

# Bone mineral density (BMD) and bone-related biochemical markers after short-term supplementation in normal and osteoporotic elders, the pilot study

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## ABSTRACT

Bone mineral density (BMD) and bone-related marker measurements in elders are necessary to provide for osteoporosis diagnosis and can be predicted supplement effectiveness. This study was aimed to evaluate and compare the effectiveness of short-term calcium and vitamin D supplements in normal and osteoporotic elders. The cross-sectional study was determined for BMD; bone turnover markers, including procollagen type 1 N-terminal propeptide (P1NP), osteocalcin, beta-crossLaps (CTX), and alkaline phosphatase (ALP); 25-hydroxy-calciferol [25-(OH)D]; and bone-related minerals in normal group ( $N = 30$ ) and in osteoporotic elders ( $N = 30$ ) before and after calcium and vitamin D3 supplements daily for 3 months. BMD was measured by calcaneal quantitative ultrasound. Serum concentrations of bone-related biochemical markers were determined by automatic analyzers. Paired *t*-test was used for the statistical analysis. BMD of both groups before and after supplementation was significantly increased. In normal group, phosphorus, ALP, and P1NP were significantly increased ( $p < 0.05$ ), and 25-(OH)D was strongly significant ( $p < 0.0001$ ). In an osteoporotic group, magnesium and 25-(OH)D were increased at a strong level of significant ( $p < 0.0001$ ). Moreover, the decrement of CTX after supplementation was strongly significant ( $p < 0.0001$ ) in the osteoporotic group. The short-term supplement increased the rate of bone formation in normal group and decreased bone resorption in osteoporotic groups.

## INTRODUCTION

Maintaining a calcium intake of at least 1,000–1,200 mg/day has long been recommended for older individuals to treat and prevent osteoporosis. Calcium supplements are commonly taken to achieve such intakes, which are considerably higher than the average intake of calcium in the diet of older people, around 700–900 mg/day (Consensus conference, 1984; IOM, 2011; Tai, *et al.*, 2015). Increasing calcium intake from dietary sources increased bone mineral density (BMD) by 0.6%–1.0% at the total

hip and total body at 1 year and by 0.7%–1.8% at these sites, and the lumbar spine and femoral neck at 2 years (Tai *et al.*, 2015). In addition, evidence supports the use of calcium, or calcium in combination with vitamin D supplementation, in the preventive treatment of osteoporosis in people aged 50 years or older. For the best therapeutic effect, we recommend minimum doses of 1,200 mg of calcium and 800 IU of vitamin D for combined calcium plus vitamin D supplementation (Tang *et al.*, 2007).

Elevating of Thai population is a trend to increase with the number of osteoporosis in elders (Pongchaiyakul *et al.*, 2003) and will be fast increase along with common chronic diseases, such as diabetes mellitus and cardiovascular diseases (Maghbooli *et al.*, 2016a; 2016b; Sudjaroen and Thongmuang, 2018). From Thai nation-wide survey, the age-adjusted prevalence of osteoporosis in Thai women age ranges from 40 to 80 years was 13.6% and 19.8% for femoral neck and lumbar spine, respectively.

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For men, the age-adjusted prevalence of osteoporosis was 12.6%, 4.6%, and 3.9% at the femoral neck, lumbar spine, and both sites, respectively. The Asian Osteoporosis Study, a multi-national research survey was documented the incidence of hip fracture in Thailand, which reported age-adjusted rates (per 100,000) were 114 and 289, in men and women, respectively (Lau *et al.*, 2001; Pongchaiyakul *et al.*, 2003). Vitamin D insufficiencies of Thai elderly women in the urban area were higher than in rural area because of the differences in lifestyle (Soontrapa *et al.*, 2009a; 2009b). Study on the multi-centers from five provinces of Thailand was covering all regions of Thailand except southern area which represented the calcidiol level of Thai premenopausal women was 29.09 ( $\pm 0.42$ ) ng/ml, and with the cut point of  $\leq 35$  ng/ml; the prevalence of vitamin D insufficiency was 77.81%, and PTH and bone resorption markers were trended to increase (Soontrapa *et al.*, 2009a). Hypovitaminosis D, and low level of 25-hydroxy-calciferol [25-(OH)D] are common occurring in Thai premenopausal women; and the risk will be increase in elderly women who living in the rural area (Soontrapa *et al.*, 2009b). The prevalence of vitamin D insufficiency among elderly males was 13.6%, while the bone turnover markers [beta-crossLaps (CTX) and procollagen type 1 N-terminal propeptide (PINP)] levels were in the normal Thai reference range (Soontrapa *et al.*, 2015).

The numbers of elders in Sumut Songkhram province are a trend to increase and approximately 16.7% of elders are home and bed-bound elders who are difficult to assist by that medical staff and equipment. The early diagnoses of osteoporosis before fractures occur and by assessing the bone mineral density and with early treatment, osteoporosis can be prevented. Therefore, increasing awareness among doctors, which, in turn, facilitates increase awareness of the normal populace, will be effective in preventing this epidemic or community level (Sözen *et al.*, 2017). In addition, BMD, vitamin D, and bone-related markers measurement of elders are necessary to provide for osteoporosis diagnosis and bone turnover or bone-related biochemical status, which are able to predict the supplement effectiveness, especially in case of mass supplement in community level. Aims of our recent study were evaluated and compare the effectiveness of short-term calcium and vitamin D supplements in normal and osteoporotic elders by determining of BMD, serum vitamin D [25-OH vitamin D (25-(OH)D)], bone turnover markers (BTMs), and bone-related minerals before and after supplementation.

## MATERIALS AND METHODS

### Participants and demographic data

The cross-sectional study was carried out from November 2017 to April 2018 (this period including public relation and health service), 150 of health customers were included from academic health service program at Amphawa district, Sumut Songkhram, which was responsible by Sumut Songkhram campus, Suan Sunandha Rajabhat University. The screening of BMD was done for classified bone density status as normal, osteopenia, and osteoporotic persons.

Normal ( $N = 30$ ) and osteoporotic female elders ( $N = 30$ ) were sampled in this pilot study for determining BMD, 25-(OH)D, BTMs, and bone-related minerals in before and after supplements. Informed consent of all participants was done, and the study

protocol was approved by the Ethical Review Committee. The inclusion criteria: (1) female elders were aged  $\geq 60$ –75 year old; (2) consciousness and interactive; and (3) had not severe medical conditions. The exclusion criteria: (1) elder was combined with metabolic bone disease (osteomalacia, Paget disease, or primary hyperparathyroidism); (2) had accident especially with fracture; (3) taking daily calcium and vitamin D supplements before joining in the program at least 1 year; and (4) pathological fracture (Gao *et al.*, 2015; Slevin *et al.*, 2014). Anthropometric data, medical history, and health behaviors (including related risks) were recorded by physical examination and interview. The elders with poor literacy and/or unable to read as well were helped for filling the form of a questionnaire.

### BMD and biochemical measurements

BMD was measured by calcaneal quantitative ultrasound (QUS), ultrasound bone densitometer (SONOST-2000, OsteoSys, Korea), and the instrument protocol and data interpretations were followed with manufacturer instruction. According to BMD status, the elders were divided into osteoporotic (T score at or below  $-2.5$ ), osteopenia (T score between  $-1.0$  and  $-2.5$ ), and normal (T score at  $-1.0$  and above) elders. Each 6ml of fasting blood sample was obtained by venepuncture from the median cubital vein during the morning (7–9 a.m.). Nearly 6ml of blood sample was drawn into clotting blood tube for centrifuged and processed within 2 hours after phlebotomy and stored at  $-20^{\circ}\text{C}$  (Young and Bemes, 1999). Each serum concentrations of BTMs including procollagen type 1 N-terminal propeptide (PINP), osteocalcin (OC), CTX, and alkaline phosphatase (ALP); 25-(OH)D; and bone-related minerals (calcium, phosphorus, and magnesium) were determined by Cobas E411, Cobas E601, and Cobas E501 automatic analyzer (Roche Diagnostics, Basel, Switzerland), respectively. The within-run and between-run CVs were  $<10\%$ . Control materials and pooled serum were also done prior sample test for accuracy checking. The supplemented and control (placebo) group were selected according to BMD screening, which was defined as osteoporotic elder group (T score at/or below  $-2.5$ ) and control or normal group (T score at  $-1.0$  and above), respectively. BMD and biochemical data of supplemented and normal groups were measured prior recruitment of participants for supplement study and were done again after supplement study was finished.

### Calcium and vitamin D supplementation

Commercial calcium and vitamin D tablet contain calcium 600 mg; vitamin D3 (cholecalciferol) 800 IU, and other minerals including, magnesium, zinc, copper, and manganese were 50, 7.5, 1, and 1.8 mg, respectively. This commercial supplement tablet had been approved by the Thai food drug administration (Thai FDA). Each participant took one tablet daily with food and with a full glass of water, respectively, for 3 months and recommendation of intake for each participant was accorded Thai recommended dietary allowance (Thai RDA) and manufacturer indication, which was informed by senior pharmacist/nutritionist. The short term of daily supplement period was 3 months. After the study program was finished, all participants from both groups will continue intervention within another 3 months by the suggestion of medical staff and receive the tablet supplement.

## Statistical analysis

The Kolmogorov–Smirnov test was used to test the normal distribution of data. Paired *t*-test was used to compare the continuous variables as appropriate between before and after supplementation of normal and osteoporotic groups. The statistical significance was judged at  $p < 0.05$ . SPSS 20.0 software was used for statistical analysis (SPSS, Chicago, IL).

## RESULTS AND DISCUSSION

Average ages of control and osteoporotic groups were  $65.4 \pm 5.4$  and  $65.4 \pm 9.8$  years old, respectively. Health behaviors and anthropometric data of both groups were seemed to be similar, such as daily food intake, no risk of smoking and alcoholics, and normal body mass index (data not shown). The consumption of both groups has sufficient calcium intake due to frequent intake of seafood diets, such as small fishes (able to eat with bone) and shrimp paste (high content of calcium); and vitamin D intake due to properly sunlight exposed. The other risks of osteoporosis may relate to increment of bone resorption and some chronic disease, such as diabetes mellitus, which recorded from medical history by interviewing. BMD of the control group was T-score =  $-0.80 \pm 0.41$  and was within normal range, while average BMD (T-score) of osteoporotic elders was  $-2.85 \pm 0.23$  and interpreted as osteoporosis. Before calcium and vitamin D tablet intake, mean of serum calcium, phosphorus, magnesium, ALP, 25-(OH)D, OC, P1NP, and CTX normal (control) group was within normal range (Table 1). However, the mean of serum calcium and magnesium in an osteoporotic group was lower than normal; and mean of CTX was higher than normal (Table 2). The BMD after calcium and vitamin D supplementation in both groups was significantly increased and strong significant was occurred in the osteoporotic group ( $p < 0.0001$ ). In normal group, serum phosphorus, ALP, and P1NP of before and after supplement were significantly increased ( $p < 0.05$ ); and 25-(OH) D was strongly significant ( $p < 0.0001$ ). In the osteoporotic group, serum magnesium, 25-(OH)D of before and after supplement were increased at a strong level of significant ( $p < 0.0001$ ) and serum calcium was slightly significant. Moreover, the decrement of CTX after supplementation was strongly significant ( $p < 0.0001$ ) in the osteoporotic group.

According to our present study, we were representing the outcome of short-term calcium and vitamin D supplementation in

normal and osteoporosis elders (post-menopausal women) during 3 months, which were indicated by BMD, vitamin D status, and bone-related biochemical markers. The age-range, dietary intake, and health behaviours between normal and osteoporosis group were similar. Age was an important determinant of risk for bone reduction. Less bone reduction in people aged 50–70 years than in those who were older than 70 years (Tang *et al.*, 2007). In our study, similar age ranges of normal and osteoporosis (~65 years old) were similar responding to supplement; and a similar pattern of dietary intake and health behaviors can reduce the interference, which may affect to this comparative study.

Significant increments of BMD in both groups, which were monitored by using calcaneal QUS, were revealed responding of short-term supplement in this study; however, BMD of the osteoporotic group was still within osteoporosis level. Calcaneal QUS is an alternative technique for assessing the bone. Compared to dual-energy X-ray absorptiometry (DXA), QUS has the advantages of being cheaper, portable, and free of ionizing radiation (Vestergaard *et al.*, 2005). The systematic review on calcaneal QUS, which is potentially useful as a pre-screen tool for assessment of osteoporosis. The calcaneal QUS in screening or stratification algorithm must be based on device-specific cut-offs that are validated in the populations for which they are intended to be used (Thomsen *et al.*, 2015). However, reliable of calcaneal QUS is reliable method, which acceptable used as alternative method for osteoporosis screening due to cost effectiveness and simple access in a rural area (Soontrapa *et al.*, 2008). The review for calcaneal QUS that is able to reflect the bone quality and should be used in the screening of osteoporosis, especially in the developing countries where DXA devices are less accessible to the general population (Chin and Ima-Nirwana, 2013). In our study, T-scores for BMD measurement were estimated the outcome of calcium and vitamin D supplementation in both groups; however, DXA may provide preferable BMD measurement rather than calcaneal QUS. Thus, the evaluation of bone-related biochemical markers including serum vitamin D or 25-(OH)D was necessary for improving the reliability of this study and providing useful for monitoring bone status before and after supplementation. BTMs are usually represented as bone formation indicators, including procollagen type 1 N-terminal propeptide (P1NP), bone-specific alkaline phosphatase, and bone resorption markers,

**Table 1.** The different bone mineral density (BMD) and biochemical parameter between before and after calcium-vitamin D supplement in normal elder group ( $N = 30$ )<sup>a</sup>.

Parameter (unit)	Normal range	Before supplement	After supplement	Paired differences	95% CI		<i>p</i> -value
					Lower	Upper	
BMD (T score)	>−1.0	$-0.80 \pm 0.41$	$-0.53 \pm 0.48$	$-0.27 \pm 0.42$	−0.43	−0.01	0.001*
Calcium (mg/dl)	8.6–10.2	$8.94 \pm 0.64$	$9.03 \pm 0.68$	$-0.08 \pm 0.26$	−0.18	0.01	0.085
Phosphorus (mg/dl)	2.7–4.5	$3.27 \pm 0.83$	$3.40 \pm 0.89$	$-0.13 \pm 0.34$	−0.26	−0.01	0.043*
Magnesium (mEq/l)	1.40–2.10	$1.92 \pm 0.30$	$1.99 \pm 0.15$	$-0.08 \pm 0.25$	−0.17	0.02	0.108
ALP (U/l)	39–105	$54.00 \pm 9.19$	$54.73 \pm 8.97$	$-0.73 \pm 0.87$	−1.06	−0.41	<0.0001*
25-(OH) D (ng/ml)	$\geq 30$	$40.43 \pm 7.17$	$41.07 \pm 7.27$	$-0.63 \pm 1.19$	−1.08	−0.19	0.007*
OC (ng/ml)	11.5–29	$16.60 \pm 2.99$	$16.70 \pm 3.08$	$-0.10 \pm 0.49$	−0.28	0.08	0.264
P1NP (ng/ml)	15.0–74.0	$47.33 \pm 14.34$	$47.90 \pm 14.47$	$-0.57 \pm 1.01$	−0.94	−0.19	0.004*
CTX (ng/ml)	0.0–0.32	$0.28 \pm 0.09$	$0.26 \pm 0.07$	$0.024 \pm 0.09$	−0.001	0.06	0.143

<sup>a</sup>Average age =  $65.4 \pm 9.8$  year old; \*Statistically significance at  $p < 0.05$ .

**Table 2.** The different bone mineral density (BMD) and biochemical parameter between before and after calcium-vitamin D supplement in osteoporotic elder group ( $N = 30$ )<sup>a</sup>.

Parameter (unit)	Normal range	Before supplement	After supplement	Paired differences	95% CI		p-value
					Lower	Upper	
BMD (T score)	T score > -1.0	-2.85 ± 0.23	-2.53 ± 0.40	-0.33 ± 0.43	-0.49	-0.17	<0.0001*
Calcium (mg/dl)	8.6–10.2 mg/dl	6.09 ± 0.81	6.21 ± 0.91	-0.12 ± 0.32	-0.24	-0.001	0.048*
Phosphorus (mg/dl)	2.7–4.5 mg/dl	2.94 ± 0.70 <sup>a</sup>	2.94 ± 0.70 <sup>b</sup>	-	-	-	-
Magnesium (mEq/l)	1.40–2.10 mEq/l	1.36 ± 0.22	1.37 ± 0.22	-0.02 ± 0.01	-0.02	-0.008	<0.0001*
ALP (U/l)	39–105 U/l	53.63 ± 9.47	53.97 ± 10.04	-0.33 ± 1.45	-0.87	0.21	0.217
25-(OH) D (ng/ml)	≥30 ng/ml	42.07 ± 7.41	43.13 ± 7.04	-1.07 ± 1.44	-1.60	-0.53	<0.0001*
OC (ng/ml)	11.5–29 ng/ml	16.76 ± 3.45	16.89 ± 3.64	-0.12 ± 0.66	-0.37	0.12	0.318
P1NP (ng/ml)	15.0–74.0 ng/ml	45.60 ± 15.34	45.23 ± 15.19	0.37 ± 1.28	-0.11	0.85	0.125
CTX (ng/ml)	0.0–0.32 ng/ml	0.496 ± 0.165	0.465 ± 0.160	0.031 ± 0.023	0.023	0.041	<0.0001*

<sup>a</sup>Average age = 65.4 ± 5.4 year old; <sup>b</sup>Before and after values are equal (cannot calculate); \* Statistical significance at  $p < 0.05$ .

including C-terminal telopeptide of type-I collagen (CTX) and OC (Lin *et al.*, 2006; Starup-Linde and Vestergaard, 2016; Vasikaran *et al.*, 2011). They may provide information on fracture risk independent of BMD and predict the rapidity of bone loss in untreated patients, which also used to predict the response to treatments. International Osteoporosis Foundation (IOF) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group evaluated BTMs in the prediction of fracture risk and for monitoring treatment; it was recommended that bone formation markers (s-PINP) and bone resorption markers (s-CTX) should be used as reference markers and measured by standardized assays in observational and intervention studies (Sözen *et al.*, 2017; Vasikaran *et al.*, 2011). In our study, vitamin D (25-OH-D), P1NP, ALP, and phosphorus levels of normal BMD group before and after supplement were significantly increased. The finding was implied that short-term supplement was increased the rate of bone formation, which was indicated by elevating of ALP and P1NP along with 25-(OH) D levels. On the other hand, significantly decrement of CTX; and increment of vitamin D, calcium, and magnesium were occurred in the osteoporotic group after supplementation. Hence, the supplement in osteoporosis has increased BMD and may relate to reducing of bone resorption, which was indicated by the reduction of CTX along with elevating of 25-(OH)D calcium and magnesium levels. This study was preliminary done only on selected normal and osteoporosis for clarifying the benefit of BTMs measurements for monitoring BMD status in the short-term supplement. However, osteopenia elders were not included; there was the limitation in this study due to unable to supplement calcium and vitamin D and to monitor BMD and BTMs for all subjects.

This result corresponded to the previous study, which was monitored BMD and bone markers during 1-year supplementation in post-menopausal women and found the decline of CTX level (Slevin *et al.*, 2014).

## CONCLUSION

The finding may provide the useful data on calcaneal QUS combined with bone-related biochemical markers measurement for osteoporosis screening and monitoring for diagnosis and management together rather than diagnosis alone, which was represented in this study that can be prevented and intervene the osteoporosis in normal and osteoporosis by calcium and vitamin

D supplement. In addition, this combined measurement is suitable for mass screening and intervention, especially in the rural area.

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## CONFLICT OF INTEREST

There is no conflict of interest related to this research study.

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