Mal J Nutr 16(2) : 233 - 242, 2010

Calcium Intake, Vitamin D and Bone Health Status of Post-menopausal Chinese Women in Kuala Lumpur

Chee WSS[1], Chong PN[2], Chuah KA[2], T Karupaiah[2], Norlaila Mustafa[3], Seri Suniza S,[4] Karuthan Chinna[5], Horcajada MN[6], Ameye L[6] & Offord-Cavin E[6]

[1] *Department of Nutrition & Dietetics, International Medical University Bukit Jalil, 57000 Kuala Lumpur*
[2] *Department of Nutrition & Dietetics, Faculty of Allied Health Sciences Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur*
[3] *Department of Medicine, Universiti Kebangsaan Malaysia Medical Center Bandar Tun Razak, 56000 Cheras, Kuala Lumpur*
[4] *Department of Obstetrics & Gynecology, Universiti Kebangsaan Malaysia Medical Centre Bandar Tun Razak, 56000 Cheras, Kuala Lumpur*
[5] *Faculty of Information Technology & Quantitative Sciences, Universiti Technologi MARA Shah Alam, Selangor*
[6] *Nestlé Research Center, Nutrition & Health Department, 1000 Lausanne 26, Switzerland*

ABSTRACT

Bone health status was investigated in 178 free-living Chinese post-menopausal women in Kuala Lumpur. Body mass index (BMI), body composition (using whole body DXA), calcium intake and serum 25-OH vitamin D status were measured along with biochemical markers of bone turnover, that is, pro-collagen Type 1 N-terminal peptide (P1NP), osteocalcin (OC) and C-telopeptide ß cross link of Type 1 collagen (CTX- β). Bone mineral density (BMD) was measured using DXA (Hologic, USA) at the lumbar spine, femoral neck and total hip. Results showed that osteopenia was present in 50% of the subjects at the spine and 57.9% at the femoral neck. Osteoporosis was diagnosed in 10% of the subjects at both the femoral neck and spine. A total of 29.3% of the subjects had high levels of CTX- ß. Mean serum level of 25-OH vitamin D was 60.4+15.6 nmol/L and 50.6% of the subjects had hypovitaminosis D (defined as <50 nmol/l). Mean total calcium intake of the subjects was 497 + 233 mg, of which only 14% met the RNI for calcium with the additional intake of calcium supplements. Body fat was also significantly correlated (r=0.181, p<0.05) with BMD at the spine but not BMD at the femoral neck. Lean body mass was positively correlated with BMD at the spine (r=0.289, p<0.001) and femoral neck (r=0.295, p<0.001). CTX-β was negatively correlated with BMD at the spine (r= -0.235, p<0.001), whereas P1NP (r=-0.215, p<0.001) and osteocalcin (r=-0.265, p<0.001) were both negatively correlated with BMD at the femoral neck. Generally, the study found that women with osteopenia had higher levels of bone turnover markers, less lean body mass and lower calcium intake than women with normal BMD. In conclusion, this study demonstrated that the majority of free living Chinese post-menopausal women in Kuala Lumpur have low calcium intake, low 25-OH vitamin D status and low bone mass and elevated biochemical markers of bone turnover.

Keywords: bone health status, calcium intake, Chinese women, Kuala Lumpur, post-menopausal woman, Vitamin D

Correspondence author: Assoc Prof Dr Winnie Chee Siew Swee; Email: *winnie_chee@imu.edu.my*

INTRODUCTION

Osteoporosis is a silent progressive skeletal disorder characterised by low bone mass and compromised bone strength which predisposes the individual to an increased risk of fractures of the hip, spine and other skeletal sites (NIH, 2001). Osteoporosis is a serious public health issue associated with increased mortality (Tanko *et al*., 2005), adverse impacts on the quality of life (Brenneman *et al*., 2006), and increased economic burden to the respective countries. Its prevalence increases with the aging of the world population.

Osteoporosis is also expected to increase in tandem with urbanisation among Asian countries. In Malaysia, the overall incidence of hip fractures was reported as 90 per 100,000 individuals (Lee & Khir, 2007), while another local study showed that the prevalence of osteoporosis amongst postmenopausal Malaysian women was 24.1% (Lim *et al*., 2005). The study of Lee & Khir (2007) had shown that prevalence of fractures was highest amongst Chinese women in Malaysia.

The skeleton is continuously being remodelled by osteclast-mediated bone resorption and osteo-blast mediated bone formation; these processes are coupled tightly (Cremers & Garnero 2006). Biochemical markers of bone turnover are substances in the blood or urine that are produced or released during bone remodelling. These markers reflect either bone resorption or formation and their levels reflect the rates of bone turnover of the entire skeleton. The majority of markers of bone resorption are degradation products of Type 1 collagen such as C-telopeptide ß cross link of Type 1 collagen (β-CTX) while markers of bone formation are expressed during different phases of osteoblast development such as procollagen type 1 N terminal peptide (P1NP). Osteocalcin (OC) is a hydroxyapatite-binding protein that is synthesised by the osteoblasts and expressed mainly during the mineralisation phase of bone formation.

In this study, investigators measured bone turnover markers in free-living post-menopausal Chinese women living in Kuala Lumpur and correlated these markers with BMD and some lifestyle indices known to contribute to bone health status. Malaysian Chinese women were selected because they have been reported to have a higher prevalence of fractures compared to other races (Lee & Khir 2007).

METHODOLOGY

Subjects

A cross-sectional study was carried out where a total of 178 Chinese free living subjects aged 50 to 70 years old were recruited from local community centres around Kuala Lumpur. Subjects were more than 4 years post-menopausal and this was confirmed by measuring serum follicle stimulating hormone (FSH) which has to be greater than 25 IU/l. Subjects were not taking medications known to affect bone health and had not sustained any fractures in the proceeding 12 months. Written informed consent was obtained from all the subjects, and the study was approved by the Research & Ethics Committee of Universiti Kebangsaan Malaysia.

Questionnaire

Demographic data was obtained through questionnaire. Current calcium intake was assessed with a previously validated semi-quantitative food frequency questionnaire (Chee *et al*., 2002).

Anthropometry

Body weight and height of the subjects were measured in light clothing. The body mass index (BMI) was calculated by dividing body weight (kg) by the square of body height (m^2). Lean body mass and fat mass was measured by total body DXA scans (Hologic, USA)

Biochemical measurements

An amount of 10 mls of fasting blood specimen was drawn by venipuncture. The serum was obtained and stored at -20 °C. Biochemical analyses were done within 1 week.

Bone turnover markers were evaluated with the Elecsys 2010 system (Roche Diagnostics, USA) based on chemi-luminescence assays for serum osteocalcin (OC), and procollagen type 1 N –terminal propeptide (P1NP) and serum C-terminal beta-cross linking telopeptide of type I collagen (CTX-β). Intra- and inter- assay coefficients of variation were below 2% and 5% for all assays.

Serum 25-hydroxyvitamin D concentration was determined by auto-mation based on competitive chemi-luminescence technology (Diasorin Liaison System, USA). We defined vitamin D deficiency as 25-hydroxyvitamin D less than 17.5 nmol/l and vitamin D insufficiency as 25-hydroxyvitamin D less than 50 nmol/l (Thomas *et al*., 1998). The inter- and intra-assay coefficient of variations based on a pooled control were 7.0 % and 4.1 % respectively.

Bone densitometry

Bone mineral density (BMD) was measured at the lumbar spine (L1-L4), femoral neck and total hip with dual-energy X-ray absorptiometry (DXA) (Hologic Discovery, USA) by a single, trained technician. BMD measurements were expressed as T-scores using the World Health Organization criteria (1994). These criteria define normal bone mass as T-scores above -1, osteopenia as a T score of -1.0 and -2.4 and osteoporosis as a T-score of -2.5 or less. T scores were calculated from the normative Asian data-base used by the manufacturers of the device.

Statistical analyses

Data analysis was performed with Statistical Package for Social Sciences (SPSS) software version 16.0 (SPSS Inc. Chicago, USA). Descriptive statistics (mean, standard deviation) was used to describe the baseline characteristics, anthropometrics, bone turnover markers, calcium intake, and bone mineral density of the subjects. The normality test by Kolmogorov-Smirnov test had shown that P1NP, Osteocalcin, CTX-β, BMD femoral neck, BMD total hip and calcium intake were not normally distributed. Therefore, the non parametric test was used. The spearmen correlation coefficient (r) was used to demonstrate associations between variables measured in the same subjects. Mann Whitney and Kruskal Wallis were used to detect differences between variables. The significance level used for all tests was $p<0.05$ at 2-tailed significance test.

RESULTS

Descriptive characteristics of subjects

Table 1 shows the descriptive characteristics of the subjects. They had a mean age of 59.7 $\pm$ 5.0 years and had menopaused for 9.8 $\pm$5.2 years. The mean age of menopause was 49.8 $\pm$ 4.1 years. The majority of the subjects (89.1%) had natural menopause. The mean body mass index was 23.3 $\pm$ 3.2 kg/m^2 and most subjects had normal weight (64.6%) while 31.4% were overweight and obese. The mean percentage of body fat (36.6 $\pm$ 4.8%) was relatively high. This phenomenon is common amongst post-menopausal women as they tend to gain body fat due to the decrease in estrogen levels (Wang *et al*., 1994).

Calcium intake

Table 1 shows that the mean calcium intakes were 497 $\pm$ 233 mg/ day, fulfilling only 49.7% of the Malaysian RNI of 1000 mg/day for this age-group (NCCFN, 2005). A total of 35.4% of the subjects took calcium supplements. Taking this into consideration, the mean calcium intake then rose to 652 $\pm$ 342 mg, fulfilling 65% of RNI. Of the 178

Table 1. Descriptive characteristics of the study population (mean ± SD)

	N=178	
Age (y)	59.7 ± 5.0	
Age of menopause (y)	49.8 ± 4.1	
Years since menopause (y)	9.8 ± 5.2	
Natural menopause (% subjects)	89.9	
Surgery induced menopause (% subjects)	10.1	
Anthropometry		
Height (cm)	155.6 ± 4.9	
Weight (kg)	56.4 ± 8.0	
Body mass index (kg/m^2)	23.3 ± 3.2	
Percent body fat (%)	36.6 ± 4.8	
BMD (g/cm^2)		
Total body	0.97 ± 0.09	
Spine L1-L4	0.86 ± 0.13	
Hip		
Femoral neck	0.66 ± 0.10	
Total hip	0.80 ± 0.11	
Biochemical markers of bone turnover		
P1NP (ng/ml)	49.0 ± 16.10	<73.87†
% subjects within normal range		
Osteocalcin (ng/ml)		
% subjects within normal range	21.45 ± 6.84	11.00-43.00†
CTX (ng/ml)		
% subjects within normal range	0.518 ± 0.210	0.025-0.573†
Calcium intake (mg/day)		
Dietary calcium	497 ± 233	
Calcium supplementation	158 ± 259	
Total calcium intake	652 ± 342	
25 -OH Vitamin D (nmol/L)	60.4 ± 15.6	60-160†
% subjects normal (> 50 nmol/l) (%)	49.4	
% subjects hypovitaminosis D (<50nmol/l)	50.6	

† Normal range as per data provided by manufacturer

subjects, almost all of them (96.6%) failed to meet daily calcium requirements from food sources alone. Calcium supplements played an important role in boosting their calcium intake enabling 14% of the subjects to meet their daily calcium requirement of 1000 mg per day.

Vitamin D status

Mean serum level of 25-OH vitamin D of the subjects was 60.4 ± 15.6 nmol/L whereby 49.4% of subject achived the acceptable level of above 50 nmol/l (Green ***et al.***, 2006). However, the majority of the subjects (50.6%) can be classified as hypovitaminosis D (<50

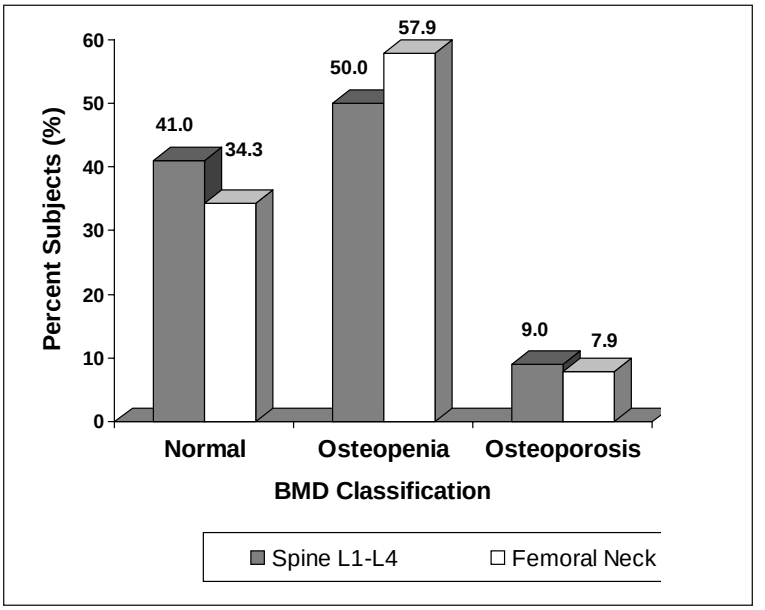

Figure 1. BMD classification of subjects

nnmol/l). No subject was vitamin D deficient or hypervitaminosis D (Table 1).

Bone turnover markers

Table 1 shows that majority of the subjects (>90%) were within the normal range for bone formation markers such as P1NP and osteocalcin. However, 29.3% subjects had high bone turnover based on their measurements of bone resorption marker, CTX-β.

Bone mineral density

Figure 1 shows that osteopenia was present in 50.0% of the subjects at the spine and 57.9% of the subjects at the femoral neck. This is not surprising given that the women were within the age range of 50-70 years, and bone loss is pravelent in this age-group. However, these groups of women may be at increased risk of osteoporosis in the future as they grow much older. Osteoporosis was already present in 9.0% of the subjects at the spine and 8% of the subjects at the femoral neck.

Associations between biochemical markers of bone turnover, BMD and related variables

Table 2 shows that subjects with osteoporosis at the spine had significantly lower BMI and lean body mass than osteopenic and normal subjects. Subjects with osteopenia at the spine had a significantly higher bone resorption marker, β-CTX than those with normal BMD. There was no significant difference in the vitamin D status of subjects with osteoporosis, osteopenia and normal BMD at the spine. The mean calcium intake of women with osteoporosis appears lower than others; however this was not statistically significant perhaps due to the small number of women with osteoporosis.

Table 3 shows similar relationships between bone variables at the femoral neck as seen in the spine. Subjects with normal BMD at the femoral neck had significantly lower levels of bone turnover markers of P1NP and osteocalcin compared to osteopenic and osteoporosis women.

Table 2. Comparison between normal, osteopenia and osteoporosis subjects for bone related variables at lumbar spine BMD (mean ± SD)

Spine BMD	*Normal N=69*	*Osteopenia N= 85*	*Osteoporosis N= 8*
BMI (kg/m^2)	23.9 ± 3.4 [a]	23.1 ± 3.1 [a,b]	21.7 ± 2.6 [b]
Fat (kg)	21.3 ± 5.1	20.8 ± 5.1	18.8 ± 4.2
Lean body mass (kg)	34.5 ± 2.9 [a]	32.9 ± 5.4 [b]	31.4 ± 3.4 [c]
Vitamin D (nmol/L)	60.7 ± 15.6	60.4 ± 16.1	58.3 ± 8.15
P1NP (ng/ml)	46.4 ± 13.5	50.7 ± 18	51.5 ± 13.4
Osteocalcin (ng/ml)	20.3 ± 5.7	22.2 ± 7.7	23.1 ± 5.0
CTX (ng/ml)	0.466 ± 0.178 [a]	0.555 ± 0.228 [b]	0.551 ± 0.191 [a,b]
Calcium intake(mg/day)	663 ± 391	660 ± 312	562 ± 247

* Different alphabets (a, b,c) indicate significant difference by Kruskal-Wallis and Mann-Whitney non parametric test (p<0.05)

Table 3. Comparison between normal, osteopenia and osteoporosis subjects for bone related variables at femoral neck BMD (mean ± SD)

Femoral neckBMD	*Normal N=59*	*Osteopenia N= 96*	*Osteoporosis N= 7*
BMI (kg/m^2)	23.7 ± 3.3	23.2 ± 3.1	22.4 ± 3.6
Fat (kg)	21.3 ± 5.1	20.5 ± 4.9	20.8 ± 4.9
Lean body mass (kg)	34.5 ± 3.2 [a]	33.4 ± 3.8 [b]	28.9 ± 9.1 [c]
Vitamin D (nmol/L)	61.4 ± 18.9	60.4 ± 13.5	51.3 ± 11
P1NP (ng/ml)	45.5 ± 12.5 [a]	50.1 ± 15.7 [b]	57.2 ± 28.1 [b]
Osteocalcin (ng/ml)	19.3 ± 5.1 [a]	22.2 ± 6.6 [b]	26.3 ± 12.0 [b]
CTX (ng/ml)	0.472 ± 0.135	0.536 ± 0.226	0.600 ± 0.328
Ca intake (mg/day)	657 ± 346	661 ± 349	573 ± 275

* Different alphabets (a, b,c) indicate significant difference by Kruskal-Wallis and Mann-Whitney non parametric test (p<0.05)

Subjects who had normal femoral neck BMD also had significantly higher lean body mass than osteopenic and osteoporosis subjects. Osteoporosis subjects had signficantly lower lean body mass than osteopenic subjects. As observed for the lumbar spine, osteoporotic women had lower mean calcium intake compared with osteopenic and normal femoral neck subjects but this was not statictically significant.

All the bone markers were significantly correlated with BMD. CTX was negatively correlated with BMD at the spine (r= -0.235, p<0.001), whereas P1NP (r=-0.215, p<0.001) and osteocalcin (r=-0.265, p<0.001) were both negatively correlated with BMD at the femoral neck (Table 4).

Body fat was significantly positively correlated (r=0.181, p<0.05) with BMD at the spine but not BMD at the femoral neck. Lean body mass was significantly correlated for both BMD at the spine (r=0.289, p<0.001) and femoral neck (r=0.295, p<0.001).

DISCUSSION

In the present study, more than 50% of the subjects were found to be osteopenic and 10% osteoporosis without the women being aware of their condition. The majority of women regarded themselves as having

Table 4. Correlation for bone related variables with BMD lumbar spine and femoral neck

	BMD lumbar spine r- value	*BMD femoral neck r-value*
P1NP	-0.159*	-0.215**
Osteocalcin	-0.174*	-0.265**
CTX	-0.235**	-0.167*
Vitamin D	0.035	-0.004
Body fat	0.181*	0.127
Fat free mass	0.289**	0.295**
Total Ca intake	-0.011	0.041

** Spearman correlation significant at $p<0.01$
* Spearman correlation significant at $p<0.05$

'normal bone' before entering this study. This implies that screening of bone density is important amongst free living post-menopausal woman as they may be having osteoporosis silently and may be aware only after the first fracture has occurred.

Low calcium intake has been found to be a risk factor amongst Asian women for hip fractures (Lau *et al*., 2001). The present study has found that almost all the subjects did not meet their calcium requirement and 29.3% of these women had high bone resorption as indicated by CTX- β levels. A previous study has also shown that brief increases of calcium intake from 500mg to above 1000mg/day was accompanied by a reduced rate of bone resorption by 33% (Shapses *et al*., 1995).

Taking into account the difficulty of achieving sufficient calcium from diet, elemental calcium supplement might be an alternative. This study has shown that amongst women who consumed calcium supplements, their mean calcium intake increased to 652 + 342 mg, fulfilling 65% of the RNI. Increasing calcium intake through supplementation can lead to decreased bone resorption (Kamel *et al*., 1998). Kenny *et al*. (2004) has shown that supplementation of 1000mg/day of Ca for 24 weeks to post-menopausal women decreased the collagen crosslink resorption markers, urine N-telopeptide (-30%), C telopeptide (-31%), free deoxypyridinoline (19%) and serum N telopeptide (-8%) (Kenny *et al*.,2004).

The present study found that subjects with normal BMD had significantly lower levels of bone turnover markers, that is, P1NP and osteocalcin compared to osteopenia and osteoporosis subjects. Similarly, women with osteopenia and osteoporosis had higher levels of bone resorption marker CTX in their blood. None of the subjects were on hormone replacement therapy and a high bone turnover rates coupled with low BMD may increase the risk of osteoporosis and fractures. A previous study by Bauer *et al.* (1999) had shown that higher levels of bone resorption markers are associated with faster bone loss at the hip in elderly women not receiving estrogen replacement therapy. Increased bone turnover rate raises bone fracture risk (Glover *et al*., 2008), particularly trabecular bone (Gerdhem *et a*l., 2004). This is because increased bone turnover accelerates bone loss, damages micro architectural skeleton and bone strength (Glover *et al*., 2008).

Vitamin D status, which is most closely reflected by the serum concentration of 25-hydroxyvitamin D, is a topic currently of interest in relation to bone health. Insufficient serum concentrations of 25(OH) D are thought to lead to secondary hyper-

parathyroidism, which is a contributing factor to the age related acceleration of bone loss (Janssen , Samson & Verhaar, 2002). However, despite Malaysia being a country with sunlight the whole year through, more than half of the subjects (50.6%) were hypovitaminosis D with the mean serum level of 60.4 + 15.6 nmol/L. A previous study amongst post-menopausal Chinese women in Kuala Lumpur reported a similar high prevalence of hypovitaminosis of 87% (Suriah *et al*., 2004). Possible explanation for low vitamin D status could be the hot weather and less preference for outdoor activities amongst these women.

Body composition factors such as fat mass and muscle mass may have protective effects on bone health. Muscle seems to exert a larger influence than fat mass, body weight and age on bone mass capacity in the whole body and lower limbs regardless of the gender and reproductive status of the individual (Capozza *et al*., 2004). Douchi *et al.* (1997) reported that total fat mass (r= 0.38, P<0.05) was the most significant determinant of lumbar BMD, while total lean mass (r=0.38, p<0.05) was the most significant determinant of total body BMD (Gjesdal *et al*., 2007). In the Hordaland health study, lean mass was generally more strongly related to BMD of the femoral neck in middle aged and elderly men and women compared to fat mass, in which a 10 kg increase in lean mass was associated with 0.083 g/cm^2 increase in BMD (Gjesdal *et al*., 2007). Our observation was consistent with the previous study, where body fat was significantly positively correlated (r=0.181, p<0.05) with BMD at the spine but not BMD at the femoral neck. Lean body mass was significantly correlated for both BMD at the spine (r=0.289, p<0.001) and femoral neck (r=0.295, p<0.001). This was because muscles exert their action through insertion into bones. The bulk of muscle and other non fat soft tissues contribute to the load carried by the skeleton, and this tends to lead to a positive relationship between the masses of these two tissues (Reid, 2002).

In conclusion, this study demonstrated that the majority of free living Chinese post-menopausal women in Kuala Lumpur have low calcium intake, low 25-OH vitamin D status and low bone mass and elevated biochemical markers of bone turnover. Appropriate lifestyle intervention is needed to decrease the risk of osteoporosis in their later years.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Nestle Research Center, Switzerland for funding this study. The cooperation of all the women who participated in this study, the bone densitometry technician, lab technicians, community leaders and many others are gratefully acknowledged.

REFERENCES

Bauer DC, Sklarin PM, Stone KL, Black DM, Nevitt MC, Ensrud KE, Arnaud CD, Genant HK, Garnero P, Delmas PD, Lawaetz H & Cummings SR (1999). Biochemical markers of bone turnover and prediction of hip bone loss in older women: the study of osteoporotic fractures. *J Bone Miner Res* 14: 1404-1410

Brenneman SK, Barrett-Connor E, Sajjan S, Markson LE & Siris ES (2006). Impact of recent fracture on health related quality of life in postmenopausal women. *J Bone Miner Res* 21: 809-816.

Cremers S & Garnero P (2006). Biochemical markers of bone turnover in the clinical development of drugs for osteoporosis and metastatic bone disease. *Drugs* 66(16): 2031-2058.

Capozza RF, Cointry GR, Cure-Ramirez P, Ferretti JL & Cure C (2004). A DXA study of muscle-bone relationship in the whole body and limbs of 2512 normal men and

pre- and post-menopausal women. *Bone* 35: 283-295

Chee SS, Suriah AR, Zaitun Y, Chan SP, Yap SL & Chan YM (2002). Dietary calcium intake in postmenopausal Malaysian women: comparison between the food frequency questionnaire and three-day food records. *Asia Pac J Clin Nutr* 11: 142-146.

Douchi T, Oki T, Nakamura S, Ijuin H, Yamamoto S & Nagata Y (1997). The effect of body composition on bone density in pre- and postmenopausal women. *Maturitas* 27(1): 55-60.

Gerdhem P, Ivaska KK, Alatolo SL, Halleen JM, Hellman J & Isaksson A, Petterson K, Vaananen HK, Akesson K & Obrant KJ (2004). Biochemical markers of bone metabolism and prediction of fracture in elderly women. *J Bone Miner Res* 19: 386-393.

Gjesdal CG, Halse JI, Eide GE, Brun JG & Tell GS (2007). Impact of lean mass and fat mass on bone mineral density: The Hordaland Health Study. *Maturitas* 59(2): 191-200.

Glover SJ, Garnero P, Naylor K, Rogers A & Eastell R (2008). Establishing a reference range for bone turnover markers in young, healthy women. *Bone* 42: 623-630.

Janssen HCJP, Samson MM & Verhaar HJJ (2002). Vitamin D deficiency, muscle function, and falls in elderly people. *Am J Clin Nutr* 75: 611-615.

Kamel S, Fardellone P , Meddah B., Lorget-Gondelmann F, Sebert JL & Brazier M (1998). Response of several markers of bone collagen degradation to calcium supplementation in post-menopausal women with low calcium intake. *Clin Chem* 44: 1437-1442.

Kenny AM, Prestwood KM, Biskup B, Robbins B, Zayas E, Kleppinger A, Burleson JA & Raisz LG (2004). Comparison of the effects of calcium loading with calcium citrate or calcium carbonate on bone turnover in post-menopausal women. *Osteoporos Int* 15: 290-294.

Lau EMC, Lee JK, Suriwongpaisal P, Saw SM, Dasde S, Khir A, Sambrook P (2001). The incidence of hip fracture in four Asian countries: the Asian Osteoporosis Study. *Osteoporos Int* 12: 239-243.

Lee, JK & Khir ASM (2007). The incidence of hip fracture in Malaysians above 50 years of age: variation in different ethnic groups. *APLAR J Rheumatol* 10(4): 300-305.

Lim PS, Ong FB, Adeed N, Seri SS, Noor-Aini MY, Shamsuddin K, Hapizah N, Mohamed AL, Mokhtar A & Wan HWH (2005). Bone health in urban midlife Malaysian women: risk factors and prevention. *Osteoporos Int* 16: 2069-2079

National Coordinating Committee on Food and Nutrition (2005). Recommended Nutrient Intakes for Malaysia. Ministry of Health Malaysia, Kuala Lumpur.

National Institute of Health (2001). Consensus development panel on osteoporosis prevention, diagnosis and therapy. *JAMA* : 285:785-795.

Reid I (2002) . Relationships among body mass, its components, and bone. *Bone* 31: 547-555.

Shapses SA, Robins SP, Schwartz EI & Chowdhury H (1995). Short term changes in calcium but not protein intake alter the rate of bone resorption in healthy subjects as assessed by urinary pyridinium cross-link excretion. *J Nutr* 125: 2814-2821.

Suriah AR, Chee WSS, Zaitun Y & Chan SP (2004). Vitamin D status among post-menopausal Malaysian women. *Asia Pac J Clin Nutr* : 13: 255-260.

Tanko LB, Christiansen C, Cox DA, Geiger MJ, McNabb MA & Cummings SR (2005). Relationship between osteoporosis and cardiovascular disease in post-menopausal women. *J Bone Miner Res* 20: 1912-1920.

Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska D, Kitch BT, Vamvakas EC, Dick IM, Prince RL, Finkelstein JS (1998). Hypovitaminosis D in medical inpatients. *N Engl J Med* 338: 777-783.

Wang Q, Hassager C, Ravn P, Wang S & Christiansen C (1994). Total and regional body composition changes in early postmenopausal women: age related or menopause related. *Am J Clin* Nutr 60: 843

World Health Organisation (1994). Assessment of Fracture Risk and its Application to Screening for Post-Menopausal Osteoporosis. Technical Report Series no. 843, Geneva WHO