

## Bone mineral density and content in adolescent girls

### *Conteúdo e densidade mineral óssea de adolescentes do sexo feminino*

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**Abstract** – The aim of the present study was to characterize bone mineral density (BMD) and content (BMC) in Brazilian adolescent girls according to age and pubertal stage. A total of 329 girls ranging in age from 10 to 20 years participated in this study. Body weight, height, body mass index, pubertal stage, race, daily calcium intake, and time spent per week performing moderate- to vigorous-intensity physical activity (MVPA) were evaluated. Lumbar spine and femoral neck BMD and BMC were assessed by dual-energy x-ray absorptiometry. One-way ANOVA with Tukey post-hoc test was used to identify differences in bone mass between ages and pubertal stages ( $p \leq 0.05$ ). The daily calcium intake reported by the adolescents was inadequate, corresponding to only 26-47% of the recommended allowance (1,300 mg/day). On the other hand, weekly MVPA was higher than that recommended for adolescents. Significant differences in BMD and BMC were observed for girls aged 10-14 years. In addition, lumbar spine and femoral neck BMD was 58 and 31% higher in postpubertal girls, respectively, when compared to prepubertal adolescents.

**Key words:** Bone mineral density; Bone mineral content; Adolescents; Puberty.

**Resumo** – O presente estudo teve como objetivo caracterizar o conteúdo mineral ósseo (CMO) e a densidade mineral óssea (DMO) de adolescentes do sexo feminino de acordo com a faixa etária e o estágio de maturação sexual. A amostra desse estudo foi composta por 329 meninas com idades entre 10 e 20 anos. Foram avaliados o peso corporal, estatura, índice de massa corporal, estágio de maturação sexual, a raça, o consumo diário de cálcio e o tempo dispendido em atividades físicas de intensidades moderada a vigorosa por semana (AFMV). A densidade e o conteúdo mineral ósseo da coluna lombar e do colo do fêmur foram avaliados pela densitometria óssea. As diferenças da DMO e do CMO, de acordo com a idade e a maturação sexual, foram avaliadas por uma análise de variância One-way ANOVA com o teste post-hoc de Tukey ( $p \leq 0,05$ ). O consumo diário de cálcio reportado pelas adolescentes é inadequado, pois representa uma variação de 26 a 47% do que é recomendado. Por outro lado, o tempo dispendido em AFMV, por semana, foi muito superior ao mínimo recomendado, em todas as idades. Ocorreram diferenças significativas tanto na DMO quanto no CMO das adolescentes no período dos 10 e 14 anos de idade. Além disso, os valores de DMO da coluna lombar e do colo do fêmur das adolescentes pós-púberes foram 58% e 31% maiores, respectivamente, quando comparados com os seus correspondentes nas adolescentes pré-púberes.

**Palavras-chave:** Densidade mineral óssea; Conteúdo mineral ósseo; Adolescentes; Puberdade.

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## INTRODUCTION

Osteoporosis is a metabolic bone disease characterized by a reduction in bone mineral density (BMD) and deterioration of bone microarchitecture, which increases skeletal fragility and the risk of fracture<sup>1</sup>. The Brazilian Health System (Sistema Único de Saúde) spent almost R\$ 81 million (US \$ 46 million) with the treatment of fractures in older people in 2009<sup>2</sup>. The standard method for the diagnosis of osteoporosis is densitometry of the lumbar spine and proximal femur (femoral neck and/or total femur).

Although osteoporosis commonly affects older people, approximately 60% of the risk of developing the disease can be explained by bone mass acquisition during childhood and adolescence<sup>3</sup>, a fact that has encouraged studies investigating the aspects of bone mass gain during this period. Actually, some factors influencing bone mass acquisition during adolescence have already been established: genetic factors<sup>4</sup> that can account for 80% of the variation in BMD; age and pubertal stage<sup>5,6</sup>, with 90 to 100% of bone mass being acquired at the end of adolescence; ethnicity<sup>3</sup>, Afro-Americans have higher BMD than Caucasians and Asians, and lifestyle factors<sup>6,7</sup> such as daily calcium intake and physical activity level.

However, there are many differences in lifestyle behaviors and cultural way between populations around the world and these differences can change significantly adolescents BMD values from a country to another. For example, Lebanese adolescents had lower BMD than Canadian and American adolescents<sup>8</sup>. In Brazil, studies investigating BMD in adolescents only started to emerge in the last decade. However, the number of publications is still very modest when compared to the international literature.

Physical fitness<sup>9</sup> and sport<sup>10</sup> have been identified as factors associated with BMD in Brazilian adolescents. However, other factors that are also important for the study of BMD, such as age and puberty, have been little investigated in Brazilian adolescents. Only two studies evaluating these aspects were found until now. One study only included adolescent boys<sup>11</sup>, whereas the other evaluated adolescents of both genders ranging in age from 6 to 14 years<sup>12</sup>. Therefore, the objective of the present study was to characterize bone mineral content (BMC) and BMD in adolescent girls according to age and pubertal stage.

## METHODOLOGICAL PROCEDURES

### Sample

The present population included sister pairs with at least one girl being enrolled in a public school in Brasília, Distrito Federal. These adolescents were first recruited to participate in a larger study that analyzed the linkage of chromosome region 1q and 11q with BMD in sister pairs. Thus, a convenience sample consisting of 329 girls ranging in age from 10 to 20 years was used. The following inclusion criteria were adopted for selection of the sample: absence of any chronic-degenerative disease, absence of a history of diseases or use of medications that could affect bone development, and no immobilization of body parts over a prolonged period of time during the year prior to the study.

For characterization of the sample, all adolescents answered questions about the regular consumption of cigarettes and/or alcoholic beverages and about the use of oral contraceptives. The participants or legal guardians (for adolescents younger than 18 years) signed a free informed consent form before any intervention. The study was approved by the Ethics Committee of Universidade Católica de Brasília (CEP/UCB No. 078/2006) according to Resolution 196/96 of the National Health Council.

### Anthropometry and Pubertal stage

Body weight and height were measured using standard procedures. Height was measured with a Seca wall-mounted stadiometer to the nearest 0.1 cm. Body weight was measured with a Plena digital scale to the nearest 100 g. Body mass index (BMI) was calculated as body weight (kg) divided by the square of the height (m). Pubertal stages was determined by self-report of pubic hair as described by Tanner<sup>13</sup>. The adolescents were classified as prepubertal (Tanner I), pubertal (Tanner II and III), and postpubertal (Tanner IV and V).

### Ethnic classification

Self-evaluation of ethnicity was used for sample characterization. The skin color and race classification system adopted in household surveys of the Brazilian Institute of Geography and Statistics (IBGE) was used: white (in Portuguese, branco), black (preto), brown (pardo), yellow (amarelo), and Amerindian (indígena).

### Estimation of daily calcium intake and time spent in moderate- to vigorous-intensity physical activity (MVPA)

A 24-h food diary was applied to estimate daily

calcium intake. Calcium intake was calculated using the Diet Pro 5.1i nutrition software. MVPA was evaluated using the short version of the International Physical Activity Questionnaire (IPAQ). This instrument presents acceptable measurement properties to monitor physical activity levels in adolescents, although some limitations have been reported for younger adolescents (< 14 years)<sup>14</sup>.

### Bone mineral density and bone mineral content

Lumbar spine and femoral neck BMD and BMC were measured by dual-energy x-ray absorptiometry (DXA) using a Lunar DPX-IQ device (software version 4.7e). The coefficient of variation obtained for measurements performed at the laboratory of Universidade Católica de Brasília (8 measurements were obtained from the same subject over 8 consecutive days) ranges from 0.7% to 2.4%<sup>9</sup> for both BMD and BMC at all bone sites. The device is calibrated daily and all measurements were performed and analyzed by the same technician.

### Statistical analysis

First, the variables were analyzed descriptively using means and standard deviations. Skewness and kurtosis were calculated to determine whether the data were normally distributed. Calcium intake and MVPA were slightly skewed (skewness > +1.0) and they were square root modified ( $\sqrt{x}$ ) before being included in the subsequent analysis. The bone parameters were classified and reported according to age and pubertal stage. One-way ANOVA

with Tukey's post-hoc test was used to determine differences between variables according to age and pubertal stage. The SPSS for Windows (version 16) package was used for analysis of the data, adopting a level of significance of  $p \leq 0.05$ .

## RESULTS

The mean and standard deviation of body weight, height, BMI and daily calcium intake were classified according to age and are shown in Table 1. Daily calcium intake and physical activity level were positively skewed. After correction, no significant differences in these parameters were observed between ages. None of the adolescents reported regular cigarette consumption and only four reported to regularly consume alcoholic beverages. In addition, 23 adolescents reported the use of oral contraceptives, but their bone parameters were similar to those not using contraceptives. The mean ( $\pm$  standard deviation) age at menarche was  $12.2 \pm 1.28$  years, corresponding to 79% of the girls studied since 67 have not had their first menstrual period. Ethnic self-identification of the participants showed the following distribution: 32.8% (n=108) white, 7.3% (n=24) black, 56.2% (n=185) brown, 1.5% (n=5) yellow, and 2.1% (n=7) Amerindian.

Mean BMD and BMC of the adolescents according to age and pubertal stage are shown in Tables 2 and 3, respectively. Lumbar spine and femoral neck BMD was 58% and 31% higher in postpubertal girls, respectively, when compared to prepubertal adolescents (Table 3).

**Table 1.** General characteristics of the sample according to age.

Age (years)	N	Body weight (kg)	Height (cm)	BMI (kg/m <sup>2</sup> )	MVPA <sup>†</sup> (min/week)	Daily calcium intake <sup>†</sup> (mg)
10	15	34.5 $\pm$ 10.5*	140.2 $\pm$ 7.9*	17.2 $\pm$ 3.3*	526.6 $\pm$ 918	415.3 $\pm$ 335.8
11	27	41.7 $\pm$ 8.7*	150.8 $\pm$ 7.2*	18.2 $\pm$ 3.2*	533.9 $\pm$ 766.7	488.8 $\pm$ 262.4
12	30	45.9 $\pm$ 10.3*	153.9 $\pm$ 6.3*	19.2 $\pm$ 3.2	553.2 $\pm$ 866.8	623.3 $\pm$ 363.3
13	32	48.9 $\pm$ 8.3	158.8 $\pm$ 7.4	19.3 $\pm$ 2.7	520.9 $\pm$ 503.9	502.9 $\pm$ 317.1
14	38	52.1 $\pm$ 9.1	159.2 $\pm$ 6.5	20.4 $\pm$ 3.1	724.6 $\pm$ 769.6	395.6 $\pm$ 287.2
15	51	53.7 $\pm$ 9.3	160.4 $\pm$ 5.3	20.8 $\pm$ 3.3	412.9 $\pm$ 512.7	417.7 $\pm$ 240.0
16	41	54.3 $\pm$ 9.4	161.1 $\pm$ 5.5	21.0 $\pm$ 3.8	495.1 $\pm$ 545.1	407.4 $\pm$ 268.6
17	51	53.0 $\pm$ 6.9	161.5 $\pm$ 5.8	20.3 $\pm$ 2.4	502.1 $\pm$ 809.9	435.2 $\pm$ 387.3
18	21	52.9 $\pm$ 6.0	160.9 $\pm$ 5.8	20.4 $\pm$ 2.0	703.6 $\pm$ 875.2	344.2 $\pm$ 236.8
19	12	53.5 $\pm$ 9.4	161.2 $\pm$ 7.6	20.6 $\pm$ 3.9	713.3 $\pm$ 1087.1	499.4 $\pm$ 293.8
20	11	54.4 $\pm$ 8.9	159.0 $\pm$ 4.4	21.5 $\pm$ 3.4	797.7 $\pm$ 1475.4	392.2 $\pm$ 229.5

Values are reported as the mean $\pm$ standard deviation.

BMI: body mass index; MVPA: time (in min) spent per week in moderate/vigorous physical activities.

\* Significant difference compared to the other ages ( $p \leq 0.05$ ).

† Uncorrected values.

**Table 2.** Bone mineral density and content of adolescent girls according to age.

Age (years)	D	Spine BMD (g/cm <sup>2</sup> )	Spine BMC (g)	Femoral neck BMD (g/cm <sup>2</sup> )	Femoral neck BMC (g)
10	15	0.657±0.17*	21.47±8.83*	0.796±0.12*	1.99±0.62*
11	27	0.792±0.12*	30.62±8.39*	0.867±0.11*	2.40±0.68*
12	30	0.883±0.11*	35.47±9.44*	0.928±0.13*	2.86±0.72
13	32	0.988±0.13*	43.95±8.68*	0.983±0.14	2.80±0.80
14	38	1.047±0.09	49.05±8.22	1.074±0.13	3.28±0.98
15	51	1.073±0.11	51.38±8.77	1.074±0.13	3.67±0.90
16	41	1.107±0.13	54.08±8.78	1.079±0.12	3.36±0.89
17	51	1.088±0.11	52.57±8.89	1.064±0.12	3.45±1.08
18	21	1.107±0.14	53.90±10.48	1.085±0.13	3.37±0.79
19	12	1.135±0.16	56.31±9.53	1.028±0.14	3.36±1.06
20	11	1.135±0.09	53.77±8.43	1.042±0.12	2.93±0.69

DMO: Densidade mineral óssea; CMO: Conteúdo mineral ósseo.

\* Diferença significativa em relação às outras idades (p≤0,05)

**Table 3.** Bone mineral density and content of adolescent girls according to pubertal stage.

Pubertal stage	N	Spine BMD <sup>†</sup> (g/cm <sup>2</sup> )	Spine BMC <sup>†</sup> (g)	Femoral neck BMD <sup>†</sup> (g/cm <sup>2</sup> )	Femoral neck BMC <sup>†</sup> (g)
Tanner I <sup>a</sup>	32	0.690±0.14	23.2±7.1	0.806±0.12	2.19±0.68
Tanner II <sup>b</sup>	25	0.970±0.14	43.5±10.7	1.014±0.14	3.04±0.90
Tanner III <sup>b</sup>	49	0.946±0.15	41.6±11.8	0.978±0.15	2.80±0.78
Tanner IV <sup>c</sup>	86	1.074±0.13	50.7±8.9	1.063±0.14	3.35±1.08
Tanner V <sup>c</sup>	137	1.089±0.12	52.4±8.9	1.059±0.12	3.42±0.87

Values are reported as the mean±standard deviation.

BMD: bone mineral density; BMC: bone mineral content.

a: prepubertal; b: pubertal; c: postpubertal.

† Significant difference between pubertal stages: a<b<c.

## DISCUSSION

In the present study, body weight and height increased significantly between 10 and 13 years of age. The mean weight and height of the sample are similar to those reported in a study of students from five Brazilian regions<sup>15</sup>. However, comparison with regional studies showed slightly higher values up to 14 years of age when compared to adolescents from the northeastern region<sup>16</sup> and similar values compared to students from the southern region<sup>17,18</sup>. These data suggest that, although body weight and height are within the Brazilian reference range, BMD and BMC can vary between adolescents from different Brazilian regions.

The daily calcium intake observed in the present study ranged from 26% to 47% of the recommended for adolescents (1300 mg/day)<sup>19</sup>. On the other hand, the time spent by the participants performing MVPA was higher (twice as high for some age groups) than the minimum time recommended for adolescents (300 min of MVPA per week)<sup>20</sup>. The relationship between physical activity, calcium intake and BMD gain is still not well understood. However, BMD can increase due to an increase in

blood estrogen levels mediated by physical activity. Estrogen reduces the activity of osteoclasts, the cells responsible for bone-resorption, which leads to an increase of bone mass, and more calcium and phosphorus is then absorbed from blood to bone<sup>21</sup>. As a consequence, inadequate calcium intake by adolescents can reduce the amount of circulating calcium in blood and thus compromise BMD gain mediated by MVPA. In this respect, since inadequate calcium intake has been observed in different Brazilian cities<sup>10,22</sup>, national food reeducation programs for adolescents are necessary to increase the consumption of foods rich in calcium.

A significant increase of femoral neck and lumbar spine BMD was observed in adolescents between the age of 10 and 14 years, with the stabilization of femoral neck BMD occurring one year earlier when compared to the lumbar spine BMD curve. In addition, BMD stabilized one year after peak growth velocity and 2 years after menarche (12.2 years). Despite the cross-sectional design of the study, the present results are similar to those reported in longitudinal studies. Peak bone mass gain occurred at 13 years of age in Canadian adolescents, approximately one year after peak height

velocity<sup>6</sup>. In American adolescents, the increase in total hip BMD reached a plateau at 14 years of age and in lumbar spine BMD at 15 years<sup>3</sup>. In contrast, in Swiss adolescents the gain in lumbar spine and femoral neck BMD was only significant up to 14 years of age, 2 years after menarche<sup>5</sup>.

On the basis of the periods of bone mass acquisition reported in longitudinal studies, the period of 10 to 14 years observed in the present investigation can correspond to the time of bone mass acquisition for physically active Brazilian adolescent girls but with inadequate calcium intake. However, longitudinal studies involving other populations of adolescents are needed to determine the true gain of bone mass.

The impact of puberty on bone mass acquisition was demonstrated by the significant differences in BMD between prepubertal, pubertal and postpubertal girls (Table 3). This fact is directly related to the increased production of sex hormones, particularly the already mentioned action of estrogen on osteoclast activity<sup>21</sup>. In addition, considering lumbar spine and femoral neck BMD values of 1.200 (g/cm<sup>2</sup>) and 0.965 (g/cm<sup>2</sup>)<sup>23</sup> during peak bone mass, postpubertal adolescent girls already reached approximately 90% and 109% of the expected values, respectively. Longitudinal studies also reported that 90% to 100% of peak bone mass is acquired at the end of adolescence<sup>5,6</sup>. Therefore, studies evaluating BMD in Brazilian adolescent girls need to control pubertal stages.

Another important factor is that the gain in BMD differs between bone sites. Lumbar spine and femoral neck BMD was increased by 58% and 31%, respectively, in postpubertal adolescents when compared to the prepubertal ones. The same was reported in studies conducted on Lebanese<sup>8</sup>, Dutch<sup>24</sup> and Australian<sup>25</sup> adolescents, where lumbar spine BMD has increased more than 60% between pre- and post-pubertal girls. This fact might be related to the effect of sex hormones, which is more pronounced in trabecular bone than in cortical bone<sup>8</sup>. Therefore, in addition to the control of pubertal stages, at least two bone sites should be used for the analysis of BMD in adolescents, especially in the period which sex hormones have great changes ( $\pm 2$  years of the age of menarche) since the use of only one bone site could produce equivocal conclusions.

The present study has some limitations. The self-reported race of the participants contributed to the characterization of the sample and cannot be

used for stratification since the number of subjects in each age group by race would be disproportional. However, in contrast to other countries, the classification of the Brazilian population according to race using only phenotypic characteristics is difficult, mainly because of the interethnic admixed between Europeans, Africans and Amerindians, in which one individual classified as white, according to phenotypic characteristics, can have African ancestry and another classified as black can have European one<sup>26</sup>. There is little information about the mechanisms by which ethnicity influences BMD, but it is known that genes related to variations in BMD are race, age and gender specific<sup>27</sup> and that BMD is influenced by genetic ancestry<sup>28</sup>. In a study evaluating BMD in Afro-American women, European genetic ancestry seen in part of the sample was negatively correlated with BMD<sup>28</sup>. Therefore, genetic markers for ancestry should be used to identify the relationship between race and BMD in the Brazilian population.

Another limitation was the cross-sectional design of the study, in which BMD and BMC were compared between different subjects and may not represent the true variation in bone mass gain of adolescent girls. Longitudinal follow-up is needed to establish and identify the rate of BMD gain during the growth spurt.

## CONCLUSIONS

The BMC and BMD of the Brazilian adolescent girls studied were characteristic of physically active individuals, but calcium intake was considered to be inadequate. Although the body weight and height of the adolescents were within the Brazilian reference range, extrapolation of the BMD and BMC results to other Brazilian regions should be done with caution.

Analysis of BMC and BMD according to age and pubertal stage showed significant increases during similar periods. Femoral neck and lumbar spine BMD increased significantly between 10 and 14 years of age. In addition, major differences in bone mass acquisition were observed during puberty. Bone mass gain differed between the two bone sites and the differences in BMD between pre- and postpubertal adolescents reached almost 60%. We therefore suggest that studies investigating BMD in Brazilian adolescent girls should control for pubertal stage and use at least two bone sites to rule out equivocal conclusions.



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