Bone mineralization in Brazilian adolescents: the years of maximum bone mass incorporation

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SUMMARY. Puberty is the fundamental period for bone mass (BM) acquisition. In this period mineralization is found to increase with levels of high bone formation. The critical years of intense bone anabolism deserve special attention, as adequate gain could minimize fracture risk in later years. The objective of this work was to study bone mineral content (BMC) and bone mineral density (BMD) in male adolescents with age bracket and maturation level. Sixty-one healthy male 10 to 19 year-olds were evaluated for calcium intake, weight, stature, BMI, puberty stage and BMC and BMD in the lumbar spine and femur. BM was measured by bone densitometry (DXA). Calcium intake was calculated by recording 3 days diet. Puberty stage was defined as per Tanner. Descriptive statistics was used with means and standard deviations, linear correlation, and analysis of variance for comparison between age groups, and the Tukey test (p<0.05). Linear correlation was positive and indicated body weight as the main correlation variable with BMD in both studied locations (p<0.01). BMC and BMD increased with age, differences were significant from 14 to 15 years, and when adolescents reached Tanner stage G4. These results showed a pronounced increase in bone mineralization, with the years after 14 to 15 being critical for BM acquisition in Brazilian adolescents.
Key words: Adolescence, pubertal events, bone mass, bone mineral density, calcium intake.

RESUMEN. Mineralización ósea en adolescentes brasileños: años de máxima incorporación de masa ósea. La pubertad es un momento fundamental para la adquisición de la masa ósea (MO). En este período, la mineralización se encuentra en aumento en los niveles de formación ósea. Los años críticos de intenso anabolismo óseo necesitan de atención, porque el adecuado aumento de la masa ósea podría minimizar el riesgo de fracturas en los años posteriores. El objetivo de esta investigación fue estudiar el contenido mineral óseo (CMO) y la densidad mineral ósea (DMO) en adolescentes según el grupo de edad y el nível de maduración. Sesenta y un adolescentes saludables de 10 a 19 años fueron evaluados cuanto a la ingesta de calcio, peso, estatura, índice de masa corpórea (IMC), etapa puberal, CMO y DMO en la columna lumbar y en el fémur. La MO fue medida por densitometría ósea (DEXA). La ingesta de calcio fue calculada mediante un recordatorio de ingesta de tres días. La etapa puberal fue definida por los criterios de Tanner. Estadística descriptiva fue utilizada con media y desviación estándar, correlación linear y análisis de varianza para comparar los grupos y test de Tukey (p<0,05). Correlación linear fue positiva e indicó que el peso corporal fue la principal variable correlacionada con la DMO en los dos sitios estudiados (p<0,01). CMO y DMO aumentaron con la edad y las diferencias fueron considerables de los 14 a los 15 años, cuando los adolescentes alcanzaban la etapa G4 de Tanner. Estos resultados muestran un aumento pronunciado en la mineralización ósea; entre los 14 y 15 años fueron críticos para la adquisición de MO en adolescentes brasileños.
Palabras clave: Adolescencia, eventos puberales, masa ósea, densidad mineral ósea, ingesta de calcio.

INTRODUCTION

The bone mineralization process begins in the fetus, extending throughout infancy, and peaks in adolescence. These years are the fundamental period for bone mass acquisition. Several researchers consider that infancy and adolescence have highest bone mineral capital increase for both sexes (1-6).

International research has been developed showing the relationship between adolescence and bone health. These studies are based on the cyclic principle involving bone mass deposition throughout life. Infancy and adolescence are marked by a very important bone formation rate with predominance of formation over reabsorption. In adulthood both processes stabilize and from 45 to 50 years, especially for females, there is a predominance of bone reabsorption.

However, bone reabsorption is not exclusive to females as both osteopenia and osteoporosis have significantly increased in males (7).

Expressive longitudinal growth during puberty has three distinct phenomena which occur sequentially. They are: growth spurt lasting about 2 to 3 years, characterized by a reduced growth velocity prepuberal phase, an accelerated growth velocity known as Peak Height Velocity (PHV), and a growth cessation phase which contributes to over 20% of final adult stature; a rapid acquisition of bone mineral content known as bone mass peak, and the skeletal maturation process which ends with epiphyseal closure (1,2,8,9).

Bone mass peak contributes to 40 - 50% of bone mineral content variation, with the incorporation of approximately 1000g mineralized bone during adolescence [10]. Research

has shown that when bone mass accumulation is potentialized during puberty and maintained in adulthood we can minimize reductions from advancing age, thus helping to prevent osteopenia/osteoporosis and consequent fractures (11).

Osteoporosis is a heterogenous disorder considered a severe public heath problem which can in part be due to inadequate bone gain during infancy and adolescence (12). Nutritional factors such as adequate calcium supplement, according to age bracket and gender [13] and physical exercise, especially with high impact show protective effects related to healthy bone tissue maintenance independent to the time in life when these measures are adopted; however they should be put into practice early in infancy and adolescence (11,14,15).

In recent years, methods have been developed to accurately evaluate bone mass allowing us to better understand bone tissue dynamics. Dual energy x-ray absorptiometry (DXA) allows very precise analysis with low radiation exposure; this is suitable for evaluating children and adolescents (16,17).

Variations in bone density during infancy and adolescence have been seen in epidemiological studies in different countries. In Brazil however, there have been few investigations on bone mineralization in healthy children and adolescents (18). The objective of this study was to determine the behavior of bone mineral content (BMC) and bone mineral density (BMD) in male adolescents in relation to age bracket and sexual maturity levels.

SUBJECTS AND METHODS

This study included 10 to 19-year-old healthy volunteer students from a private school (Associação Brasileira de Educadores Lassalistas, Colégio La Salle, Botucatu, São Paulo State). The research was approved by the Research Ethics Committee of Botucatu School of Medicine – UNESP, and the volunteers and their parents/guardians gave informed written consent.

Inclusion criteria were weight between the 10 and 90 percentiles, and height between the 10 and 97.5 percentiles for each age bracket (19), with adequate body mass index (BMI) for their age (20), and with daily dairy product intake. They had to be non-smokers and non-drinkers, could not be involved in any extra-curricular sporting activity, only the school's physical education classes. Control of normal physical activity was not necessary as investigations indicate that it is programmed sporting activities that produce higher increases in bone mass (1,2,15).

Exclusion criteria were: history of prematurity or low birth weight, prolonged corticoid therapy, or calcium or iron supplement in the twelve months prior to research. Other exclusion criteria were: diabetes mellitus, acute or chronic malnutrition, congenital or acquired bone diseases, gastrointenstinal diseases followed by malabsorption, history of nephropathy with or without chronic renal insufficiency, endocrinopathies, precocious and delay puberty, chronic drug consumption, cystic fibrosis, celiac disease, and use of drugs negatively affecting bone metabolism such as anticonvulsants and antacids with aluminum. Exclusions related to diet were: vegetarianism, high fiber, caffeine, or soft drinks consumption, and no daily dairy product intake.

Data collection started at school; randomly selected students without any dysfunction or disorder exclusions were invited for weight and height measurements. When weights and heights were within the proposed limits, they were asked about drinking and smoking habits. Those fulfilling the criteria were then invited to participate in the study. Their parents/guardians were then contacted to explain the methods used and seek consent. Students or parents could withdraw from the study at any time.

From 497 students, 61 who fitted the inclusion criteria participated in all evaluations. A private school was chosen because it represents a socially privileged population thus ensuring the most favorable conditions to achieve full bone gain potential. As far the ethnic question is concerned, the extent of miscegenation in Brazil is very high, however none of our adolescents were children of exclusively African or Asian origin parents.

Volunteers fitting the criteria were then invited with their parents to attend the Adolescent Outpatient Clinic at Botucatu University Hospital School of Medicine – UNESP where they were interviewed with their parents and submitted to a general and specific physical examination to detect any physical alterations. Secondary Sexual Characters were evaluated, and compared to Tanner Criteria (21). To assess the impact of puberty stage on bone mineralization, maturation level by visual inspection of genitals was compared to BMC and BMD results from dual energy x-ray absorptiometry (DXA). Skeletal maturity (bone age) was obtained by the GP method (22), where hand and wrist x-rays are compared with the Atlas.

Diet characterization then followed using a three-day dietary record completed by participants and analyzed by the authors to obtain information on food intake, preferences, refusals, the main meals involving calcium and any other factors that could possibly interfere in the bio-availability of this mineral (23). Centesimal quantification of food data was by a computerized nutritional analysis system developed by São Paulo University School of Public Health Nutrition Department (24).

BMC (g) and BMD (g/cm^2) were determined for each adolescent by a DXA with Hologic QDR 2000-Plus densitometer. Bone mass evaluation was performed on the lumbar spine between L1-L4 and the femoral neck.

Data were analyzed using Statistica Version V. Age brackets (AB) between 10 and 19 years were defined as follows: 10y complete to 11y, 11m, 29d (AB 1); 12y to 13y, 11m, 29d (AB 2); 14y to 15y, 11m, 29d (AB 3); 16y to 17y, 11m, 29d (AB 4); and 18y to 19y, 11m, 29d (AB 5). Means

and standard deviations were used to characterize weight, height, BMI, and three-day mean calcium intake. Pearson simple linear correlation coefficients were calculated between bone mass and morphological aspects, and puberty stage (p<0.01). Analysis of variance was used to compare all ABs and maturation levels with BMC and BMD, and the Tukey test was used to locate significant differences (p<0.05).

RESULTS

Table 1 shows general characteristics: body weight, height, BMI, and mean daily calcium intake calculated by recording diet over three days for each age bracket.

We observed increased body weight, height, and BMI with advancing age; these were significant from 14 to 15 yrs (AB 3) on (Table 1).

TABLE 1

General characteristics of the adolescents studied: body weight (kg), stature (m), BMI (kg/m^2), and total calcium intake (mg/day)

Age Bracket Age (years)	Weight (kg) X ± SD	Stature (m) X ± SD	BMI (kg/m2) X ± SD	Calcium (mg/day) X ± SD
(AB1)10-11 (n=14)	35.75 ± 4.57 c	1.432 ± 0.07 bc	17.39 ± 1.43	783 ± 236
(AB2)12-13 (n=14)	43.49 ± 8.47 ac	1.550 ± 0.08 ac	17.91 ± 1.63	740 ± 198
(AB3)14-15 (n=14)	57.71 ± 7.53 ab	1.710 ± 0.06 ab	19.67 ± 1.83 a	887 ± 228
(AB4)16-17 (n=12)	62.71 ± 7.48 ab	1.731 ± 0.07 ab	20.91 ± 1.99 ab	894 ± 275
(AB 5) 18-19 (n=7)	70.34 ± 3.20 abc	1.803 ± 0.05 ab	21.65 ± 1.16 ab	1073 ± 434 b

ANOVA analysis of variance and Tukey's test for differences between age groups.
Letters show the differences between age groups (p<0.05).
[a] Difference between AB1 and the other groups.
[b] Difference between AB2 and the other groups.
[c] Difference between AB3 and the other groups.
BMI= body mass index;   AB= Age brackets;   SD= standard deviations

After analysis of the dietary diaries records completed by the adolescents, we observed that calcium intake for the different age groups were from 740±198 mg/day to 1,073±434 mg/day, with an average of 863±280 mg/day.

Pearson's simple linear correlation coefficient was used to investigate the impact of body dimension and nutritional changes related to genital maturation stage classification over bone mass. Table 2 shows significant and positive differences for simple linear correlation between all variables; significance level was less than 1%.

TABLE 2

Simple correlation coefficients between variables related to bone mass, morphological aspects, and secondary sexual characteristics

Bone Mass	Age (years)	Bone Age (years)	Weight (kg)	Stature (m)	BMI (kg/m^2)	Genitals (stage)
Spine area (cm^2)	0.774*	0.765*	0.860*	0.890*	0.602*	0.789*
Spine BMC (g)	0.831*	0.830*	0.881*	0.860*	0.687*	0.807*
Spine BMD (g/cm^2)	0.827*	0.840*	0.852*	0.803*	0.714*	0.797*
Femur area (cm^2)	0.782*	0.803*	0.891*	0.922*	0.648*	0.843*
Femur BMC (g)	0.822*	0.831*	0.919*	0.882*	0.743*	0.814*
Femur BMD (g/cm^2)	0.758*	0.748*	0.805*	0.725*	0.711*	0.706*

*Significant values (p<0.01).

More precise analysis reveals that, from all the studied indicators, BMI had the poorest correlation to adolescent bone mass. The highest correlation was between BMC and body weight; the score for lumbar spine was r= 0.88, and for femural neck was r=0.91. The highest correlation for BMD was also to body weight; the score for lumbar spine was r=0.85, and femural neck r=0.80. The correlation between skeletal and sexual maturation indicators was greater than 0.70, showing a strong participation from biological maturation in relation to bone mass increase for these adolescents.

Table 3 shows BMC and BMD values for lumbar spine and femoral neck with adolescent age. Significant differences (p<0.05) were seen from 14 to 15 yrs (AB 3) for both BMC and BMD in these regions.

TABLE 3

Mean and standard deviations of bone mineral content and bone mineral density
in the lumbar spine and femoral neck for age groups

Age (Years)	BMC- Spine (grams)	BMD-Spine (g/cm^2)	BMC-Femur (grams)	BMD-Femur (g/cm^2)
(AB-1)10–11	28.71± 3.95 [c]	0.618 ± 0.049 [c]	26.01 ± 3.69 [c]	0.800 ± 0.07 [c]
(AB-2)12–13	36.08 ± 10.16 [c]	0.708 ± 0.116 [c]	32.85 ± 9.73 [c]	0.844 ± 0.08 [c]
(AB-3)14–15	51.54 ± 13.28 [ab]	0.836 ± 0.132 [ab]	48.87 ± 9.40 [ab]	0.978 ± 0.14 [ab]
(AB-4)16–17	59.26 ± 11.38 [ab]	0.937 ± 0.113 [ab]	54.54 ± 11.65 [ab]	1.095 ± 0.17 [ab]
(AB-5)18–19	76.72 ± 10.46 [abc]	1.094 ± 0.132 [abc]	62.15 ± 7.35 [abc]	1.205 ± 0.11 [abc]
Total (n=61)	47.16 ± 18.50	0.806 ± 0.187	42.59 ± 15.54	0.956 ± 0.18

ANOVA analysis of variance and Tukey's test for differences between age groups
Letters show differences between age groups (p<0.05).
[a] Difference between AB1 and the other groups.
[b] Difference between AB2 and the other groups.
[c] Difference between AB3 and the other groups.
BMC= bone mineral content;   BMD= bone mineral density

Bone mineralization parameters were compared with sexual maturation level, particularly genital development, to see which stages of puberty had the highest increase in bone mass (Table 4). Significant differences (p<0.05) were seen in G4 and G5 for both BMC and BMD; there were no significant alterations in mineralization parameters between G1 and G3.

TABLE 4

Mean and standard deviations of bone mineral content and bone mineral density
in the lumbar spine and femoral neck according to sexual maturity levels

Genital Development	BMC- Spine (grams)	BMD-Spine (g/cm2)	BMC-Femur (grams)	BMD-Femur (g/cm2)
G 1 (n=6)	25.98 ± 2.86	0.584 ± 0.045	24.53 ± 3.43	0.786 ± 0.089
G2 (n=12)	29.61 ± 4.26	0.639 ± 0.053	26.48 ± 3.71	0.806 ± 0.066
G3 (n=8)	34.48 ± 6.77	0.694 ± 0.102	33.13 ± 3.93	0.842 ± 0.062
G4 (n=15)	51.55 ± 13.46 ab	0.844 ± 0.121 ab	50.00 ± 13.35 abc	0.999 ± 0.178 b
G5 (n=20)	65.03 ± 13.51 abc	0.989 ± 0.138 abc	55.90 ± 8.97 abc	1.109 ± 0.151 abc

ANOVA analysis of variance and Tukey's test for differences between age groups
Letters show differences between age groups (p<0.05).
a Difference between G1 and the other groups.
b Difference between G2 and the other groups.
c Difference between G3 and the other groups.

Figure 1 shows BMD variation in ABs. Increased growth can be seen from 10 to 19 yrs, with significant differences in femur neck and lumbar spine BMD between 14 and 15 yrs. Femoral neck values are higher than lumbar spine at all ages.

FIGURE 1

Variation in bone mineral density according to age group
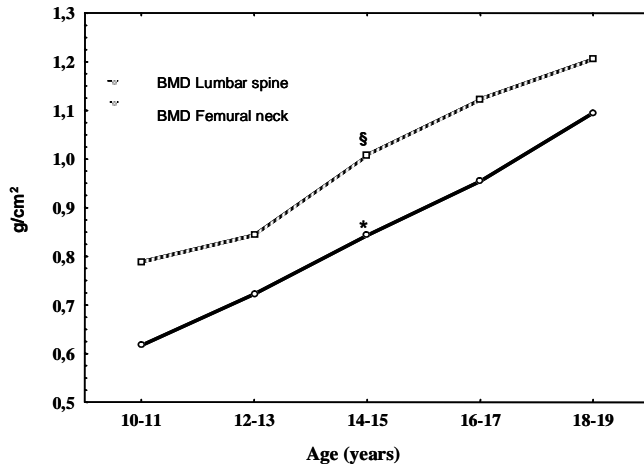
* > significant in BMD – Lumbar spine (p<0.05).
§ > significant in BMD – Femural neck (p<0.05).

Bone mineralization behavior was similar and increased with sexual maturation (Figure 2), indicating significant differences in G4 and G5 for BMD in both regions. However in G3, we can clearly see pronounced increases in BMD.

FIGURE 2

Variation in bone mineral density according to sexual maturity levels
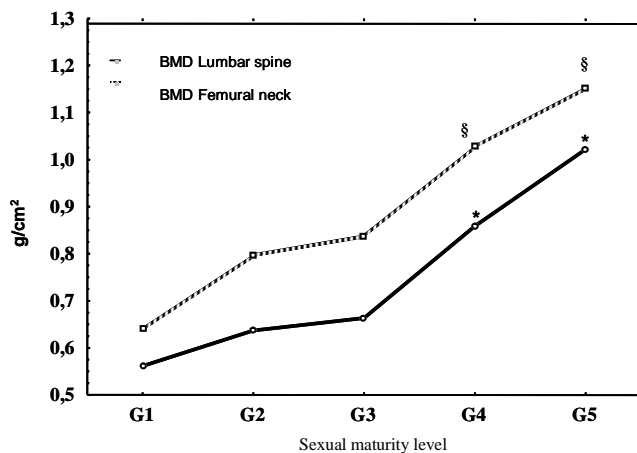
* > significant in BMD – Lumbar spine (p<0.05).
§ > significant in BMD – Femural neck (p<0.05).

DISCUSSION

Bone mineralization is a complex process with several factors affecting bone mass acquisition, the most important being genetic factors; body dimension, weight, and height alterations; hormonal profile which leads to sexual and skeletal maturity; physical exercise; and an adequate calcium intake at this age which is reflected in strong bone mineralization (7,12,25,26).

This study showed mean nutrition indicator values for body weight, stature and BMI for each age bracket similar to those presented by the National Center for Health Statistics (NCHS) data, as inclusion criteria were similar to the methods chapter (19,20).

In this group, the calcium supplement intake did not reach minimum recommended levels. Intake ranged from 713 ± 292 to 1451 ± 334mg/day, when ideal intake for adolescents of either sex should be 1300mg/day (13). Literature shows that maximum intake should not exceed 2500mg/day; this was not reached by any adolescent in this study. However values in this study were higher than in other Brazilian studies for the same age bracket (27-29). Transverse studies on children and adolescents indicate the beneficial effects of adequate calcium intake on bone mass peak (12). Apparently, low calcium intake during child and adolescent growth results in lower bone mineralization than with same age bracket individuals who had adequate intake (30).

According to Abrams et al. (31) there is no doubt that adolescents may adapt to very low calcium intakes (<500mg/day) by increasing fractional absorption and decreasing both urinary and endogenous fecal calcium excretion, but the exact contributions to "calcium economy" are not known at this time. They suggest that those on very low calcium intakes are at substantial risk of low calcium retention. However, a double blinded controlled study on calcium carbonate supplementation over 13 months in 143 male adolescents showed a significant increase in spinal (+2.5%), proximal femur (+2.3%), and whole body (+1.3%) bone mineral content. The authors also emphasized that bone mass potentialization is associated with an increase in stature (0.4%) equivalent to 7mm (32).

The mean for calcium intake in our study was 863±280mg/day, values under the dietary intake references for calcium (DRI) (13), but superior to those considered as very low calcium intake (31).

The beneficial impact of calcium intake on child and adolescent bone mineralization was reported in a longitudinal study by Lee et al. (33). Calcium carbonate (800mg/day) was given for 18 months to children of both sexes with a mean age of 8.5 years. The results showed a significant increase in lumbar spine BMC in relation to controls.

The potential benefits of a calcium rich diet and systematic

physical exercise during infancy and adolescence have been reported by several authors (2,4,11). These behaviors are the basis of a healthy lifestyle related to bone mass. Literature shows that adequate habits started in pediatric populations tend to last throughout adult life and minimize the risk of fractures later on (2,12,15).

The results in Table 2 are similar to other investigations correlating BMD with anthropometric variables such as body weight, height, and alterations in sexual and bone maturation (12,18,29).

In a Brazilian study, Pessoa et al. (34) evaluated BMC and BMD in pre-pubescent children, 7–8 years old, and found high and positive correlations between lumbar spine BMC and BMD and bone age, body weight, and height. They suggest that interpretation of bone mass in pre-pubescence should be linked to body weight and bone age variation (34). Similarly, Klein reported a significant correlation between total body BMD and chronological and bone age in children of approximately 10 years old and of both sexes (35).

In relation to age, this study shows significant differences from 14 to 15 years, both in BMC and BMD in the lumbar spine and femoral neck regions. Rubin evaluated BMD in 299 children and adolescents of both sexes between 6 and 18 years old (12). The results indicated a major acceleration in lumbar spine BMD from 13 years in males, which stabilized around 15 to 16 years, similar to our study. In a study with 207 Caucasian children and adolescents of both sexes between 9 and 17 years, there was a pronounced difference in males in both lumbar spine and femoral neck between 13 and 17 years [36]. According to Theintz, the period between 13 and 17 years was fundamental for BMD increase in the lumbar spine and femoral neck (37).

Everything indicates that the period from 14 to 16 years old is critical for bone mineralization. These data are in agreement with several studies where there a linear increase was seen in bone mass during infancy, with an exponential increase during puberty in several bone sites (12,18,38).

Although age is a major temporal indicator for alterations occurring in adolescence, it is limited in relation to the constant modifications occurring in puberty due to maturation level variability in individuals of the same age. More recently researchers have reported that puberty stage and bone age should be considered when interpreting bone mass measurements (26).

Male adolescents significantly increase bone mass between the ages of 14 and 15, and between G4 and G5 maturation levels; this is reflected in the bone mineral content and bone mineral density gains seen in our study in the lumbar spine and femoral neck regions. Therefore, to be between 14 and 15 years old and over the G3 maturation stage, corresponds to a critical gain in bone mass acquisition which impacts high mineralization rate.

This study shows that the increase in bone mineralization during puberty occurs at the same time as significant increases in body dimensions and is related to secondary sexual characters.

It is important to emphasize that the adolescents in this study come from a socially differentiated stratum with adequate weight and height, and higher calcium intake than other Brazilian studies (27-29). The results indicate variations in BMC and BMD in healthy adolescents. Currently, this is the only study that considers rigorous inclusion and exclusion criteria for the Brazilian population. When compared with international published data they indicate great similarity.

ACKNOWLEDGEMENTS

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