Peak bone mass as measured by phalangeal bone mineral density and its association with nutritional status, socioeconomic status and physical activity: A community based-cross-sectional study in Galle district

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Abstract

Current knowledge on bone mineral density (BMD) changes during adolescence is based on the studies done in Western populations. Obvious differences in determinants of bone health between Asians and Europeans would not allow such a comparison. Knowledge of the age in which BMD attains its peak is important in planning health promotional activities in a country. Current recommendations on bone health in Sri Lanka are based on studies conducted in Western populations but geographical variations in BMD accrual would limit such an application. This project examines phalangeal BMD (pBMD) in subjects selected from the Galle district to ascertain the timing of BMD peak and its associations with nutritional status, socioeconomic status and physical activity, in a cross-sectional manner. The age at which the peak phalangeal BMD is achieved was determined in 657 healthy men and women, aged between 20-49 years, selected by stratified randomization from the Galle District. The peak phalangeal BMD was seen in men and women between 30-39 years. Females of this age group (i.e. 30-39 years, n=582) were further studied to examine the associations of their bone mineral density and bone mineral content (BMC)with anthropometry, physical activity (current and past), socioeconomic status, dietary intake and biochemical markers of bone health.

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Introduction

Peak bone mass (PBM) can be defined as the amount of bony tissue present at the end of skeletal maturation. The bone mass of a given part of the skeleton is directly dependent upon both its volume (size) and the density of the mineralized tissue contained within the periosteal envelope (1). It has been generally accepted that peak bone mass at any skeletal site is attained in both sexes during mid-thirties (1). It is considered that bone mass increases rapidly during childhood and adolescence to 90% of adult levels and reaches the peak in the third decade (1). Thereafter, bone mass starts to decline where minerals and collagen matrix are removed from bone more rapidly than new bone tissue is added. The rate of bone loss, however, varies between two genders and in different age groups (1).

Peak bone mass and the subsequent rate of loss would determine the amount of bone mass left in old age. From youth to old age, women typically lose half of trabecular and one-third of cortical bone, whereas men lose only one-third of trabecular bone and one-fifth of cortical bone (2). Therefore, Frankie, in 2004 (2) suggested that optimizing PBM at the point of skeletal maturity is one method of ensuring higher bone mass in later life as it means that loss begins at a higher point and it would take a longer time before bone mass becomes critical.

All the studies on PBM have used either BMD or BMC as the in-vivo methods of quantifying bone mass and despite subtle differences they have used these terms interchangeably in studies on PBM (3,4). The importance of achieving an ideal peak bone mass for bone strength in later life has been suggested by previous observational and longitudinal studies (2,4). They have suggested that individuals with a high peak bone mass are likely to have a high bone mass in old age, so that, osteoporosis-prone individuals could be identified much earlier in life if bone density was low for age. Osteoporosis is a systemic skeletal disease

that is mainly characterized and quantified by low BMD. Although a great emphasis has been placed on understanding the mechanisms of bone loss in postmenopausal women and older men, it is now known that early life events are equally important in the pathogenesis of osteoporosis (4).

Several variables, more or less independent, are supposed to influence bone mass accumulation during growth; heredity, sex, dietary components, endocrine factors, mechanical forces, and exposure to risk factors (5). Quantitatively, the most prominent factor appears to be the genetic determinant, as estimated by studies comparing monozygotic and dizygotic twins (7). With respect to nutrition, the quantitative importance of calcium intake in bone mass accumulation during growth, particularly at sites prone to osteoporotic fractures, remains to be clearly determined; the same can be said for the impact of physical activity. Finally, the crucial years when these external factors will be particularly effective on bone mass accumulation remain to be determined by longitudinal prospective studies in order to produce credible and well targeted recommendations for the setting up of osteoporosis prevention programs aimed at maximizing peak bone mass (6).

The mass of bone tissue present at any time during adult life is the difference between the amount achieved at maturity and the loss due to aging. Hence there is a growing interest among researchers to investigate how bone mass evolves during development and to identify the important factors that influence its rate of accumulation (8). Experimentally, bone mass is assessed by burning a piece of bone and estimating the weight of ash. Using pork carcasses, Michell *et al.*, in 1998 (9) found a strong correlation between the ash content and both BMD and BMC measured by Dual Energy Xray Absorptiometry (DXA). Nagy *et al.*, in 2002 (10), found 100% accuracy of DXA in estimating bone mass when compared with weight of ash. Hence, both BMD and BMC are used as surrogate measurements of bone mass in clinical applications and in-vivo studies.

There are many methods available for bone mass measurement. They are not much used in clinical practice. Most of them are used only for research purposes. For example, single-proton absorptiometry (SPA), dual proton absorptiometry (DPA), single energy X-ray absorptiometry (SXA) and radiographic photodensitometry (11,12) The most widely used technique to measure bone density is DXA.

Measurement of bone mineral content and bone mineral density

BMC is measured in grams of bone size where as BMD is expressed as the BMC over the antero-posterior projected bone area (BA) measured in square centimetres.

$$\text{BMD} = \frac{\text{BMC}}{\text{BA}} \ \text{g/cm}^2$$

There are several different machines that measure bone density. Central machines measure density in the hip, spine and total body. Peripheral machines measure density in the finger, wrist, kneecap and heel.

Dual energy X-ray absorptiometry

Radioigraphic absorptiometry is the modern-day descendent of radiographic photodensitometry (11). The underlying principle of Dual – energy X-ray absorpotiometry (DXA) is to determine the bone density in *vivo* by passing a monochromatic or dual energy photon through bone and soft tissue (11). The amount of mineral encountered by the beam could be quantified by subtracting the beam intensity after passage through the region of interest from the initial beam intensity. In radiology, attenuation refers to a reduction in an X-ray beam's intensity. The differences in tissue densities are responsible for creating the images seen on an X-ray. When the beam was passed through a region of the body containing both bone and soft tissue, attenuation of the photon beam occur at both energy peaks (12).

The regions most often measured with DXA are the hip (proximal femur) and lumbar spine (second lumbar vertebra to fourth lumbar vertebra), but it can also be used for measurement of other peripheral sites such as radius, calcaneous or even the total body. From a total body scan, lean mass, fat mass and even regional bone masses like arms, legs, head, and trunk can be obtained. Hip measurement is the best site for predicting hip fracture where as the spine measurement is the best site for the prediction of vertebral fracture. Central DXA (lumbar spine and proximal femur) is used for research and making therapeutic decisions, whereas peripheral DXA (finger and or heel), is used for research and screening purposes (12). Dual energy X-ray technology is also being employed in portable devices dedicated to the measurement of one or two appendicular sites. As such, these devices are characterized as peripheral DXA devices (12).

Method

Investigation 1

The eligible men and women were subjected to the measurement of BMD and BMC of the middle phalanx of the middle finger of the non-dominant hand (14) using the accuDXA (Shick technology, New York, USA), portable densitometer with DXA. Its scan time

is less than one minute and gives BMD as g/cm^2 and BMC in grams. Machine has a precision of less than 1.0% with an inbuilt quality control procedure, hence no user intervention is required (12). It initially obtains a digital picture of the middle phalanx to make sure the proper positioning of the region of interest in the scanning area and then measures both bone mineral content and bone mineral density. All scans were done with the help of one technician who had training on accuDXA and the accuracy and precision of the machine was tested on each scanning day using the in built software in the machine. Machine was kept in a cool environment during scanning to ensure the accuracy of estimations.

Seven hundred and forty five (745) individuals of 20 to 49 years of age were invited and 702 subjects (response rate 94.2%) attended the interview (352 males and 350 females). Forty five subjects (6%) were excluded from the sample due to the presence of diseases or having received medications, which could affect bone metabolism.

Investigation 2

After analyzing the results of the investigation 1, (table 1.1 & 1.2 above) it was found that both men and women reached peak bone mass between 30-39 years. BMD among women in this age group was further studied to examine the association of BMD with physical activity, social status, dietary intake, nutritional status assessed by anthropometry and biochemical markers of bone health.

Table 1.1 Mean BMD (SD) of 324 males and 333 females according to the age categories[1]

	20-29 yrs	30-39 yrs	40-49 yrs	*P-value
Male	0.595 (0.057)	0.603 (0.061)	0.591 (0.066)	0.32
Female	0.495 (0.057)	0.506 (0.062)	0.502 (0.064)	0.42

[1]There were 108 males and 108 females in 20-29 yrs category, 105 males and 110 females in 30-39 yrs category and 111 males and 115 females in 40-49 yrs category

*p compares the mean BMDs in three age groups and calculated using ANOVA

Measurements of the BMD and BMC

Measurements of the BMD and BMC were done by using AccuDXA (Shick technology, New York, USA). Measurements were taken by the same technician who participated in the investigation 1, and precautions described earlier were taken to ensure the accuracy of the measurements.

Table 1.2 Mean BMC (SD) of 324 males and 333 females according to the age categories[1]

	20-29 yrs	30-39 yrs	40-49 yrs	*P-value
Male	2.15 (0.34)	2.21 (0.36)	2.17 (0.39)	0.48
Female	1.48 (0.28)	1.55 (0.31)	1.55 (0.31)	0.15

[1]There were 108 males and 108 females in 20-29 yrs category, 105 males and 110 females in 30-39 yrs category and 111 males and 115 females in 40-49 yrs category

*p compares the mean BMCs in three age groups and calculated using ANOVA

Biochemical analysis

The subjects were instructed to be present at designated places at a given time (i.e. 0800hours in the morning) in batches with overnight fasting to obtain a sample of venous blood (5ml) for the biochemical assessment of serum vitamin D, parathyroid hormone and alkaline phosphatase. Blood was drawn from the median cubital vein under sterile conditions using disposable syringes. Specimens were brought immediately to the radioimmuneassay (RIA) laboratory of the Nuclear Medicine Unit, Faculty of Medicine in a box containing ice packs and serum separation was done without a delay and the separated sera were stored in a freezer at -80°C.

Results

Bone mineral density (BMD) and bone mineral content (BMC)

Mean (SD) BMD of the study sample was 0.493 (0.06) g/cm^2 and mean BMC was 1.49 (SD 0.28) g. BMD in the sample ranged from 0.230 to 0. 660 g/cm^2 while BMC ranged from 0.85 to 2.36 g. characteristics of study subjects were shown in Table 2.1

Effect of anthropometry on BMD/ BMC

Simple linear regression was performed with BMD as the dependent variable and all anthropometric measurements separately as independent variables. When all measures were included in the model and

Table 2.1 Characteristics of the subjects under study (n=582)

Measurement	Unit	Mean	SD
Weight	kg	51.06	8.9
Height	cm	154	5.6
BMI[1]	kg/m^2	21.61	3.6
Abd girth[2]	cm	74.4	8.9
Chest circum[3]	cm	86	8.3
Hip circum[4]	cm	89.97	8.3
SFT[5]	mm	15.72	6.4
Foot length	cm	23.48	7.9

[1]BMI – body mass index (weight / height^2)
[2]Abd girth - Abdominal girth
[3]Chest circum -Chest circumference
[4]Hip circum -Hip circumference
[5]SFT- Skin fold thickness

Table 2.2 The association between BMD and anthropometric measurements[1]

Measurement	r	r^2	Regression Co-efficient	p-value
Weight (kg)	0.29	0.08	0.002	<0.001
Height (cm)	0.34	0.12	0.004	<0.001
BMI (kg/m^2)	0.15	0.02	0.003	<0.001
Abd girth (cm)	0.2	0.04	0.001	<0.001
Chest circum (cm)	0.2	0.04	0.001	<0.001
Hip circum (cm)	0.18	0.03	0.001	<0.001
SFT (cm)	0.01	0.0001	0.0001	p=0.80
Foot length (cm)	0.03	0.001	0.0002	p=0.49

[1]n= 582

weak associations were excluded in step-wise fashion, height remained the strongest predictor of BMD (regression coefficient 0.004; SE 0.0004; p<0.001). In this model, height alone explained 12% of variation in BMD (Table 2.2).

Other measurements such as weight; abdominal circumference, chest circumference, and hip circumference, when taken individually were able to explain only 3-8% of variation in BMD. A one centimeter difference in height was associated with 0.004 g/cm^2 difference in BMD. Similarly, a change of one kilogram was associated with 0.002 g/cm^2 change in BMD. A unit increase in BMI was associated with an increase in BMD of 0.003 g/cm^2 (Table 2.2).

Socioeconomic status on BMD/ BMC

Classification of subjects according to the current socio-economic status is shown in Table 3. None were qualified to be included in Class I while 242 women were in social Class 2. There was no. difference in BMC/ BMD according to their social class indicating that socioeconomic status of these women had no significant influence on the peak bone mass observed

Physical activity on BMD/ BMC

They were categorized according to their physical activities as very active, moderately active, and less active depending on the type and duration of their physical activities. None of the participants were qualified to be named very active based on their current physical activity. BMD and BMC results according to their physical activity are presented in Table 4.

Women who were very active in their school days had the highest BMD irrespective of their current activities (mean BMD of 0.533 in very active women in the past). Women who were less active both in the past and at present had the lowest BMD (mean level of 0.448). In general, there was a positive association between BMD and the intensity of physical activity, in which BMDs of the moderately active women in the past were in between those of less and very active women irrespective of their current activities (p<0.001).

Dietary intake on BMD/ BMC

The intake of macro and some micro nutrients were assessed by a 24-hour dietary recall on three non consecutive days. The dietary survey data sheets that lacked information on any of the three days were excluded (n=5). Furthermore, incomplete recalls (n=3) or unrealistic data (n=53) that could not be corrected reliably were also rejected. Exclusion of unrealistic data was done with the agreement of the specialist on nutrition who oversaw the data collection. 524 study subjects or 90.0% of the total sample were able to provide complete dietary records. After recording and summarizing the recalls, the food intake data were converted into nutritional values with the help of Food Composition Tables (15). Daily energy and nutrient intakes were calculated in the three-day intake and presented as the average intake (mean, SD, median and inter-quartile range) in Table 5.

Table 3 BMC and BMD according to the socioeconomic status[1]

Category		n	BMC mean	BMC SD	BMD mean	BMD SD
Social Class[2]	Class- 2	242	1.485	0.269	0.49	0.057
	Class -3	136	1.483	0.285	0.489	0.059
	Class -4	155	1.523	0.298	0.497	0.063
	Class- 5	50	1.512	0.298	0.501	0.062
			(f=0.87; p=0.46)		(f=0.74; p=0.53)	
Marital status	Unmarried	202	1.507	0.231	0.496	0.052
	Married	380	1.492	0.308	0.491	0.064
			(f=0.90; p=0.34)		(f=0.38; p=0.54)	
Education[3]	Upto O/L	198	1.55	0.368	0.504	0.074
	A/L	324	1.45	0.221	0.484	0.051
	Above A/L	60	1.572	0.208	0.509	0.045
			(f=10.35; p<0.001)		(f=9.85; p<0.001)	
Occupation[4]	Professional	254	1.444	0.227	0.482	0.052
	Clerical	133	1.541	0.225	0.502	0.048
	Skilled	132	1.333	0.24	0.463	0.053
	Unemployed	63	1.961	0.142	0.58	0.032
			(f=121.55; p<0.001)		(f=85.86; p<0.001)	
Income[5]	< Rs.5,000.00	20	1.784	0.369	0.548	0.078
	Upto Rs.10,000.00	162	1.601	0.278	0.515	0.058
	> Rs. 10,000.00	400	1.44	0.26	0.481	0.056
			(f=32.25; p<0.001)		(f=28.98; p<0.001)	

[1]n= 582, p and f -values were calculated from analysis of variance (ANOVA)
[2]Classification of social classes were given in Chapter 2, section 2.2.2.4, page 24
[3]Educational level was grouped as those who did not complete Advanced Level (A/L) examination, an upto Ordinary level (O/L), those who had at least one attempt in A/L to A/L group and those who had either a diploma or University degree in above A/L group 4 Income was calculated for the gross earnings of the family

Table 4 Mean BMD (SD) by past and present physical activities

		Past activities: Very active	Past activities: Moderately active	Past activities: Less active
Present activities	Moderately active	0.533 (0.050)[a] (n=127)	0.491 (0.06)[b] (n=134)	0.471 (0.05)[c] (n=97)
	Less active	0.533 (0.04)[a] (n=40)	0.477 (0.05)[b] (n=142)	0.448 (0.07)[c] (n=42)

[a b c] values with different superscript in a row are significantly different (ANOVA, p<0.001)

Table 5 The average daily energy and nutrient intakes[1]

Parameter	Mean intake	SD	Median	IQR
Energy (Kcals/day)	2268.6	515.8	2285.57	1910 -2600
Protein (g/day)	27.72	14.5	24.39	20.60 -28.40
Fat (g/day)	43	26.8	38.03	30.10 - 50.40
Calcium (mg/day)	783.49	461.6	659.33	450.00 - 1028.00
Phosphate (mg/day)	1042.39	289.3	1009.42	841.00 -1216.00
Iron (mg/day)	20.04	12.3	18.38	14.30 -23.40
[2]Vitamin D (µg/day)	4.98	5.53	2.5	1.5 - 25.96
[3]Vitamin K (µg/day)	0.88	1.43	4.03	0.21 - 2.42
[4] Vitamin A (µg/day)	266.9	1.72	275.62	192.10 - 387.60

[1] calculated from the 3-day intake and presented as mean intake, standard deviation (SD), median and inter quartile range (IQR); n=524
[2] data on vitamin D intake obtained from 363 subjects
[3] data on vitamin K intake obtained from 78 subjects only
[4] data on vitamin A intake obtained from 413 subjects

Table 6 Daily intake of nutrients (according to % Recommended Daily Energy Intake)[1]

	Below 75% (n=68)	75-100% (n=190)	above 100% (n=324)	F-test[2]	P-value[2]
Energy intake (Kcals)	1406.55 (165.8)	1949.02(150.6)	2636.90(327.2)	809.91	<0.001
Protein intake (gm)	23.63(12.6)	25.47(13.6)	29.89(14.9)	8.89	<0.001
Fat intake (gm)	32.64(20.4)	37.76(25.1)	48.17(27.7)	15.52	<0.001
Calcium intake (mg)	468.72(251.5)	718.86(410.3)	856.30(487.2)	27.84	<0.001
Phosphate intake (mg)	722.97(170.6)	911.37(196.0)	1186.27(264.1)	154.27	<0.001
Iron Intake (mg)	13.94(8.2)	17.03(6.0)	23.08(14.5)	26.25	<0.001
Vitamin D(µg)	6.55(36.16)	24.68(43.5)	20.82(41.6)	0.021	0.487
Vitamin K(µg)	2.63(1.4)	2.04(3.1)	1.48(1.7)	1.104	0.337
Vitamin A (µg)	318.64(165.0)	291.49(137.6)	306.03(143.4)	0.794	0.453

[1]The recommended daily intake (RDI) of energy was taken as 2200 Kcals/day based on recommendations of WHO/FAO expert panels and published in Nutrition Guide by Department of Health Services in 2000 (16)
[2] The f-test and p-value from analysis of variance (ANOVA)

Table 7 BMC/BMD in different categories of energy intake(n=524)

	Subjects who met daily energy requirement				
	<75% (n=68)	75-100% (n=190)	>100% (n=324)	F-test	P-value
BMD	0.484 (0.05)	0.488 (0.06)	0.497 (0.06)	2.22	0.11
BMC	1.433 (0.27)[a]	1.468 (0.28)[a]	1.527 (0.28)[b]	4.62	0.01

F-test and p-value from analysis of variance
[a b] values with different superscript in a row are significantly different (p<0.01)

Table 8 Mean BMC/ BMD of different categories determined by consumption of calcium from dairy products [1]

	Daily (n=343)	2-5 times per week (n=206)	Less than 2 times per week (n=30)	F-test	P-value
BMC	1.498 (0.015)[a]	1.512 (0.020)	1.373 (0.053)[b]	3.114	0.045
BMD	0.492 (0.003)	0.497 (0.004)	0.472 (0.011)	2.317	0.099

[1] corrected for calcium intake from non-dairy products; results expressed as mean (SE)
[a b] values with different superscript in a row are significantly different (ANOVA; p<0.01)

Table 9 Mean BMC/ BMD of different categories determined by consumption of calcium from non-dairy products[1]

	Daily (n=172)	2-5 times per week (n=304)	Less than 2 times per week (n=102)	F-test	P-value
BMC	1.508 (0.022)	1.507 (0.016)	1.446 (0.029)	1.881	0.164
BMD	0.495 (0.005)	0.495 (0.003)	0.482 (0.006)	1.87	0.155

[1] corrected for calcium intake from dairy products; results expressed as mean (SE); f-test and p-value from analysis of variance (ANOVA)

Table 10 Mean BMD (SD) according to the frequency of dairy and non-dairy calcium consumption [1]

		Dairy calcium		
		Daily	2-5 times wk	< 2 times wk
Non dairy calcium	Daily	0.493 (0.07 n=171	0.497 (0.07) n=169	0.508 (0.07) n=101
	2-5 times wk	0.495 (0.06) n=323	0.496 (0.06) n=250	0.484 (0.06) n=125
	<2 times wk	0.476 (0.05) n=222	0.495 (0.06) n=154	0.452 (0.05) n=66

[1]BMD of women who consumed less calcium (either in the form of dairy or non dairy) was significantly lower (p<0.05 by ANOVA)

Table 11 Mean values (SD) of serum 25(OH) D, i-PTH and ALP in 434 subjects

Parameter	unit	mean	SD
Serum parathyroid hormone	pg/ml	49.97	24.64
Serum 25(OH) D	nmol/L	35.32	24.71
Serum Alkaline Phosphatase	IU/L	64.18	27.51

Table 12 Pearson correlation coefficient (r) between BMD/BMC and serum measurements[1]

Measurement	BMD		BMC	
	r	p-value	r	P-value
25(OH) D	0.127	0.008	0.124	0.01
i-PTH	-0.164	0.001	-0.152	0.002
ALP	-0.016	0.74	-0.03	0.52

[1] n=434; serum 25(OH) D has shown a significant positive correlation with BMD/ BMC whereas intact parathyroid hormone has shown a significant negative correlations ($p<0.05$)

Table 13 Anthropometry, BMD and other serum measurements (PTH, ALP) in the thirds of 25(OH) D concentrations[1]

Measurement	Lower third of 25(OH)D	Middle third of 25(OH)D	Upper third of 25(OH)D	f-test	P-value
BMC	1.475 (0.29)	1.489 (0.28)	1.542 (0.27)	2.147	0.12
BMD	0.485 (0.06)[a]	0.493 (0.05)[a]	0.503 (0.06)[b]	3.449	0.03
Weight	50.57 (9.2)	51.17 (9.2)	51.02 (8.1)	0.111	0.9
Height	155.28 (5.0)[a]	153.50 (6.0)[b]	152.59 (5.2)[b]	8.047	0.03
BMI	20.98 (3.7)	21.75 (3.8)	21.93 (3.3)	2.262	0.11
Foot length	23.28 (1.3)	24.46 (1.7)	23.13 (1.0)	0.725	0.49
Abd girth	74.22 (8.8)	74.90 (9.2)	74.43 (9.2)	0.186	0.83
Chest circum	85.78 (7.9)	86.29 (8.5)	86.30 (8.1)	0.162	0.85
Hip circum	90.36 (8.0)	90.75 (8.3)	88.84 (8.8)	1.93	0.15
SFT	15.21 (5.9)	16.01 (6.4)	15.86 (6.9)	0.533	0.59
Serum i-PTH	69.61 (16.7)[a]	53.10 (21.6)[b]	30.11 (17.7)	141.91	<0.001
Serum ALP	61.72 (24.6)	66.52 (25.7)	64.50 (31.2)	1.119	0.328

[1] there were 145 subjects in each thirds of serum vitamin D
[a, b,c] values with different superscript in a row are significantly different (ANOVA; $p<0.05$)

The intake of different nutrients and the recommended daily intake (RDI) for Sri Lankan (16) in this age group were examined. It was evident that only 324 women (55.7%) meeting the recommended daily energy intake of 2200 Kcals/ day. 514 women (88.3%) met up to 75% of daily energy requirement and in 11.7% of women's diet contained below 75% (Table 6) of the requirement. It was also shown that the intakes of other dietary components (protein, fat, calcium, iron and phosphate) were significantly improved with the energy intake except in the case of dietary vitamin A, D and K (Table 6). In the categorical analysis (Table 7), mean (SD) BMD and BMC values in the three categories of energy intake, defined as a percentage of total energy intakes, showed a positive trend where women in the highest energy category had the highest BMD/BMC while women in the lowest energy intake category had the lowest BMD and BMC values. Difference of BMD (2.6%) between the lowest and the highest was not statistically significant while difference of BMC (6.6%) was significant.

Effect of calcium on BMD/BMC

In addition to the survey by 24-hour recall method, the calcium intake among women was assessed by using food frequency questionnaire. Among the good sources of calcium, dairy products (milk, yoghurt, curd and cheese) as well as non dairy calcium sources such as green leaves, legumes, and small fish were considered and the frequency of their intake was documented. For the analysis, subjects were categorized as those who consumed these foods daily, 2-5 times per week and less than 2 times per week.

Effect of the consumption of dairy products on BMD/ BMC was examined after adjusting for non dairy calcium intake. Consumption of dairy calcium daily or more than 2-5 times per week was associated with significantly higher BMC (p=0.05) when compared with those who consumed dairy calcium less than 2 times per week (Table 8). A Similar relationship was found with BMD but the difference did not reach statistical significance. Further, **effects of the consumption of non dairy calcium on BMD/ BMC were examined after adjusting for dairy calcium. Even though it was evident that BMC/ BMD levels were low in those who consumed non dairy calcium less than 2 times per week, the difference was not statistically significant (Table 9).**

The effect of both dairy and non-dairy calcium on BMD was examined in 3x3 table using ANOVA with categories of dairy and non-diary calcium as fixed factors (Table 10). There was no significant difference in BMD in women who consumed any source of calcium more than 2 times a week. However, women who consumed both dairy and non-diary calcium less than 2 times per week had the lowest BMD.

Biochemical measurements

Serum samples obtained from study subjects were analyzed for 25-hydroxy vitamin D {25(OH) D}and intact-parathyroid hormone (i-PTH) using radioimmunoassay technology. Serum total alkaline phosphatase (ALP) levels were also estimated using kinetic method. Blood was not able to collect from 62 subjects as they did not present for blood drawing sessions. Furthermore, 86 serum samples that were spoilt during storage had to be discarded. As a result, 434 serum samples were finally analyzed for 25(OH) D, i-PTH, ALP and the results are illustrated in Table 11. Mean (SD) level of 25(OH) D was 35.32 (24.7) nmol/L while median and IQR were 30.84 and 15.75-52.36 respectively. **Severe vitamin D deficiency below 12.5**nmol/L **defined according to the Lips (2001) classification (127) (below 12.5**nmol/L**) was seen in 21.4% of subjects. 19.1% subjects had moderate (12.5- 25.0**nmol/L) and 15.7% had mild (25.0-35.0nmol/L) vitamin D deficiency.

Mean (SD) i-PTH concentration of the study sample was 49.97 (24.64) while median and IQR were 52.00 and 30.87 -70.00 pg/mL respectively. Elevated serum i-PTH concentrations (defined as a serum i-PTH concentration >65.0pgm/L) was observed in 142 (33.5%) subjects. Mean (SD) serum ALP concentration was 64.18 (27.51) while median and IQR were 61.75 and 44.99-78.00 IU/L respectively. Elevated serum ALP (defined as serum ALP>95.00 IU/L) was observed in 51 (12.1%) subjects.

Effect of serum 25(OH) D and i-PTH on BMC/BMD

Serum 25(OH) D showed a significant positive correlation with BMD (r=0.13, p=0.008) and BMC (r=0.124, p=0.010) (Figure 3.1) and a significant negative correlation with i-PTH (r= -0.624, p=0.000). Serum i-PTH showed a significant negative correlation with BMD(r= -0.164, p=0.001 and BMC(r= - 0.152, p=0.002) (Figure 3.2). Although ALP had negative correlations with BMD and BMC, they were not statistically significant (Table 12).

Correlation of serum 25(OH) D and i-PTH

Serum 25(OH) D had a negative correlation with i-PTH. Regression model was fitted with i-PTH as the dependent variable and 25(OH) D as the independent variable to asses the relationship of these two variables and the following formula was developed..

$Y = a + bc$ (a = intercept = 73.08; b = slope = -0.62)

When i-PTH value of 65.0pg/ml was considered as the cut-off value which demarcates the elevated serum parathyroid response, above formula was used to calculate the serum 25 (OH) D values that would initiate the rise of PTH level: Where

Y = cut-off for elevated i-PTH (65pg/ml); c = 25(OH) D levels i-PTH rise

When the results were applied to the above formula:

$Y=a+bc$ $(65 = 73.08 + (-0.62 \times c)\ c = 13.02)$

The initiation of rise in i-PTH in the sample was seen at 25(OH) D level of 13.02nmol/l

Hence this level would demarcate 25 (OH) D insufficiencies among the subjects in the sample. The present study revealed that only 22.1% (n=96) of subjects had 25(OH) D levels below 13.02nmol/l. In this subgroup, 76% of subjects (n=73) had elevated i-PTH (above 65pg/ml). Other 24 % (n=23) had mean i-PTH concentration of 51.64pg/ml (median of 58.00pgm/l).

Further, BMC/BMD, anthropometric indices and other serum measurements were analyzed in the tertiles of 25 (OH) D concentrations (Table 13). In contrast to women in the lower tertile of 25(OH) D, women in the upper tertile were shorter, had higher BMD and lower i-PTH level. BMC, weight and BMI showed no difference in the tertiles of 25(OH) D. Women with lower 25(OH) D had higher i-PTH level and women with higher 25(OH) D had lower i-PTH level. Furthermore, i-PTH showed a trend across the tertiles of 25(OH) D. ALP which was tested as a surrogate of hypovitaminoses D did not show a difference in the tertiles of 25(OH) D(Table 13).

Discussion

This study provides information on BMD of appendicular skeleton in a group of healthy Sri Lankan men and women. Results of the present study indicate that phalangeal peak bone mass is achieved between 30 and 39 years in both sexes. Compared to women of the same age category, men had a higher (19%) bone mineral density (17).These findings are comparable with the Third National Health and Nutrition Examination Survey (NHANES-III) conducted in the USA, which showed higher BMD in men than in women in all age groups (17). Puberty is the period during which the gender differences in BMD both in the axial and appendicular skeleton begin to appear (19). It is also well known that men have bigger bones than women (24). Gender difference in BMD partly depends on the bone size as the differences in BMD were minimized when they were adjusted for bone volume (19). Furthermore, the volumetric bone mineral density appears to be similar in the female and male newborns (19),

According to Bonjour *et al*, in 1994 (1) the age at which peak bone mass is achieved has been shown to vary in different study populations and in different skeletal sites. In the present study, men and women reached the peak bone mass between 30 and 39 years. In the USA, all three major ethnic groups (non-Hispanic White, non-Hispanic Black and, Mexican American) reached the peak hip BMD between 20 and 30 years (17). Delays in reaching the peak bone mass in populations outside the USA have been reported. Saudi women reached peak spine BMD around 35 years (18). Similarly in Greek women, while spinal BMD reached its peak between 30 and 35 years, femoral neck peak BMD was seen between 25 and 30 years (20). In Chinese women, although peak BMD in proximal femur was seen between 20-24 years, the peak BMD in the forearm bones was not seen until 40-44 years (21). The exact reasons for the delay in reaching the peak bone mass in certain populations are not known. As suggested by Bonjour *et al.*, 1994 (1) variations in genetic, social, and nutritional states in different populations may have played a role. It is also possible that BMD trends in different skeletal sites are under different genetic control mechanisms. A recent study conducted in Southern Sri Lanka (22) among a group of community dwelling healthy women, showed an age discrepancy in achieving PBM in two central skeletal sites. Although this study was not reassigned to study the time of PBM, the maximum spine BMD was observed between 30-39 years while hip BMD reached the maximum between 40-49 years. This delay in PBM in Sri Lankan subjects may be due to several reasons. Genetic factors and nutrition appear to be the most plausible explanation. The average daily income in Sri Lanka is below SLR 500.00 (USD 3.88) and it is well below the figures of other countries in the region and outside. According to the population census in 2004, nearly 20% of households were below the National Poverty Line (23). Nutrition and poverty are linked in many ways and poor nutrition may play a role in timing of PBM in Sri Lankan population. The results of this study could not be compared quantity wise with the PBM observed elsewhere as there are no data available on pBMD from other countries. However when calculations were done based on BMD and T scores of two individuals, the mean and SD used as the reference values in the machine were estimated to be 0.512 and 0.06 g/cm2 respectively. Our results were highly comparable with these figures. While SD's were the same, mean BMD of the reference population was only 2.0% higher.

Conclusions

This project examines the peak bone mass in a peripheral skeletal site which is rich in cortical bone, with regards to its timing and association with historical determinants of bone health in a group of subjects from the Galle district selected by stratified randomization method. The main aim of the present study is to determine the most appropriate interventions which can be used in the community level to improve peak bone mass among Sri Lankans. The study was conducted in the Galle district which has most of the characteristics that describe the cross-section of normal Sri Lankan population. The rural-urban mix, the full spectrum of socio-economic strata, and agricultural base with supplementary industries are some of these key characteristics.

It is hoped that findings of this project would help health policy makers when formulating guidelines and recommending interventions in promoting bone health especially among adolescents and preadolescents in the country. Findings of this study can be used as a baseline for future studies in this area.

Based on the findings of this project, the following conclusions can be made

Phalangeal BMD/BMC reaches its peak between 30-39 years in both males and females.

Males have a higher BMD/BMC when compared with females in the same age group.

In women, height, weight, body mass index, hip circumference, chest circumference and abdominal girth showed significant associations with BMD and BMC

Of the anthropometric indices examined, height was the best predictor of the phalangeal BMD in women participated in this study.

In women, physical activity during adolescence has a positive and long lasting effect on bone mineral density.

Dietary calcium has no linear relationship with peak BMD/ BMC in women. However, phalangeal BMD was lower among women who consumed both dairy and non-dairy calcium rich food infrequently.

The peak BMD/ BMC showed a variation in different socio-economic classes partly due to inequalities of income and degree of physical activity

In contrast to the widely held belief, hypovitaminosis D is prevalent among these community living healthy women. Hypovitaminosis D was a significant determinant of BMD/ BMC in this group of women

i-PTH among women in this study showed an inverse correlation with vitamin D levels. The rise of i-PTH occurs when 25 (OH) D levels is reduced below 13.02 nmol/L.

Serum alkaline phosphatase, the widely used surrogate marker of vitamin D deficiency in clinical evaluation of patients, showed poor correlation with vitamin D and PTH levels in women in this study indicating that it is not reflective of serum vitamin D level.

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