2).

Bone mineral density

Total body, lumbar spine (L2-4), femoral neck, distal and mid-shaft of radius BMD and total bone mineral content (BMC) were measured using a dual-energy X-ray absorptiometry (DXP-IQ, Lunar Corp, USA). Each woman was classified as having "osteoporosis" if her BMD T-score was $\leq$ -2.5, or "osteopenia" if her BMD T-score was between -2.5 and -1 or "normal" if her BMD T-score $\geq$ -1 at any sites according to the reference range for Thai women[15,16].

Laboratory analysis

Overnight fasting blood samples from 125 women were collected between 8.00 a.m. and 9.00 a.m. All sera were stored at -20° c before analysis. Serum total alkaline phosphatase (ALP) and serum calcium were measured by automated enzymatic method (Dimension RxL Clinical Chemistry System, Dade Behring Inc., USA). Serum N-terminal mid fragment osteocalcin (N-mid OC) and serum carboxy terminal telopeptide fragment of type I collagen (CTX) were measured by electrochemiluminescence immunoassay (Roche Diagnostics, Germany). The coefficient of variation of serum total ALP, N-mid OC and CTX of intra-assay were 1.4%, 3.6%, 3.4% and of inter-essay were 3.1%, 3.4% and 4.1%, respectively.

Statistical analyses

Statistical analysis was performed with SPSS, version 11.5, (SPSS Inc, Chicago). The effect of age on dietary calcium intake as well as bone parameters and biochemical bone turnover markers were tested using one-way analysis of variance (one-way ANOVA). The dietary calcium intake of the study subjects were further categorized into quartiles and the influence of level of calcium intake on bone parameters and biochemical markers were tested by analysis of covariance (ANCOVA) and using the values of age and BMI as covariates. To identify the characteristics of the group at risk for osteopenia and osteoporosis, the diagnostic guidelines proposed by World Health Organization were adopted[15] and comparisons between groups were performed by one-way ANOVA. The significance level for test statistics was set at $p < 0.05$.

Results

Of 255 women were recruited in the analysis. The mean age was 50.6 years old. In the present study, 7 and 37.6% of these women were classified by BMI value as underweight (< 18.5 kg/m^2) and obese (> 25.0 kg/m^2), respectively. Very few of these women (< 1%) were current smokers and alcohol drinkers and none of them had a history of regular milk drinking habit during childhood which indicated that consumption of milk is uncommon eating habit among rural Thai population.

The usual calcium intake of the study participants is shown in Table 1. Mean dietary calcium intake was 265 mg/day and most of participants had a calcium intake lower than the Thai recommended value (800 mg/day). More than half of participants had an average daily calcium intake less than 400 mg/day, and only 2.8% (n = 7) could meet the Thai RDA level. The average calcium intake decreased with advancing age and lowest in $\geq$ 70 years age group (Table 1).

In the analysis of the relationship between dietary calcium intake, BMD and biochemical bone turnover markers, the authors categorized the level of calcium intake into quartile and during statistical analysis the authors controlled for the effect of age and BMI by including them into the analysis model as covariates. In Table 2, the authors observed a significant effect of high dietary calcium intake on the BMD at all sites as well as the total BMC. Women with highest quartile of calcium intake had the values of all biochemical bone markers (Total ALP, N-mid OC and CTX) significantly lower than those the lowest quartile of calcium intake, while serum calcium was not significantly different.

In the present study, there were 140 women (70 in each group) who were classified as at risk for osteopenia and osteoporosis at lumbar spine site. For femoral neck, 56 and 26 women were classified as at

Table 1. Daily dietary calcium intake in rural Thai women

Dietary calcium intake (mg/day)	
Mean	265.57
Median	211.54
Standard deviation	208.42
Age group	
20-29 yr	281.57 $\pm$ 156.27*
30-39 yr	358.06 $\pm$ 343.31
40-49 yr	290.57 $\pm$ 187.79
50-59 yr	256.16 $\pm$ 147.41
60-69 yr	229.29 $\pm$ 217.66
$\geq$ 70 yr	181.94 $\pm$ 103.02
Number of subjects by level of intake (%)	
< 200 mg/day	119 (46.9)
200-400 mg/day	104 (40.9)
401-800 mg/day	24 (9.4)
> 800 mg/day	7 (2.8)

* Significant difference among group at $p < 0.05$

Table 2. Comparison of bone parameters and biochemical bone turnover markers by quartile of daily dietary calcium intake

	Calcium quartiles, mg/day			
	1 *< 155*	2 *155-211.5*	3 *211.5-315.9*	4 *> 315.9*
Bone parameters				
Total BMC, g	1979 ± 473	2166 ± 397	2203 ± 465	2225 ± 415*
Total BMD, g/cm^2	1.04 ± 0.13	1.10 ± 0.12	1.12 ± 0.14	1.13 ± 0.12*
FN BMD, g/cm^2	0.78 ± 0.19	0.88 ± 0.18	0.91 ± 0.22	0.91 ± 0.16*
L2-4 BMD, g/cm^2	0.91 ± 0.23	1.01 ± 0.22	1.04 ± 0.22	1.06 ± 0.18*
Distal radius BMD, g/cm^2	0.27 ± 0.07	0.30 ± 0.06	0.32 ± 0.07	0.32 ± 0.07*
Mid shaft radius BMD, g/cm^2	0.57 ± 0.13	0.61 ± 0.12	0.63 ± 0.12	0.65 ± 0.10*
Biochemical markers				
Total ALP, U/L	92.50 ± 20.90	80.04 ± 29.20	92.00 ± 24.30	82.60 ± 21.20*
N-mid OC, ng/ml	28.10 ± 23.60	16.80 ± 7.60	19.50 ± 7.50	20.00 ± 7.50*
CTX, ng/ml	0.42 ± 0.24	0.30 ± 0.17	0.39 ± 0.23	0.33 ± 0.14*
Serum calcium, ng/ml	2.30 ± 0.10	2.40 ± 0.10	2.40 ± 0.10	2.40 ± 0.09*

* Significant difference between quartile of intake by ANCOVA at $p < 0.05$

risk for osteopenia and osteoporosis, respectively. Women who were classified as having osteopenia and osteoporosis at both femoral neck and lumbar spine were significantly older, lower weight, consumed less dietary calcium than those who had normal BMD. A high difference in the bone markers was also observed. The women with osteopenia and osteoporosis had significantly higher value of total ALP, CTX and N-mid OC than women with normal BMD. However, there was no significant difference in the serum calcium level (Table 3).

Discussion

The dietary habit of the participants who were considered as a rural population in the northeast of Thailand reflected the traditional eating habit with limited food sources high in calcium. Milk drinking was uncommon eating habit in this population. Their main food sources for calcium reflected the lifestyle of the study population. Hunting and gathering forest foods were considered as a tradition. Moreover, the amount of consumption was minimal as reflected by the average daily calcium intake far less than the recommended value. The mean daily calcium intake was 265 mg. More than 87% of participants consumed calcium less than 400 mg/day and only 3% consumed dietary calcium at the level of 800 mg/day. The results were consistent with previous study in adult Thai subjects living in urban area (Bangkok metropolitan) and in the Chinese and Hong Kong population[6-8], confirmed that a low dietary intake of calcium persists in an Asian population especially in those who lived in rural area. Moreover, decreasing in calcium intake was also observed with advancing age. The low calcium intake may compromise the rate of bone loss especially in postmenopausal women and in the elderly.

Results from the present study indicated that dietary calcium seems to be independently related to BMD because when age and BMI were adjusted, the significant relationship between level of calcium intake and BMD values at all sites as well as the BMC were still observed. These findings reflect the potentially beneficial effect of habitual high consumption of dietary calcium on BMD at all age groups. The present results agreed with those reported in other cross-sectional studies[6,17-21]. Reports by other investigators, however, failed to demonstrate a statistically significant effect of dietary calcium on parameters of bone health, including BMD of the spine[22,23], radius[22,24-26] and femur[23,24]. Lack of significant relationship between dietary calcium and bone health of these various studies may partly explained by the study design which dealt only with a fairly narrow range of calcium intake within study population and also method of dietary assessment used to estimate calcium intake. Dietary intake estimated from recall technique over a short period of time may not be a good representation of usual or long-term calcium intake. This may be true in the case of dietary calcium, given to the fact that BMC may

Table 3. Comparison of age, BMI, daily dietary calcium intake and biochemical bone turnover markers in among women with normal BMD, osteopenia and osteoporosis

	L2-4 BMD			FN BMD		
	Normal (n = 115)	Osteopenia (n =70)	Osteoporosis (n = 70)	Normal (n = 172)	Osteopenia (n = 56)	Osteoporosis (n = 26)
Age, y	38.84 ± 10.5	55.44 ± 13.42	65.17 ± 9.81*	43.27 ± 12.73	63.32 ± 10.07	71.42 ± 6.49*
BMI, kg/m^2	24.90 ± 3.85	24.99 ± 3.67	21.81 ± 3.85*	24.94 ± 3.75	23.21 ± 3.77	20.30 ± 4.02*
Calcium intake, mg/d	303.04 ± 238.13	275.11 ± 218.15	193.43 ± 102.57*	299.19 ± 232.79	199.12 ± 122.04	185.71 ± 112.04*
Biochemical markers	n = 57	n = 34	n = 34	n = 84	n = 26	n = 15
Total ALP, U/L	74.65 ± 20.61	91.06 ± 25.47	104.32 ± 17.61*	80.06 ± 23.73	100.92 ± 18.9	103.27 ± 20.95*
N-mid OC, ng/ml	14.63 ± 5.46	21.04 ± 7.09	31.94 ± 20.86*	16.56 ± 6.62	28.06 ± 8.66	34.00 ± 30.57*
CTX, ng/ml	0.26 ± 0.14	0.42 ± 0.21	0.47 ± 0.22*	0.31 ± 0.17	0.49 ± 0.2	0.46 ± 0.27*
Serum calcium, U/L	2.37 ± 0.1	2.38 ± 0.11	2.36 ± 0.1	2.38 ± 0.11	2.37 ± 0.07	2.32 ± 0.11

* Significant difference among groups by one-way ANOVA at $p < 0.0$

be affected more by early nutrient intake than by current calcium intake, as suggested in other previous studies[27-29]. Studies in which habitual calcium intake rather than current calcium intake was used for analysis often reported a positive association between dietary calcium and BMD[27-29]. Furthermore, identification of major food sources of calcium of the study population should also be taken into account. The response of the body to dietary calcium varies according to the calcium bioavailability. It is assumed that calcium absorption from milk and dairy products is markedly higher than that from other foods or inorganic calcium salts[30]. Indeed, calcium absorption from vegetables is generally considered low because they contain substances such as phytate, oxalate and other dietary fiber components which bind calcium in unabsorbable compounds. Therefore, assessment of calcium intake from the diet without accounting for its sources may confound the potential effect of dietary calcium on bone parameters.

A significant independent effect of calcium intake level on bone turnover markers was also observed in the present study. Studied participants in the lowest quartile of calcium intake had significantly higher values of all biochemical bone turnover markers than those who were in higher quartiles of calcium intake indicating that inadequate intake of calcium has a significant effect on the degree of bone turnover.

The results from the present study demonstrated that women who had osteoporosis consumed less calcium and had increased level of bone markers. It was suggested that elevated bone turnover appeared to have a negative influence on BMD. In postmenopausal women, the biochemical bone turnover markers were negatively correlated with BMD at the hip, spine, and forearm[31]. Another study also confirmed that all biochemical bone turnover markers performed fairly comparably in predicting BMD of the hip, spine, radius and total body[32].

A previous study demonstrated the elevation of bone turnover markers were identified as an independent factor associated with fracture risk at spine, hip and forearm at all ages particularly in postmenopausal women[31]. It has been suggested that biochemical markers are able to estimate the rate of bone formation and resorption, and perhaps provide unique information about rapid bone loss. Moreover, biochemical bone turnover marker measurement combined with BMD testing may provide a better prediction of the future risk of osteoporosis and fractures than BMD alone[31-33]. In the present study, the authors observed a significant increase in biochemical bone turnover

markers; total ALP, CTX and N-mid OC in women who were classified as having osteopenia and osteoporosis at femoral neck and lumbar spine, which was consistent with a previous study[31]. When comparing premenopausal women with postmenopausal women whose lumbar spine BMD was more than 2SD below the young normal mean, they observed that all of a panel of markers of bone formation, except for serum carboxy-terminal propeptide of type I collagen, and all of the markers for bone resorption, except for type I collagen cross-linked C-telopeptide, were elevated among the postmenopausal women with low BMD.

There are a number of limitations to generalize the present findings. In a cross-sectional study, correlations cannot be taken as a definitive evidence of causal relationships. Measurement of dietary calcium intake at a single time point may not reflect long-term effects. The authors did not examine other dietary factors e.g., protein intake, vitamin intake, salt intake which may be associated with calcium and bone homeostasis[34-37]. Finally, these results from subjects of Thai ethnicity may not be generalizable to other populations. Despite these potential limitations, the present study has the advantage of being based on random sample and direct observation of dietary calcium intake, which allow a more accurate assessment of the relationship among this factor, bone turnover markers and BMD.

In conclusion, Thai population especially those in rural area is more at risk of having inadequate intake of calcium from their habitual diet that is needed for bone retention and for prevention of bone loss during old age. Dietary calcium was proved to be an independent risk factor of lower BMD at all sites. Low intake of dietary calcium also has a negative impact on bone formation and bone resorption as indicated by an elevated level of biochemical bone turnover markers in those who were in the lowest quartile of calcium intake. Low dietary calcium intake, advancing age, low BMI, and high biochemical bone turnover markers could determine the risk of having osteopenia and osteoporosis in our study population. Modification of eating pattern by promotion of increased consumption of local calcium rich foods with high bioavailability together with adoption of other protective healthy lifestyle may be beneficial in prevention of osteoporosis among this population.

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การศึกษาความสัมพันธ์ระหว่างแคลเซียมจากอาหารกับความหนาแน่นของกระดูกและดัชนีทางชีวเคมีของการสร้าง และการสลายกระดูกในผู้หญิงไทยที่อาศัยอยู่ในเขตชนบท

ฉัตรเลิศ พงษ์ไชยกุล, วงสวาท โกศัลยวัฒน์, สมศรี เจริญเกียรติกุล, ละออ ชัยเลอกิจ, นิภา รุ่งโรจน์วศินกุล, รัชตะ รัชตะนาวิน

วัตถุประสงค์: *เพื่อศึกษาความสัมพันธ์ระหว่างแคลเซียมจากอาหารกับความหนาแน่นของกระดูกและดัชนีทางชีวเคมีของการสร้าง และการสลายกระดูกในผู้หญิงชนบทไทย*

วัสดุและวิธีการ: *เป็นการศึกษาแบบตัดขวางในผู้หญิงไทยที่อาศัยอยู่ในเขตชนบทจำนวน 255 คน ปริมาณแคลเซียมจากอาหารประเมินโดยบันทึกเป็นเวลา 3 วันร่วมกับแบบสอบถามความถี่ของอาหาร ทำการวัดความหนาแน่นของกระดูกด้วยเครื่องวัดความหนาแน่นของกระดูก (DXA) สำหรับดัชนีทางชีวเคมีของการสร้างและการสลายกระดูกได้ทำการวัด serum total alkaline phosphatase, serum N-mid osteocalcin และ type I collagen C-telopeptide รวมทั้งวัดปริมาณแคลเซียมในเลือดในผู้หญิงเหล่านี้จำนวน 125 ราย*

ผลการศึกษา: *ปริมาณแคลเซียมเฉลี่ยที่ได้จากอาหารเท่ากับ 265 มิลลิกรัมต่อวัน ร้อยละ 87 (233 คน) ได้รับแคลเซียมจากอาหารน้อยกว่าครึ่งหนึ่งของปริมาณที่ควรได้รับ และเพียงร้อยละ 3 เท่านั้นที่ได้รับแคลเซียมจากอาหารมากกว่า 800 มิลลิกรัมต่อวัน ผู้ที่ได้รับปริมาณแคลเซียมจากอาหารสูงจะมีความหนาแน่นของกระดูกทุกตำแหน่งสูงกว่าโดยไม่ขึ้นกับผลของอายุและดัชนีมวลกาย พบว่ากลุ่มของผู้ที่มีระดับแคลเซียมจากอาหารต่ำสุด (lowest quartile) จะมีระดับ biochemical bone marker สูงที่สุด ผู้ที่มีกระดูกบางและกระดูกพรุนจะมีอายุมากกว่า ดัชนีมวลกายน้อยกว่า ได้รับปริมาณแคลเซียมจากอาหารต่ำกว่าและมีระดับดัชนีทางชีวเคมีของการสร้างและการสลายกระดูกสูงกว่าผู้ที่ความหนาแน่นของกระดูกปกติอย่างมีนัยสำคัญทางสถิติ*

สรุป: *การศึกษานี้พบว่าผู้หญิงชนบทในภาคตะวันออกเฉียงเหนือได้รับปริมาณแคลเซียมจากอาหารไม่เพียงพอในการส่งเสริมความแข็งแรงของกระดูกและอาจทำให้สูญเสียมวลกระดูกได้ การปรับเปลี่ยนพฤติกรรมการบริโภคอาหาร โดยการเลือกรับประทานอาหารที่มีแคลเซียมสูงที่หาได้จากท้องถิ่นน่าจะช่วยในการป้องกันโรคกระดูกพรุนในประชากรกลุ่มนี้ได้*