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Axial and peripheral bone mineral acquisition: a 3-year longitudinal study in Chinese adolescents

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Abstract We performed a 3-year longitudinal study of a group of 179 healthy Chinese adolescents (92 boys and 87 girls) aged from 12 to 16 years to determine the effects of puberty, physical activity, physical fitness, and calcium intake on the acquisition of bone mass. At yearly intervals for 3 consecutive years we recorded nutrition, calcium intake and anthropometric measurements, and assessed pubertal status according to Tanner. Bone mass of the lumbar spine was determined by dual-energy X-ray absorptiometry and radial bone mass by single-photon absorptiometry. Physical fitness and level of physical activity were assessed and muscle strength and power determined by isokinetic testing. Peripheral bone mass correlated with axial skeleton bone mass. Age, pubertal staging, physical fitness and muscle strength were significantly associated with bone mass increments on cross-sectional univariate and regression analysis. Longitudinal regression analysis showed that the most important factor affecting bone mass accretion in adolescents in both sexes was their pubertal stage. In boys, bone mass increment throughout the study was greater in children who were already in the advanced pubertal stages on entering the study than in those who started puberty in year 2 or 3 of the study. The percentage change in bone mineral content of the forearm and in bone mineral density of the lumbar spine was greater than 25% in the advanced pubertal group as compared to around 20% in the less mature group. For girls, the reverse was true. The increment of bone mass during the study period was significantly greater in those who presented in the earlier pubertal stages than in those who were at the more advanced stage of puberty on entry into the study. There was no significant effect of calcium intake and physical activities on the bone mass accretion.

Conclusion In Chinese adolescents, bone mineral accretion at adolescence is not influenced by exercise, level of physical fitness and calcium intake. In both sexes, and especially in girls, to optimally increase bone mass, regular physical exercise programmes should be instituted well before the onset of puberty rather than at or after it. Once puberty starts, these interventions may have no or only limited effect.

Key words Adolescents · Axial bone mass · Bone mineral density · Longitudinal study · Puberty

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Abbreviations *BMC* bone mineral content · *BMD* bone mineral density · *DEXA* dual-energy X-ray absorptiometry · *PE* physical education

Introduction

The main determinants of peak bone mass are related sex and race [12, 27]. Bone density is higher in black than in white subjects, and lower still in Asian girls [6, 22]. Environmental factors, such as dietary calcium, physical activities and hormonal effects, can contribute significantly to the variance observed [3, 20].

During skeletal maturation, two distinct processes occur: bone growth and bone modelling [1]. The first increases bone volume, achieving adult body size. Modelling is under the influence of local factors, such as mechanical load, and alters specific growth patterns and their organisation of tissue components to produce macro-architectural features [11]. To maximise bone mass during skeletal maturation, bone modelling must be optimised. Appropriate mechanical load during rapid skeletal growth and modelling in children would therefore appear important for skeletal health [15].

The effects of different levels and types of mechanical loading on bone mineral density (BMD) and bone mineral content (BMC) in adolescent and pre-adolescent children have rarely been investigated [10]. The limited data available suggest that impact loading and weight bearing exercise are associated with greater BMD and BMC in the loaded skeletal elements [24].

We recently studied, in a cross-sectional fashion, the association of puberty, physical activity, physical fitness, and nutritional factors on axial and peripheral bone mass in a group of healthy Chinese children of homogeneous age [8]. In the present study, we report the results of a 3-year prospective study aimed at evaluating the determinants of axial and peripheral bone mineral mass in healthy Chinese adolescents.

Materials and methods

Subjects

The study was approved by the Ethical Committee for Research on Human Subjects of the Medical School of the Chinese University of Hong Kong. Informed consent was obtained from the parents and from each of the 179 healthy adolescents (92 boys and 87 girls) enrolled in the 1st year of the Tii Junior High School in Shatin, Hong Kong. Children underwent physical examination to exclude any condition known to affect bone metabolism. No child was excluded on this criterion, and none was taking medications known to alter bone and/or calcium metabolism. All measurements were taken once a year during 3 consecutive years (1991–1993).

Dietary nutrition and calcium intake data

Each pupil was interviewed separately by a fully trained dietitian. A quantitative food frequency questionnaire modified and validated for the Hong Kong Chinese population [7] was used to assess individual dietary intake in the previous 2 months. Nutrient intake of the various food items was calculated from a comprehensive computerised food database compiled from previously published sources.

Level of sports participation

Pupils had been assigned to the Physical Education (PE) or to Arts classes according to their own and their parents' choice and to school matching procedures, based on the past performance of each pupil in the major subject chosen. Approximately 50% of the pupils enrolled (94) were in the PE major class, and the other 85 were in the Arts major class. Each pupil was interviewed by one of the investigators to determine the type (ball games, water sports, track and field, and other) and the level (national/school team, or recreational) of sport practised. In the PE group, 71.7% of the boys belonged to the national/school team, while none of the boys in the Arts group did ($P < 0.001$).

Anthropometric measurements and pubertal staging

Height without shoes was measured to the nearest 0.1 cm using a portable Harpenden Stadiometer [35] (Holtain, Crosswell, UK). Weight in light vest and knickers was measured using a Seca digital physician's scale (Seca Ltd., Bonn, Germany) [34, 36]. Pubertal staging was performed according to Tanner's criteria [34]. Limb dominance was determined by asking the children which hand they wrote with and with which leg they kicked a ball.

Bone mass measurements

In this study, BMC is the total bone mineral content values of all pixels within the area of interest outlined on dual-energy X-ray absorptiometry (DEXA) scanning (see below). BMD is the ratio between the total BMC and the detectable bone area. BMC and the width of both distal radii was measured using the Norland 2780 single beam photon absorptiometer (Norland Corporation, Fort Atkinson, Wisconsin, USA). The BMD of the L2 to L4 lumbar vertebrae was measured using DEXA (Model XR-26 Norland Corporation, Fort Atkinson, Wisconsin, USA). The reproducibility in evaluating the radius in two successive scans without repositioning of the subjects was 2.09% and 2.06% respectively for the BMC and bone width of the distal radius. Reproducibility of the DEXA scan results was within 1.4% for the L2 to L4 lumbar vertebrae. Repositioning the subject, reproducibility in evaluating the radius in two successive scans was 4.1% and 4.8% respectively for the BMC and bone width of the distal radius. For the L2 to L4 lumbar vertebrae, reproducibility of the DEXA scan results after repositioning of the subjects was within 3%.

Physical fitness assessment

All tests were conducted after the children had performed stretching exercises for 5 min and cycled for a further 5 min on a commercially available cycle ergometer (Monark model 818 E, Monark AB, Stockholm, Sweden) at a load setting of zero. The dominant limb was tested first, and the non-dominant limb was tested after a 3 min rest (handgrip test) or a 5 min (isokinetic test) rest. A 3 min rest was allowed between each test. Children were verbally encouraged to produce maximal effort.

1. Recovery index after a 3 min step-test [2]
2. Sit-and-reach test [1] using the Sit-and-Reach 5111 testing kit (Takei, Tokyo, Japan)

3. 1 min sit up test
4. Bilateral handgrip [30] using the Grip D 5101 dynamometer (Takei, Tokyo, Japan)
5. Vertical jump [1]
6. Bilateral isokinetic quadriceps and hamstring peak flexion and extension torque test at 60°/s and 180°/s [23].

Physical activity assessment

To assess physical activity over the past 6 months, each pupil was individually interviewed, and information gathered through self-report on: (1) type of major regular sports: ball games, track and field athletics, water sports, others, and (2) level of sports participation: PE group – national/school team members, PE group – recreational purpose, Arts group.

Statistical analysis

Data were analysed using SPSS/PC$^+$ V. 4.0. Descriptive statistics was calculated. Univariate analyses by Mann-Whitney test, Student's *t*-test, chi-squared test, ANOVA and Kruscal Wallis analysis, Pearson and Spearman correlation coefficients were used to identify significant variables. These were later used for stepwise multiple regression analysis. The analyses were carried out separately for the two sexes to allow for sex differences and different measures of the pubertal stages. In the longitudinal analysis of the factors predictive of BMC and BMD, all independent variables were given the same priority to be entered in the model. To avoid co-linearity problems due to the strong correlation between the results of the measurements at the beginning and the end of the study, the averages of some variables were used in the analysis. When applicable, Bonferroni correction was applied.

Results

Puberty stages

At the beginning of the study, just above 25% of the boys had reached stage 4 or 5 of genital development, with less than 10% of then being at stage 4 or 5 for pubic hair, and 40.9% having a testicle size greater than 12 ml. A larger percentage of the girls were at a more advanced pubertal stage: at the beginning of the study 65% had reached a breast development stage of 4 or 5, nearly 35% had stage 4 or 5 of pubic hair development, and 58% had menarche. By the end of the study, over 60% of the boys and practically all the girls had reached the more advanced stages of puberty.

Calcium intake

Calcium intake was 722 mg/day (256.8) among the boys. and 560 mg/day (219.7) among girls at the beginning of the study. At the last visit, there were no significant changes (700.8 mg/day (344.9) among the boys, and 608.8 mg/day (269.3) among the girls).

Frequency and level of sport

At the beginning of the study, the boys spent on average 9.22 h/week and the girls spent 8.77 h/week practising sport. By the end of the study, the girls' average weekly sports participation had dropped significantly to 4.81 h ($P < 0.01$). The percentage of children who played in a national team or school team did not change significantly between the beginning and the end of the study (boys: 44.2% vs 48.5%, girls 37.5% vs 31.9%) in either sex.

Isokinetic strength and physical fitness

In the boys there was a significant increase in such variables from the 1st to the 3rd year. Girls reached a plateau in their physical capabilities at an earlier age than boys.

BMC of the forearm and BMD of the lumbar spine

During the study period, the mean BMC of the dominant forearm increased from 0.61 to 0.75 ($P < 0.001$) in the boys (25%) and from 0.64 to 0.73 ($P < 0.001$) in the girls (12.3%). Similarly, the mean BMD of L2-L4 increased for both sexes, from 0.63 to 0.77 ($P < 0.001$) in the boys (24.2%) and from 0.75 to 0.87 ($P < 0.001$) in the girls (14.5%). Female students had higher BMD of the lumbar spine at all times, and higher BMC of the forearm in the 1st and 2nd year of the study. Female students were taller and heavier than male students at the beginning of the study, but shorter and lighter than male students by the end of the study. There was a significant correlation between the BMC of the forearm and BMD of the lumbar spine and age, weight, height, pubertal stage, bone width of distal forearm, and muscle strength in both sexes. The level and frequency of sports participation were associated the BMD of the lumbar spine more significantly than the BMC of the forearm. Interestingly, calcium intake and the type of sport did not significantly affect BMC of the forearm and BMD of the lumbar spine (Table 1).

Regression analysis

A number of independent variables were used to predict the changes in BMC of the dominant forearm and the BMD of the lumbar spine over the study period (Table 2). The data at the beginning and at the end of the study were used in four models. Models 1 and 2 pertained to the boys forearm BMC (A) and the L2-L4 BMD (B), and models 3 and 4 studied the girls forearm BMC (C) and the L2-L4 BMD (D), respectively (Table 3).

Knee flexion torque in the girls was a significant predictor of BMD of the lumbar spine. Dietary calcium intake and differences in sports participation and physical activity were not significantly associated with BMD of the lumbar spine and BMC of the forearm. Pubertal staging was the most important predictor of the changes in BMC of the forearm or BMD of the lumbar spine

Table 1 Univariate analysis of bone mineral data and correlated variables. *a* Pearson correlation, *b* Mann-Whitney, *c* Kruskal-Wallis, *BMC* bone mineral content of distal radius, *BMD* bone mineral density of lumbar spine L2–L4

	Boys						Girls					
	BMC			BMD			BMC			BMD		
	r	*P*	Test	*r*	*P*	Test	*r*	*P*	Test	*r*	*P*	Test
Age year 1 (1991)	0.542	<0.001**	a	0.685	<0.001**	a	0.534	0.010*	a	0.313	0.003**	a
Height year 1 (1991)	0.652	<0.001**	a	0.647	<0.001**	a	0.479	<0.001**	a	0.462	<0.001**	a
Height year 3 (1993)	0.593	<0.001**	a	0.626	<0.001**	a	0.309	0.012*	a	0.176	0.153	a
Height (average)	0.533	<0.001**	a	0.598	<0.001**	a	0.383	0.001**	a	0.365	0.002**	a
Height (% change)	−0.338	0.006**	a	−0.501	<0.001**	a	−0.535	<0.001**	a	0.647	<0.001**	a
Weight	0.671	<0.001**	a	0.591	<0.001**	a	0.479	<0.001**	a			
Calcium	0.105	0.320	a	0.099	0.349	a	−0.264	0.014*	a	−0.142	0.189	a
Bone width	0.764	<0.001**	a	0.558	<0.001**	a	0.714	<0.001**	a	0.340	0.001**	a
Bone length	0.657	<0.001**	a	0.702	<0.001**	a	0.471	<0.001**	a	0.618	<0.001**	a
Frequency of sports	0.499	<0.001**	a	0.491	<0.001**	a	0.162	0.311	a	0.073	0.650	a
Level of sports		0.057	c		0.007**	c		0.059	c		0.006**	c
3 min step test	0.059	0.580	a	0.064	0.547	a	0.007	0.946	a	0.098	0.368	a
Mean score in flexion test	0.526	<0.001**	a	0.340	<0.001**	a	0.250	0.020*	a	0.336	0.001**	a
1 min sit-ups	0.200	0.056	a	0.219	0.036*	a	0.309	0.004*	a	0.341	0.001**	a
Upper body strength	0.777	<0.001**	a	0.763	<0.001**	a	0.539	<0.001**	a	0.555	<0.001**	a
Vertical jump	0.368	<0.001**	a	0.506	<0.001**	a	0.149	0.168	a	0.280	0.009**	a
Pubertal stage early/advanced												
Genitalia/breast	0.358	<0.001**	b	0.433	<0.001**	b	0.511	<0.001**	b	0.386	<0.001**	b
Pubic hair	0.682	<0.001**	b	0.540	<0.001**	b	0.676	<0.001**	b	0.657	<0.001**	b
Size of testis/menarche	0.311	<0.001**	b	0.552	<0.001**	b	0.561	<0.001**	b	0.443	<0.001**	b

Table 2 Cross-sectional stepwise regression models for the prediction of BMC and BMD. Input variables used in the analysis were: height, genitalia/breast, pubic hair, size of testis/menarche, group, level of sports, 3 min step test, mean score in flextest, 1 min sit-up, upper body strength (grip), vertical jump and calcium intake

Independent variables	BMC				BMD			
	Boys		Girls		Boys		Girls	
	Coefficients (B)	*P*	Coefficients (B)	*P*	Coefficients (B)	*P*	Coefficients (B)	*P*
Age	0.056	<0.0001	0.033	0.0096	0.090	<0.0001	0.045	0.0069
Weight	0.004	0.0002	0.003	0.0088	0.007	<0.0001	0.009	<0.0001
Bone width	0.332	0.0001	0.516	<0.0001				
Flex-test	0.003	0.0028						
Pubic hair			0.046	0.0012			0.060	0.0013
Jump					0.004	<0.0001		
Sit up test							0.005	0.0001
Constant	−0.699		−0.425		−0.948		−0.321	
r-square	0.780		0.670		0.740		0.670	

Table 3 3-year longitudinal analysis of determinants of bone mass (multiple regression models). Input variables used in the stepwise model: age, weight, height, bone width, genitalia/breast, pubic hair, size of testis/menarche, level of sports, 3 min step test, mean score in flextest, 1 min sit-up, grip strength, vertical jump, calcium intake

	Boys		Girls	
	Model A ΔBMC	Model B ΔBMD L2–L4	Model C ΔBMC	Model D ΔBMD L2–L4
Variable	Testis size year 1	Pubic hair year 3	Menarche year 1	Pubic hair year 1
(Coefficient)	(0.060)	(0.054)	(−0.048)	(−0.070)
P	0.0013	0.0016	< 0.0001	< 0.0001
Variable	Testis size year 3	Genitalia year 3	Age year 1	Peak flexion torque
(Coefficient)	(0.036)	(0.044)	(−0.028)	(0.0015)
P	0.0472	0.0127	0.0007	0.0023
Constant	0.098	0.085	0.476	0.006
r-square	0.32	0.40	0.38	0.419
F	20.50	18.30	16.20	14.03
Df	1, 55	2, 54	2, 53	2, 39
P(F)	< 0.001	< 0.001	< 0.001	< 0.001

during the study. The direction of influence was, however, different for boys and girls. In the boys, advanced puberty stages in the whole period of the study were associated with a larger increase in BMC of the forearm or BMD of the lumbar spine. For girls, a higher age and an advanced pubertal stage at the beginning of the study resulted in a significantly ($P = 0.03$) slower rate of increase in BMC of the forearm and BMD of the lumbar spine when compared with those girls with a less advanced pubertal stage at the beginning of the study. Comparing BMC of the forearm and BMD of the lumbar spine between boys and girls, the significant differences detected at the beginning of the study remained by the end of the experimental period. The rates of change in the boys were significantly higher than in the girls ($P < 0.01$).

Discussion

The determinants of bone mass acquisition during adolescence include genetic [32, 33] and racial factors [12], mechanical factors and physical activity [24], nutritional factors [21, 39], sex hormones [5], and other even less well defined factors such as drugs. All these factors interact to influence bone accretion during adolescence, but the relationship between physical activity, calcium intake, and bone accretion during growth and development is not well understood [4].

A low peak bone mass at skeletal maturity is a risk factor for osteoporosis in later life [19, 29]. In Caucasian and black subjects, bone density is positively correlated with muscular strength both in adolescent males [9] and females [28]. Competitive adolescent weightlifters have greater bone density than age matched controls [9, 38], and college-aged female body builders have greater bone density than inactive controls and athletes involved in other types of training [17, 18].

In children, bone mass increase with age, there is a well defined correlation with calcium intake, body weight and height, and there are significant differences between boys and girls [13, 25,]. However, detailed studies on the effect of physical exercise and the effect of puberty on bone mass acquisition are relatively few [16, 37].

The present study focused on adolescents to study various factors affecting bone mass acquisition. We found that BMD of the lumbar spine is a more reliable reflection of the effect of physical exercise than BMC of the distal forearm [14, 31], but calcium intake and the level and frequency of sport participation did not significantly affect BMC of the forearm and BMD of the lumbar spine.

In our subjects, puberty was the single greatest predictor of the change in BMC of the forearm or BMD of the lumbar spine during the study. The changes, however, were different between the sexes. Although the mean age of boys and girls were similar throughout the study, girls were pubertally more advanced, and had a higher BMD of the lumbar spine. By the end of study, girls had increased their BMD of the lumbar spine, but much less than boys. This would indicate that puberty is the most significant influence in the attainment of BMD of the lumbar spine. Moreover, by the time girls achieve menarche, they would have passed their growth spurt. In boys, growth spurt occurs at a more advanced pubertal stage [34], explaining the significantly greater increase in BMD of the lumbar spine in boys over the 3 years of the study. Boys who reached a higher pubertal stage showed greater increase in BMC of the forearm and BMD of the lumbar spine, while older girls showed a lower increase in BMC of the forearm. Also, girls who had already had menarche at study entry had a smaller increase in BMC of the forearm when compared to those who had not. Similarly, girls with higher pubic hair stage in year 1 would be expected to have a smaller increase in BMD of the lumbar spine when compared to those girls with a lower pubic hair stage in year 1.

Muscle strength was significantly associated with lumbar spine BMD in girls only, and higher peak torques were associated with increase in BMD of the lumbar spine, again only in girls. All the other variables studied, including weight, height, frequency of sports, weight, height and the other fitness variables, become non-significant once the puberty variables (and age or peak torque of the knee flexors in girls) were entered into the model. This would suggest that developmental stage is the strongest predictors of the increase in BMC of the distal forearm or BMD of the lumbar spine. Such findings are consistent with some recent studies [5, 26]. One twins study has shown that, in adolescents, calcium and exercise have doubtful additional effect on the bone mass acquisition, and pubertal staging overrides all these effects [20]. Frequency of sports plays an important role in affecting BMD of the L2-L4 vertebrae in girls, but not in boys. While boys were generally more physically active, pubertal staging was the main determinant of BMD. However, with the wider range and lower level of physical activity in girls, frequency of sports participation became a significant factor.

Other studies have also shown that the rate of bone mineral acquisition in girls will dramatically fall after the age of 15–16. In boys, the increase bone mineral acquisition continues at least up to age 17–18 [5, 25].

The results of this longitudinal study are in contrast with that reported by Haapasalo et al [16]. In a cross-sectional study, they showed that, in a majority of female junior tennis players, unilateral sports activity does not produce increases in BMD until adolescent bone spurt. However, Haapasalo et al [16] studied Northern European subjects in a cross-sectional instead of a longitudinal fashion, and included subjects with a wide age range (7–17 years).

In conclusion, it appears that in Chinese adolescents, bone mineral accretion at adolescence is not influenced by exercise, level of physical fitness and calcium intake. Instead, external factors are overridden by puberty. We speculate that, in both sexes, and especially in girls, to optimally increase bone mass, regular physical exercise programmes should be instituted well before the onset of puberty rather than at or after it. Once puberty starts, these interventions may have no or only limited effect.

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