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Bone mass in Chinese premenarcheal girls: the roles of body composition, calcium intake and physical activity

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The association of growth and anthropometric characteristics and lifestyle factors with bone mass and second metacarpal radiogrammetry parameters was evaluated in 373 healthy Chinese premenarcheal girls aged 9–11 years. Bone mineral content (BMC) and density (BMD) and bone area (BA) of distal forearm, proximal forearm and total body, bone mineral-free lean (BMFL) mass and fat mass were measured by dual-energy X-ray absorptiometry. Metacarpal bone periosteal and medullary diameters were measured. Dietary intakes were assessed by 7 d food record and physical activity (PA) by questionnaire. BMFL and fat mass together explained 6·3 and 51·6 % of the variation in total body BMC and BMD, respectively. BMFL mass contributed to a substantial proportion of the variation in forearm BMC and BMD and periosteal diameter (10·4–41·0 %). The corresponding BA explained 14·8–80·4 % of the variation in BMC. Other minor but significant predictors of total body bone mass were Ca intake, height, age and PA score (BMD only), and of forearm bone mass were PA score, bone age, height and fat mass. Nevertheless, after adjusting for bone and body size and for age or bone age, subjects with Ca intake above the median (417 mg/d) had 1·8 % greater total body BMC ($P<0·001$), and subjects with PA scores above the median had 2·4–2·5 % greater distal and proximal forearm BMC ($P<0·05$) than those below. Vitamin D intake negatively associated with medullary diameter (partial R^2 1·7 %). The results indicate that premenarcheal girls should be encouraged to optimise nutrition and Ca intake and exercise regularly to achieve maximum peak bone mass.

Bone mass: Body composition: Calcium intake: Physical activity: Premenarcheal girls

Peak bone mass attained at skeletal maturity is considered to be inversely related to the risk of developing osteoporotic fractures in later life. There are several factors which are known to influence peak bone mass. These include racial and genetic factors, body weight, soft tissue composition, endocrine factors, nutrient intake and physical activity. Studies relating physical activity during childhood to adult bone mass in Australian and Finnish females have indicated that the premenarcheal period is an important time for bone mineral acquisition (Kannus *et al.* 1995; Bass *et al.* 1998; Khan *et al.* 1998).

The people of China represent 25 % of the world's population, and have characteristic dietary patterns and lifestyles. Although the incidence rate of hip fractures in Beijing in 1988 was low, 4 years later, in 1992, it had increased by 33 % (Xu *et al.* 1996). As the numbers of elderly people in Asian populations increase, the prevalence of osteoporotic fractures, by the year 2050, has been predicted to become greater than that in Western countries (Cooper *et al.* 1992). Adolescents in China are known to have both low dietary Ca intakes (Du *et al.* 2001) and little physical exercise outside school hours (Tudor-Locke *et al.* 2003). These two characteristics may put adolescents in China at increased risk of suboptimum peak bone mass at maturity. Only a few studies have examined the influence of physical and lifestyle factors on bone mass in adolescents in China (Cheng *et al.* 1999; Du *et al.* 2002; Afghani *et al.* 2003). In a cross-sectional study of 649 Beijing adolescent girls aged 12–14 years, milk intake and school physical activity score were found to be important predictors of bone mineral content (BMC) (Du *et al.* 2002). On the other hand, a longitudinal investigation over 3 years of ninety-two male and eighty-seven female Chinese adolescents in Hong Kong from a starting age of 12–13 years found that bone mineral accretion was not affected either by exercise or by the level of Ca intake. It was concluded that the influence of such external factors was less important for bone development than the effects of puberty (Cheng *et al.* 1999). In a study of 300 male and 166 female Chinese adolescents aged 12–16 years in Wuhan City, Afghani *et al.* (2003) reported that lean body mass was the primary determinant of bone mass, and that the participation in team sports was a predictor of bone mass in boys but not in girls. High prevalence

Abbreviations: BA, bone area; BMC, bone mineral content; BMD, bone mineral density; BMFL mass, bone mineral-free lean mass; DRIs, dietary reference intakes; 25(OH)D, 25-hydroxyvitamin D.

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of low body weight was also found to be a major public health problem in Beijing adolescent girls aged 12–14 years, and girls with low body weight were reported to have delayed development and low bone mass (Du *et al.* 2003). There has been no investigation of the correlates and determinants of bone mass in premenarcheal girls in China.

Because the dietary intervention study of Du *et al.* (2004) had found significant effects of increased milk consumption on bone growth and development, it was decided to analyse the data of these same subjects before they took part in the dietary intervention study and while they were still at the premenarcheal stage of development. The aim of the present research, with Chinese premenarcheal girls aged 9–11 years in urban Beijing, was to determine whether BMC and/or bone mineral density (BMD) are influenced by anthropometric and growth characteristics (height, weight, body composition, age, bone age), or by their habitual intakes of Ca and vitamin D, or by their level of physical activity. Metacarpal morphometry measurements were also made, as these could be an indicator of cortical bone growth in children.

Materials and methods

Subjects

A random sample of 414 girls was drawn for total body bone mineral measurements from a cohort of 757 healthy girls participating in a dietary intervention programme from nine randomly selected primary schools in urban Beijing (Du *et al.* 2004). Of these 414 girls, baseline data (before dietary intervention) for 373 girls (mean age 10·1 (SD 0·4) years) who were premenarcheal and at Tanner breast stage 1 or 2 are reported here. None of the subjects had medical conditions, or were taking medications, known to influence bone mineral metabolism. The investigation was carried out with the approval of the ethics committees of the University of Sydney, Australia, and the Institute for Nutrition and Food Safety, China. Written informed consent was obtained from the parents of each subject.

Bone mass and body composition

BMC, BMD and bone area (BA) of the distal and proximal forearm and the total body, the bone mineral-free lean (BMFL) mass, and the fat mass were measured by dual-energy X-ray absorptiometry, using a Norland XR-36 densitometer in pencil-beam mode (Norland Medical Systems, Inc., Fort Atkinson, WI, USA) from March to April 1999. Forearm measurements were made on the non-dominant arm. The starting point of the distal forearm measurement scan for each subject was determined by XR software using the minimum BMD value found during the first forearm scout scan. A numeric value for 33 % of the total length of the ulna (the distance from the ulnar end plate) was calculated by XR software, and was determined as the starting point for the proximal forearm measurement scan. The precision error (CV) for this method was less than 1 % in children. A daily quality assurance test was performed using a manufacturer-supplied phantom, and the accuracy error was less than 1 %.

Bone age and metacarpal morphometry

Postero-anterior X-ray radiographs of the non-dominant hand and wrist were taken using a Toshiba KXO-152 X-ray apparatus (Toshiba, Tokyo, Japan) at an average setting of 5 mAs and 46 kV. The film focus distance was 110 cm and the central beam was focused on the wrist. Periosteal diameter (outer width) and medullary diameter (inner width) of the midshaft of the second metacarpal were measured by one examiner with a digital caliper (Mitutoyo, Kanagawa, Japan). The intra-observer reliability was assessed by repeating the measurements of twenty-eight hand radiographs at different times during 1 month. The CV were less than 1 % for periosteal diameter and less than 2 % for medullary diameter. The combined cortical thickness was calculated as (periosteal diameter – medullary diameter). Bone age (to the nearest 0·1 year) was determined from these radiographs by assessing the development stages of metacarpal, phalanges and carpal bones according to the Chinese standard (National Sports Committee, 1992).

Anthropometry and pubertal staging

Subjects were weighed (wearing light clothing and without shoes) to the nearest 0·1 kg with an electronic digital scale (THINNER, Gays Mills, WI, USA). Height was measured in bare feet to the nearest mm with a body height measuring device (TG-III Type, No. 6 Machinery Plant, Beijing, China). Breast development and pubic hair development were ascertained during interview according to Tanner's definitions of the five stages of puberty (Tanner, 1962). All the measurements were taken by trained investigators following a standardised procedure.

Dietary intake

Dietary intakes were assessed from 7 d food records in which subjects recorded all items of food and drink consumed over 7 d. The size of food portions was estimated using household measures and three-dimensional food models. The average weight of a food item either in household measures (bowls, plates and spoons) or as a standard portion of the food item (small, medium or large) was determined and standardised. Nutrient intakes were calculated using a data entry and nutrient calculation program named CAVD and the Chinese food composition tables (Institute of Nutrition and Food Hygiene, 1991; He *et al.* 1997). The vitamin D content of food was estimated from the UK food composition tables with a downwards adjustment in the values for vitamin D in eggs, cakes, and fortified fresh milk, by replacing the vitamin D content of these food with local Chinese analyses data in the database (Holland *et al.* 1991). The vitamin D contents of eggs and cakes were lower in China, probably because chicken feed in China is not wholly fortified with vitamin D.

Physical activity

A physical activity questionnaire was answered by all subjects. They were instructed to report firstly the type, frequency and duration of physical exercise undertaken during class breaks, at the lunchtime recess and outside school hours during the previous 6 months and secondly the extent of any training at sports clubs or in sporting teams over the previous 12 months. The validity of this part of the questionnaire had been studied in Chinese school children, and had shown high reliability (correlation coefficients between two questionnaires at 1 month's interval in physical activity energy expenditure and weekly activity time were 0·46 and 0·43, respectively; $P<0·05$) but relatively low validity (partial correlation coefficient between physical activity energy expenditure estimated from the questionnaire and Caltrac accelerometer was 0·24; $P>0·05$) in Chinese school-girls (Liu *et al.* 2003). To improve efficiency of the questionnaire in assessing physical activity level, we also included the following items in the questionnaire: (1) the school physical activity score awarded by teachers for the previous semester, which had been previously shown to be a predictor of bone mineral status in Beijing adolescent girls (Du *et al.* 2002); (2) the pattern of spare time allocation, as indoor sports facilities are not common in China, therefore a child spending more spare time outdoors is generally more active; (3) the means of travel to school.

Habitual physical activity level was then assessed with a sum score method modified from the habitual physical activity estimation method of Telama *et al.* (1985). Variables were first categorised according to the following: (a) total leisure time activity hours (1 = less than 1 h per week; 2 = 1–2 h per week; 3 = more than 2 h per week); (b) participation in training (0 = no; 1 = yes); (c) training frequency (1 = once per week; 2 = more than once per week); (d) school physical activity score (1 = pass; 2 = good; 3 = excellent); (e) allocation of spare time (1 = mainly indoor; 2 = half indoor and half outdoor; 3 = mainly outdoor); (f) means of travel to school (1 = by car or by bus; 2 = sometimes by car or bus and sometimes by foot or bicycle; 3 = by foot or by bicycle). Then a sum physical activity score, as a qualitative physical activity indicator, was calculated. A minimum value of four indicated that a subject was very inactive. The higher the score, the more physically active a subject was deemed to be.

Biochemical measurements

Overnight fasting blood samples were collected in March and April 1999 (late winter). Plasma 25-hydroxyvitamin D (25(OH)D) concentration was measured in 291 subjects by a competitive protein-binding assay, modified after Mason & Posen (1977). The inter-assay and intra-assay CV were 12·4 and 4·2 %, respectively.

Statistical analyses

Descriptive statistics are expressed as the means (SD) for the actual measurement values and as the means (SE) for the calculated differences unless otherwise indicated. Bivariate correlation analyses were conducted to identify variables associated with bone measurements. Any correlations were further evaluated by stepwise multiple regression analysis. To determine the power relationship between continuous variables independent of the unit of measurements and the proportional effects of discrete variables, all continuous variables, except age and bone age, were transformed to natural logarithms (Prentice *et al.* 1994; Parsons *et al.* 1996). BMFL mass and fat mass were entered instead of body weight, because the latter includes the weight of the skeleton. Corresponding BA were force-entered into each BMC regression model to correct for the bone size. Collinearity was tested in each regression model, and a variance inflation factor value larger than 10 was considered as showing the existence of collinearity or near collinearity (Kleinbaum *et al.* 1998). Further analyses were made by coding Ca intake or physical activity score as factors with two ranges of values in the multiple regression analysis (above the median coded as 1, and below the median values coded as 0), then the percentage differences in BMC between the above-median and below-median groups were assessed. BMC values rather than areal BMD were used in the comparison as BMC is considered to be the best indicator of bone change during growth (Prentice *et al.* 1994; Heaney, 2003). A P value of less than 0·05 was considered significant. All data were analysed by SPSS for Windows version 10.0 (SPSS Inc., Chicago, IL, USA).

Results

The descriptive characteristics of the subjects are presented in Table 1. The mean bone age (9·9 (SD 1·0) years) was similar to the mean chronological age (10·1 (SD 0·4) years), indicating the skeletal maturity of our subjects was proportional to their chronological age. Mean values for height, weight and BMI were in the normal range for Chinese urban girls from high-income families of this age group (Chinese Student Fitness and Health Research Group, 1995). There were 176 (47·2 %) subjects at Tanner stage 1 for breast development and 197 (52·8 %) subjects at Tanner stage 2. Using the Chinese standards (Ministry of Health and National Education Committee, 1993), 32·2 % subjects were underweight (BMI $<15·2$ kg/m^2), 51·7 % subjects of ideal body weight (BMI = 15·2–18·5 kg/m^2), 8·3 % subjects overweight (BMI = 18·5–20·2 kg/m^2) and 7·8 % subjects obese (BMI $>20·2$ kg/m^2). Underweight subjects had significantly lower bone age than girls with ideal weight (9·7 (SD 0·9) *v.* 9·9 (SD 1·0) years; $P=0·02$), and a lower percentage of subjects at Tanner breast stage 2 (45·1 *v.* 54·9 %; $P<0·001$), although they were similar in chronological age to girls with ideal weight (10·1 (SD 0·3) *v.* 10·1 (SD 0·4) years; $P>0·05$). The average daily Ca, protein and vitamin D intakes were 436 (SD 170) mg, 53·1 (SD 15·3) g and 0·95 (SD 0·75) μg, respectively. These values represented 54·5 % of the recommended adequate intake for Ca, and 81·7 and 9·4 % of the reference nutrient intake for protein and vitamin D, respectively, of the Chinese dietary reference intakes (DRIs) for this age group (Chinese Nutrition Society, 2000). About 28 % dietary Ca was from

Table 1. Growth, anthropometric, lifestyle and bone mineral characteristics of subjects (*n* 373)

(Mean values, standard deviations and ranges)

	Mean	SD	Range
Age (years)	10·1	0·4	9·1–11·7
Bone age (years)	9·9	1·0	6·5–12·7
Height (cm)	140·1	6·2	122·3–159·2
Weight (kg)	32·5	6·2	19·1–57·8
BMI (kg/m^2)	16·5	2·4	11·9–25·8
BMFL mass (kg)	20·8	3·1	14·3–33·0
Fat mass (kg)	10·5	4·3	2·9–26·6
Subjects at Tanner breast stage 1: *n*	176		1–2
%	47·2		
Subjects at Tanner breast stage 2: *n*	197		
%	52·8		
Ca intake (mg/d)	436	170	137–1161
Protein intake (g/d)	53·1	15·3	21·9–102·6
Vitamin D intake (μg/d)	0·95	0·75	0·01–4·43
Milk intake (g/d)	123	92	0–446
PA score	9·2	2·3	4–15
25(OH)D (nmol/l)*	19·7	8·3	4·4–52·2
Metacarpal morphometry			
Periosteal diameter (mm)	6·41	0·56	4·84–8·14
Medullary diameter (mm)	2·97	0·64	1·32–5·20
CCT (mm)	3·44	0·49	2·03–5·03
Total body			
BMC (g)	1317	180	827–1820
BA (cm^2)	1920	159	1503–2385
BMD (g/cm^2)	0·684	0·049	0·548–0·823
Proximal forearm			
BMC (g)	0·950	0·120	0·692–1·348
BA (cm^2)	2·012	0·130	1·585–2·520
BMD (g/cm^2)	0·472	0·047	0·339–0·624
Distal forearm			
BMC (g)	0·645	0·090	0·423–0·921
BA (cm^2)	2·826	0·211	2·158–3·404
BMD (g/cm^2)	0·228	0·027	0·156–0·313

BMFL mass, bone mineral-free lean mass; PA, physical activity; 25(OH)D, 25-hydroxyvitamin D; CCT, combined cortical thickness; BMC, bone mineral content; BA, bone area; BMD, bone mineral density.

* *n* 294.

milk. In these subjects the major dietary source of Ca (>50 % of total) was from plant foods. The physical activity scores were normally distributed, ranging from a minimum value of 4, which represents total passivity, to a maximum value of 15, with a median of 9.

Univariate analysis

Correlations between bone parameters and physical, developmental and lifestyle characteristics are presented in Table 2. Anthropometric and body composition parameters and physical activity score showed better correlations with bone mass of proximal forearm than that of distal forearm. There was no correlation between milk or protein intake and any of those bone variables. None of the components of the total physical activity score had better correlation with BMC or BMD than the total score. The correlation coefficient between weight and BMFL mass was 0·754 ($P<0·001$) and between weight and fat mass was 0·868 ($P<0·001$). The physical activity score was positively correlated with BMFL mass (r 0·109; $P=0·043$), but was not correlated with fat mass. There was a significant partial correlation between dietary vitamin D intake and plasma 25(OH)D concentrations after controlling for dietary Ca intake (r 0·139; $P=0·018$).

Multivariate analysis

From 39·3 to 87·5 % of the variation in BMC, 14·9–54·6 % of the variation in BMD and 4·0–29·7 % of the variation in metacarpal morphometry measures could be explained by independent variables entered in each model (Tables 3–6). Tanner stage did not meet the criteria for inclusion in any of these regression models.

Bone mineral-free lean mass and fat mass

Fat mass and BMFL mass appear to be important predictors of total body bone mass. Together they explained 6·3 and 51·6 % of the variation in total body BMC and BMD, respectively (Table 3). BMFL mass also contributed to a significant proportion of the variation (10·4–41·0 %) in proximal and distal forearm BMC and BMD (Tables 4 and 5) and accounted for 1·4–19·1 % of the variation in metacarpal morphometry measurement variables (Table 6).

Calcium and vitamin D intakes

Ca intake accounted for 0·4 and 1·4 % of the variation in total body BMC and BMD, respectively (Table 3). Vitamin D intake was found to be the most important predictor of medullary diameter of the second metacarpal, and explained 1·7 % of the variation (Table 6). Further analysis was done by dividing all the subjects into two groups: above and below the median Ca intake of 417 mg/d. After adjusting for bone and body size and for age, the total body BMC was 1·8 (SE 0·5) % greater in the higher Ca intake group (Ca intake: 564 (SD 139) mg/d) than in the lower Ca intake group (Ca intake: 307 (SD 72) mg/d; $P<0·001$).

Physical activity

Physical activity score was a minor but significant predictor of BMD at all three sites measured, BMC of the distal and proximal forearm, and periosteal diameter of the second metacarpal, and accounted for 0·3–0·9 % of the variation in these measures (Tables 3–6). Further analyses were carried out by dividing all the subjects into two groups, above and below the median physical activity score. After adjusting for bone and body size and for bone age, in comparison with subjects with physical activity scores of 9 and below, subjects with physical activity scores of 10 and above had 2·5 (SE 0·9) % greater BMC in the proximal forearm ($P=0·005$) and 2·4 (SE 1·2) % greater BMC in the distal forearm ($P=0·04$).

Bone area and bone age

From 14·8 to 80·4 % of the variation in BMC could be explained by corresponding BA (Tables 3–5). Bone age was the most important predictor of combined cortical thickness, and accounted for 25 % of the variation (Table 6). Bone age also entered the regression models

Table 2. Pearson's correlation coefficients between bone parameters and physical, developmental and lifestyle characteristics of subjects

	Total body		Distal forearm		Proximal forearm		Metacarpal morphometry		
	BMC	BMD	BMC	BMD	BMC	BMD	Periosteal diameter	Medullary diameter	CCT
Age	0·139†	NS	0·112*	NS	0·208‡	0·228‡	0·114*	NS	NS
Bone age	0·553‡	0·362‡	0·279‡	0·184‡	0·380‡	0·408‡	0·318‡	−0·109*	0·506‡
BA	0·893‡	0·541‡	0·523‡	NS	0·615‡	0·137†	NA	NA	NA
Height	0·728‡	0·390‡	0·371‡	0·205‡	0·460‡	0·450‡	0·383‡	NS	0·423‡
Weight	0·841‡	0·704‡	0·396‡	0·334‡	0·510‡	0·449‡	0·309‡	NS	0·333‡
BMFL mass	0·740‡	0·498‡	0·515‡	0·372‡	0·643‡	0·549‡	0·443‡	NS	0·457‡
Fat mass	0·668‡	0·647‡	0·172†	0·186‡	0·250‡	0·234‡	0·123*	NS	0·129*
Tanner stage	0·428‡	0·311‡	0·226‡	0·185‡	0·277‡	0·251‡	0·222‡	NS	0·264‡
PA score	0·113*	NS	0·154†	0·147†	0·206‡	0·178†	0·152†	NS	NS
Ca intake	0·154†	0·186†	NS	NS	NS	0·133*	NS	NS	0·125*
Vitamin D intake	NS	NS	NS	NS	NS	NS	NS	−0·124*	0·119*

BMC, bone mineral content; BMD, bone mineral density; CCT, combined cortical thickness; BA, bone area; NA, not applicable; BMFL mass, bone mineral-free lean mass; PA, physical activity.
* $P<0·05$.
† $P<0·01$.
‡ $P<0·001$.

Table 3. Multiple regression analysis of total body bone mineral status

	Ln (total body BMC)			Ln (total body BMD)		
	Regression coefficient	*P* value	Attributable percentage (partial R^2)	Regression coefficient	*P* value	Attributable percentage (partial R^2)
Ln (fat mass (kg))	0·099	<0·001	5·1	0·107	<0·001	42·9
Ln (BMFL mass (kg))	0·190	<0·001	1·2	0·198	<0·001	8·7
Ln (Ca intake (mg/d))	0·023	0·001	0·4	0·022	0·002	1·4
Ln (height (cm))	−0·317	0·010	0·2	−0·201	0·032	0·5
Age (years)	−0·018	0·013	0·2	−0·018	0·016	0·8
Ln (total body BA (cm^2))	1·105	<0·001	80·4	NA	NA	NA
Ln (PA score)	–	–	–	0·021	0·049	0·3
Constant	−0·364	0·378		−0·231	0·561	
Total			87·5			54·6

BMC, bone mineral content; BMD, bone mineral density; BMFL mass, bone mineral-free lean mass; BA, bone area; NA, not applicable; PA, physical activity; –, excluded by the regression model.

Table 4. Multiple regression analysis of proximal forearm bone mineral status

	Ln (proximal forearm BMC)			Ln (proximal forearm BMD)		
	Regression coefficient	*P* value	Attributable percentage (partial R^2)	Regression coefficient	*P* value	Attributable percentage (partial R^2)
Ln (BMFL mass (kg))	0·339	<0·001	41·0	0·300	<0·001	28·5
Ln (proximal forearm BA (cm^2))	0·831	<0·001	14·8	NA	NA	NA
Ln (PA score)	0·048	0·007	0·7	0·043	0·015	0·9
Bone age (years)	0·012	0·030	0·5	0·014	0·009	1·1
Constant	−1·885	<0·001		−1·897	<0·001	
Total			57·0			30·5

BMC, bone mineral content; BMD, bone mineral density; BMFL mass, bone mineral-free lean mass; BA, bone area; NA, not applicable; PA, physical activity.

for proximal forearm BMC and BMD, and periosteal diameter of the second metacarpal and was associated with 0·5–1·1 % of the change in R^2 (Tables 4 and 6).

Discussion

This investigation showed the contributions of fat mass and BMFL mass to total body bone mass, and BMFL mass to forearm bone mass and the periosteal diameter of the second metacarpal were substantial. Significant positive relationships were also found between dietary Ca intake and total body bone mass, and between physical activity level and forearm and total body bone mineral status and the periosteal diameter of the second metacarpal. A negative relationship was found between dietary vitamin D intake and medullary diameter of the second metacarpal. Tanner stage of pubertal development was not a determinant of any of the bone variables measured in the present study, probably because of the narrow range of pubertal development of the subjects. Anthropometric and body composition parameters

Table 5. Multiple regression analysis of distal forearm bone mineral status

	Ln (distal forearm BMC)			Ln (distal forearm BMD)		
	Regression coefficient	*P* value	Attributable percentage (partial R^2)	Regression coefficient	*P* value	Attributable percentage (partial R^2)
Ln (distal forearm BA (cm^2))	0·773	<0·001	27·3	NA	NA	NA
Ln (BMFL mass (kg))	0·417	<0·001	10·4	0·391	<0·001	11·7
Ln (height (cm))	−0·542	0·011	0·7	−0·636	0·003	1·5
Ln (PA score)	0·051	0·029	0·4	0·052	0·028	0·9
Ln (fat mass (kg))	0·032	0·047	0·5	0·038	0·018	0·8
Constant	−0·017	0·985		0·274	0·760	
Total			39·3			14·9

BMC, bone mineral content; BMD, bone mineral density; BA, bone area; NA, not applicable; BMFL mass, bone mineral-free lean mass; PA, physical activity.

Table 6. Multiple regression analysis of metacarpal morphometry measurement

	Ln (periosteal diameter)			Ln (medullary diameter)			Ln (CCT)		
	Regression coefficient	*P* value	Attributable percentage (partial R^2)	Regression coefficient	*P* value	Attributable percentage (partial R^2)	Regression coefficient	*P* value	Attributable percentage (partial R^2)
Ln (BMFL mass (kg))	0·259	<0·001	19·1	0·247	0·012	1·4	0·261	<0·001	3·9
Ln (PA score)	0·035	0·050	0·6	–	–	–	–	–	–
Ln (vitamin D intake (μg/d))	–	–	–	−0·036	0·005	1·7	–	–	–
Bone age (years)	–	–	–	−0·045	0·002	0·9	0·056	<0·001	25·0
Age (years)	–	–	–	–	–	–	−0·042	0·027	0·8
Constant	0·991	<0·001		0·750	0·002		0·308	0·143	
Total			19·7			4·0			29·7

CCT, combined cortical thickness; BMFL mass, bone mineral-free lean mass; PA, physical activity; –, excluded by the regression model.

and physical activity score showed better correlation with BMC and BMD of the proximal forearm than with those of the distal forearm. As the proximal forearm is predominantly cortical bone, compared with a greater proportion of trabecular bone in the distal forearm, these variables may have more influence on the development of cortical bone rather than trabecular bone. In contrast, Slemenda *et al.* (1994) concluded that puberty had a stronger influence on trabecular bone than cortical bone.

In our multiple models, fat mass and lean mass appear to be the most important predictors for total body bone mass when controlled for BA. Studies in Western children also showed that both lean mass and fat mass were independent predictors of total body mineral (Young *et al.* 1995; Ilich *et al.* 1998). Furthermore, Young *et al.* (1995) reported that BMFL mass is a better predictor of total body BMC than fat mass in both pre- and post-menarche female twins. However, in contrast, the present study found that fat mass was a more important predictor of total body BMC and BMD than BMFL mass. Whether this divergence of results is due to racial differences or whether it is related to the high percentage of underweight subjects in our study population requires further investigation. It was notable that 32·2 % of subjects in the present study were underweight. Chinese girls aged 12–14 years with low body weight (using the same standard as in the present study) were reported to have delayed pubertal development and lower BMC and BMD at the distal 1/3 radius and ulna (Du *et al.* 2003). The present results also indicate that BMFL mass was a more important predictor than fat mass for peripheral bone mass, because the lean mass was the major independent determinant of bone mass at the distal and proximal forearm and periosteal diameter of the second metacarpal. Lean mass could affect bone mass by direct mechanical loading on the skeleton. The positive association between lean mass and bone mass may be due also to a mutual increase in muscle and bone mass in response to external stimuli, such as physical activity, and to internal stimuli, such as changes in growth hormone secretion (Slemenda, 1995). A positive correlation was found between the level of physical activity and BMFL mass in the present study. As BMFL mass and fat mass are important determinants of bone mass, and about one-third of our subjects had low body weight, Chinese premenarcheal girls should be encouraged to optimise nutrition and participate in regular exercise programmes to maintain healthy body weight and build up lean mass.

Dietary Ca intake was found to be a significant predictor of total body BMC and BMD. This observation is consistent with the conclusions from supplementation studies with girls aged 11–12 years, which reported that increased Ca intakes had beneficial effects on increasing total body bone mineral (Andon *et al.* 1994; Chan *et al.* 1995; Lloyd *et al.* 1996; Cadogan *et al.* 1997). In a cross-sectional study with 456 pre-adolescent Caucasian girls (Ilich *et al.* 1998), it was found that Ca intake was one of the most significant predictors of BMC and BMD in the total body and in the radius, even though the average Ca intake of the subjects in that study (965 (SD 381) mg/d) was much higher

than that in the present investigation (436 (SD 170) mg/d). Although this group's earlier cross-sectional survey of Chinese adolescent girls showed that milk was the only nutritional factor with significant partial correlation with BMC (Du *et al.* 2002), the present study failed to find any correlation between milk intake and any of the bone variables measured. This discrepancy between the two studies may have its origin in the different dietary intake estimation methods used (food frequency *v.* 7 d food record), the different stage of development of the subjects (pubertal *v.* premenarcheal), or the fact that the subjects of the present study were all urban girls and had higher and more homogeneous milk intake than the subjects (girls from urban, suburban and rural areas) of the earlier study (123 (SD 92) *v.* 50 (SD 68) g/d).

Although Ca intake only accounted for a small variation in total body BMC and BMD (0·4–1·4 %), any increase in intake is likely to lead to significant increases in total body bone mass because the average intake, at about 54·5 % of the defined adequate intake (800 mg/d) of the Chinese DRIs for children aged 7–11 years (Chinese Nutrition Society, 2000), was so low. Indeed, the present study showed that those subjects with Ca intakes above the median (417 mg/d), when compared with those below the median, had a 1·8 % greater ($P<0·001$) total body BMC after adjustment for bone and body size and for age. Although girls with Ca intakes above the median had significantly higher protein intakes than those below the median (58·6 (SD 14·4) *v.* 46·1 (SD 11·2) g/d; $P<0·001$), Ca intake is more likely to be a specific mediator of bone development than a surrogate of general nutrition. Firstly, a low Ca intake is a more serious problem in the study subjects (Ca and protein intakes represented 54·5 and 81·7 % respectively of the adequate intake/reference nutrient intake of the Chinese DRIs for this age group (Chinese Nutrition Society, 2000)), and secondly there was no correlation between protein intakes and any BMC and BMD measured.

The level of physical activity was found to be a minor but significant predictor of distal and proximal forearm BMC and BMD and of total body BMD in Chinese premenarcheal girls. This was also consistent with the findings of retrospective studies in Australian and Finnish females (Kannus *et al.* 1995; Bass *et al.* 1998; Khan *et al.* 1998) and cross-sectional studies in Australian and American pre-pubertal children (Jones & Dwyer, 1998; Scerpella *et al.* 2003) which found that physical activity had a significant influence on the accumulation of bone mineral during the years before puberty. The present results also showed a positive relationship between the physical activity score and the periosteal diameter of the second metacarpal, indicating a positive effect of physical activity on periosteal apposition. It has been suggested that during the time of net bone formation, mechanical loading would increase the deposition of mineral at the bone surfaces (Garn, 1970; Bass *et al.* 1999). It has also been proposed that periosteal expansion could lead to greater increases in the mechanical strength of bone than endocortical contraction (Hayes & Gerhart, 1985). The present results provide modest support for the view that the pre-pubertal period provides an opportunity for bone mass to be enhanced by exercise (Bass, 2000).

In the present study, a sum score was devised which reflected total physical activity. The advantage of this score system was that it could easily be applied by school health workers and by parents and students to assess the level of physical activity and to identify those where bone growth could benefit from increased exercise. The disadvantage of the sum score method is that it does not take into account the intensity of each kind of activity and whether an activity is weight-bearing. Nevertheless, those with physical activity sum scores of 10 and higher had significantly greater (2·4–2·5 %) distal and proximal forearm BMC (after adjusting for bone age and bone and body size) than those with physical activity scores of 9 and below.

In contrast to the cross-sectional survey of Beijing adolescent girls which reported that vitamin D intake was a predictor of BMC (Du *et al.* 2002), in the present study vitamin D intake was not correlated with BMC and BMD at any of the sites measured. In an investigation of Finnish pre-pubertal children, vitamin D supplementation for 13 months showed no effect on distal radius BMC (Ala-Houhala *et al.* 1988). However, in a 3-year prospective study in Finnish peripubertal girls (Lehtonen-Veromaa *et al.* 2002), a positive association was found between vitamin D intake and bone mineral accretion but only in girls with advanced sexual maturation. This association was not found in less-mature girls. However, the present study found an association between vitamin D intake and endocortical bone apposition and cortical thickness. As during growth, bone mass accretion of long bone is proportional to the increase in cortical thickness with little change in volumetric BMD, and endocortical bone apposition is stimulated during puberty in females (Garn, 1970; Seeman, 1997, 2003), these findings suggest that vitamin D status may be important for bone mineral accretion after the onset of puberty. Except for a few foods, the present study assessed dietary vitamin D intakes with vitamin D content of foods from UK food tables. The uncertainties in determining the very low content of vitamin D in food may also have contributed to the absence of any association between dietary vitamin D intakes and BMC and BMD. Although the vitamin D intake of our subjects, averaging about 1 μg/d, was very low, the present study showed a positive association between vitamin D intake and plasma 25(OH)D concentration of blood samples taken during later winter. Because inadequate exposure to sunlight and the low intensity of UV light in winter in Beijing would limit vitamin D synthesis in the skin, an adequate intake of vitamin D from the diet would be important for the vitamin D status of Beijing Chinese girls.

Bone mineral acquisition is influenced both by exogenous factors (nutrition, lifestyle) and endogenous factors such as genetics and hormones. Although nutrition and lifestyle factors play a less important role than endogenous factors, they can be readily modified to improve bone mineral status. From the results of the present study, as a provisional recommendation, Chinese girls should be encouraged to optimise nutrition, Ca intake and vitamin D status and to participate more actively in regular exercise programmes (especially weight-bearing exercise) before the onset of puberty to help achieve maximum peak bone mass.

Of course, cross-sectional surveys, such as this, have limitations. Further retrospective studies, as well as intervention studies for diet and exercise in pre-pubertal children, are needed to investigate the relationship between adult bone mass and such variables as growth rate, dietary intake and physical activity during childhood. Such investigations would clarify the role of pre-pubertal growth, nutrition and physical activity on subsequent peak bone mass.

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