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ORIGINAL ARTICLE

Osteometabolic profile and bone mass in the transition phase: ethnic differences in Brazilians treated with somatropin during childhood

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Abstract

Objective: The main growth hormone action is to promote linear growth increasing protein synthesis stimulation and osteoblastic activity. Peak bone mass extends from adolescence to 30 years of age. Several factors may influence this acquisition and prevent fracture risk in adulthood, such as genetic potential, GH, ethnicity, and lifestyle factors. This study aims to compare bone mass and osteometabolic profile of white and Afro-descendant Brazilian adolescents in the transition phase, who were treated with human recombinant growth hormone in childhood.

Methods: The authors selected 38 adolescents from the Transition Outpatient Clinic of the University of São Paulo. Lumbar spine and total body bone mineral density (BMD) and bone mineral content (BMC), serum calcium, 25-OH-vitamin D and bone markers were analyzed at the rhGH withdrawal.

Results: The mean age was 16.8 ± 1.6 years. There were 21 Afro-descendant and 17 whites. Thirty-four percent of the sample presented vitamin D insufficiency, 66% inadequate calcium intake and 44.7% physical inactivity. The Afro-descendants showed a lower lumbar spine and total body Z scores than those of the whites ($p = 0.04$ and $p = 0.03$, respectively), as well as their mean body weight ($p = 0.03$). There were no differences in the remaining osteometabolic parameters.

Conclusion: As most adolescents had vitamin D insufficiency, low calcium intake, and physical inactivity, calcium, and cholecalciferol supplementation and lifestyle changes should be encouraged. The Brazilian Afro-descendant may be a vulnerable group for low bone mass, requiring

Study conducted at the Universidade de São Paulo (USP), Faculdade de Medicina, Instituto da Criança, São Paulo, SP, Brazil.

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special strategies to increase bone accrual and body weight. More studies are necessary to compare ethnic differences in this population.

Introduction

Osteoporosis is a systemic disease that compromises bone microarchitecture, leading to bone fragility. It is one of the main public health problems, as 30% of the population will suffer from some type of fracture after the age of 50, with a high mortality rate.[1]

Childhood and adolescence are fundamental periods for the development of peak bone mass, as it is at this time that the gradual increase in bone tissue occurs, and bone formation predominates over resorption.[2]

The main growth hormone action is to promote linear growth increasing protein synthesis stimulation and osteoblastic activity.[3] The recombinant human growth hormone (rhGH) was initially approved by the 'Food and Drug Administration' department for the treatment of GH deficiency in children. Until a few years ago, many pediatric endocrinologists discontinued treatment as soon as these children reached puberty and their final height.[4] This is the transition phase, which corresponds to the period that extends from puberty to the age of 30 when the bone mass peak occurs.[5] However, at this time the treatment should be withdrawn to reassess the persistence of this hormonal deficiency, which, in adults, can manifest as osteoporosis.[4] There is robust evidence that peak bone mass acquired in childhood and adolescence is the greatest determinant of fracture risk in adulthood.[6]

Bone mineral gain can be influenced by modifiable factors (such as daily intake of nutrients, calcium, vitamin D, and physical exercise), and non-modifiable factors, such as genetics and ethnicity.[7] However, little attention has been paid to modifiable factors during treatment with rhGH and immediately after its discontinuation. In addition, there are few studies that evaluated the ethnic differences in relation to bone mass in Brazil, which is relevant, given the great miscegenation that exists in the studied country. The importance of this research lies in identifying those at higher risk of low bone mass in the transitional phase, in order to intervene on modifiable factors early and maximize bone mineral accrual, preventing osteoporotic fractures in adulthood. Therefore, the aim of the study is to compare the bone mass and the osteo metabolic profile of white and Afro-descendant Brazilian adolescents in the transition phase, who were treated with rhGH during childhood.

Methods

Cross-sectional study, which included 38 individuals from 14 to 20 years of age, of both sexes, from the Transitional Outpatient Clinic of the Instituto da Criança, Hospital das Clínicas, University of São Paulo, who were treated with rhGH from childhood to their interruption, in the transition phase. Recruitment took place between May 2017 and April 2019. The selected individuals underwent regular treatment with rhGH for at least three consecutive years prior to entry into the study. Patients with chronic diseases that could alter bone mass, such as chronic renal failure, type 1 *diabetes mellitus*, osteopathy, complex syndromes that accompany GH deficiency, or affect body composition (such as Prader Willi syndrome) and chronic use of corticosteroids were excluded.

The transition phase was defined when these youngsters completed their pubertal development and reached their final height, i.e., with growth velocity less than 2 cm/year, bone age equal to or greater than 14 years in girls, and 16 years in boys. Puberty was defined as spontaneous menarche and Tanner V pubertal stage in boys. In cases where puberty was induced, girls were treated with cyclic combined therapy with estrogens and progestins, and boys with 200 mg of testosterone monthly. All were treated with rhGH at an initial dose of 0.10 IU/kg/day, 6 times a week. The dose was titrated every 3 months in order to maintain IGF-I concentrations close to the mean of their reference values for age and sex.[8] Once the growth period ended, the patients were referred to the Transition outpatient clinic. Treatment with rhGH was interrupted for at least one month, so that serum dosages of somatomedin C (IGF-I), C-terminal collagen type I peptide (CTX-I), calcium, phosphorus, alkaline phosphatase (AF), and 25(OH)D could be performed. Bone mineral density (BMD), bone mineral content (BMC), lumbar spine, and total body Z scores were measured by dual-emission X-ray absorptiometry (DXA), adjusted for sex, age and height. (Hologic, Discovery W, software 13.5.2,1) The risk of low bone mass was considered to have a spine and total body Z score between -1 and -2, and low bone mass, $\leq$2 standard deviations for sex, age and height.[9] The following anthropometric data were collected: age (years), sex, height, weight, ethnicity (Afro-descendant or white), and body mass index (BMI, in kg/m^2) were calculated. All were submitted to clinical examination, evaluating: body weight in kg, measured with light clothing, without shoes and feet juxtaposed, on a Filizola adult scale, with 100g precision; height in centimeters (cm), measured in a Harpenden Holtain wall stadiometer, with 1mm precision.[10] All were asked about their daily calcium intake (mg/day), estimated from the amount of milk and dairy products, fruits and vegetables eaten per day, and regular physical activity (yes or no). The authors considered adequate a daily calcium intake of 1300 mg/day and serum concentrations of 25(OH)D $>$ 20 ng/mL,[11] and then, the authors classified adolescents as having adequate or inadequate calcium intake, and sufficient or insufficient vitamin intake. D.

Participants were instructed to carry out blood collection until 8:30 am, after an 8-h fasting period for the measurement of the osteo metabolic profile and IGF-I. Alkaline phosphatase, calcium, and phosphorus were measured by the colorimetric method (Cobas C, Roche Hitachi). 25(OH)D was

Table 1 Demographic and anthropometric data of Brazilian adolescents of African descent and whites, after interruption of treatment with rhGH, in the transition phase.

Data	Group A[a]	Group B[b]	*p* value
Female	43%	52.9%	0.79
Weight (kg)	47.3 + 10.2	57.9 + 15 kg	0.03
Height (cm)	157.3 + 9.4	161.4 + 9.9	0.21
BMI (kg/m^2)	19.8 + 3.4	21.3 + 3.2	0.13
Adequate daily calcium intake	23.8%	41.2%	0.1
Vitamin D insufficiency	28.6%	41.2%	0.4
N	21	17	

[a] Group A, Afro-descendants.
[b] Group B, Whites.

measured by chemo-immunoassay (Cobas E-411, Abbott Park), IGF-I by chemiluminometric (IDS, Immunodiagnostics Systems), and CTX-I by electro chemiluminometric (Elecsys beta-cross Laps/Cobas serum E-411, Roche Diagnostics).

Subjects were divided according to ethnicity into Afro-descendent and whites.[12] The following data were compared: age, weight, height, BMI, sex ratio, BMD, spine, and total body BMC with respective Z scores, physical activity (yes or no), calcium intake, serum values of calcium, phosphorus, 25(OH)D, AF, and CTX-I. Their identity was preserved and they only participated in the study after the adolescents and guardians signed the terms of assent and informed consent and approval by the Research Ethics Committee of the University of São Paulo (1.511,705/2016).

Statistical analysis

Student's t-test was performed for comparisons between variables with normal distribution, and the Mann-Withney test for those without normal distribution. Fisher's test was used to assess the difference in the proportion between the sexes, and the Chi-square test to verify the differences between the proportions of calcium intake, physical activity practice (computed as yes or no), and vitamin D sufficient and insufficient subjects. For the analysis of correlations between body weight with BMD, CMO and Z scores of the spine and total body, Pearson's correlation was used. The significance level was considered to be the value of $p < 0.05$. The software used for data analysis was MedCalc 20.015.

Results

38 individuals participated in the study. The mean age was 16.8 $\pm$ 1.6 years, 17 females and 21 males. Ninety percent of the sample came from public schools and attended high school (32/38), and three did not study. Twenty-five individuals (66%) had inadequate calcium intake, 13 (34%) had vitamin D insufficiency, and 17 (44.7%) did not do exercise. Twenty-one participants were of African descent (group A) and 17 were white (group B). Only 20% of the sample (5/38) came on cholecalciferol supplementation, 3 in group A, and 2 in group B. The proportion between the sexes was similar between the groups ($p = 0.79$), with 43% (9/21) females in group A, and 52.9% (8/17) in group B. There was no difference between the proportions of individuals who adequately ingested calcium, were insufficient in vitamin D and practiced physical exercise. The mean weight of group A was significantly lower than that of group B (47.3 + 10.2 kg vs 57.9 + 15 kg, $p < 0.03$), but the mean height was similar ($p = 0,21$). Demographic and anthropometric data are shown in Table 1.

As shown in Table 2, there was no difference between the mean serum values of vitamin D ($p = 0.92$), calcium ($p = 0.14$), phosphorus ($p = 0.95$), CTX-I ($p = 0.31$) nor FA ($p = 0.76$). The mean spine z-scores of group A were significantly lower than those of group B, being, respectively, -1.5 + 1.2 vs -0.9 + 0.6 ($p = 0.04$), as well as the mean of the total body z scores, with the respective values for groups A and B of -1.6 + 1.0 vs -0.82 + 0.9 ($p = 0.03$).

Discussion

The present study's results showed that most young people treated with rhGH since childhood reach the transition phase with vitamin D insufficiency, inadequate calcium intake, and physical inactivity. Afro-descendants were more likely to be at risk for low bone mass.

GH is one of the main regulators of bone homeostasis, and with sex steroids, participates in bone remodeling under ideal conditions of nutrition and physical activity. In GH-deficient children, hormonal treatment aims to normalize growth rate, but in clinical practice, there is no specific concern about bone mineral gain during treatment with rhGH. Although there are some studies on the subject,[13,14] it is recommended to assess bone mass from the transition, and then, every 2 to 5 years.[15] Furthermore, most studies in this phase have assessed bone mineral gain between sufficient and insufficient GH subjects.[16,17] The originality of ours is to compare the ethnic differences related to osteo metabolic profile and bone mass assessed by DXA in Brazilian adolescents who used rhGH, since, in Brazil, most research were carried out in healthy teenagers through ultra sonometry.[18–20]

The osteo metabolic profile found was similar to that of Assumpção et al, who observed an inadequate calcium intake in 88% of healthy adolescents in Campinas.[21] In India, this prevalence reached 95% in low-income girls, some having, hypocalcemia.[22] This has been a universal problem among youngsters,[23] which has been attributed to the

Table 2 Bone metabolic profile of Afro-descendant and white Brazilian adolescents, after interruption of treatment with rhGH, in the transitional phase.

Data	Group A[a]	Group B[b]	*p* value
LS BMD (g/cm^2)[c]	0.825 ± 0.1[g]	0.875 + 0.08[g]	0.14
LS BMC (g)[d]	41.6 ± 9.6[g]	46.5 + 8.5[g]	0.12
TB BMD (g/cm^2)[e]	0.850	0.885 (0.85−0.9)[h]	0.16
TB BMC (g/)[f]	1166.8	1443.7 (1157.7−1604.7)[h]	0.09
LS z score[i]	-1.5 ± 1.2[g]	-0.9 + 0.6[g]	0.04
TB z score[j]	-1.6 ± 1.0[g]	-0.82 + 0.9[g]	0.03
Calcium (mg/dL)	9.6 ± 0.5[g]	9.6 + 0.4[g]	0.14
Phosphorus (mg/dL)	4.1 ± 0.4[g]	4.2 + 0.4[g]	0.95
Alkaline phosphatase (U/L)	137	104.0 (70−79)[h]	0.76
CTX-I (ng/mL)	1.21	1.14 (0.5−2.95)[h]	0.31
IGF-I (ng/dL)	218	176 (41−391)[h]	0.12
25 (OH)D (ng/mL)	24.7 ± 10.5[g]	24.5 + 7.4[g]	0.92

[a] Group A: Afro-descendants.
[b] Group B: whites.
[c] LS BMD: lumbar spine bone mineral density.
[d] LS BMC; lumbar spine bone mineral content.
[e] TB BMD: total body bone mineral density.
[f] TB BMC; total body bone mineral content.
[g] Values expressed as mean + standard deviation.
[h] Values expressed as median with 95% confidence intervals.
[i] LS Z score.
[j] TB Z score.

reduction in the intake of milk and dairy products, vegetables, and fruits. The prevalence also increases in unfavorable socioeconomic conditions and students from public schools,[21] as occurred with the participants in this study. Calcium is a critical element for skeletal mineralization, strength, and muscle contractility, requiring a minimum daily intake of 1000 mg/day to allow the peak bone mass.[24] This alarming data demonstrates the ignorance of parents and schools about the importance of adequate calcium in the diet, requiring dissemination strategies and changes in school meals, and encouraging the consumption of fruits and dairy drinks. In the case of children treated with rhGH, the authors also suggest routine monitoring of calcium intake during consultations, and supplementation when necessary.

Vitamin D plays a facilitating role in the intestinal and renal absorption of calcium and phosphorus and in bone mineralization. About 80% of the production occurs mainly through the action of UV rays on the skin, and the remaining through food intake and supplementation. Even in sunny regions, such as India, the prevalence of vitamin D insufficiency was observed in 70% of the population[22] higher than that observed by us. In a meta-analysis, which included studies from 2006 to 2017 and more than 50,000 Brazilian adolescents from the Southeast, where the authors carried out the survey, it was the one with the highest prevalence, at 55%.[25] The fact that 20% was supplemented with cholecalciferol during treatment with somatropin may explain the lower prevalence observed in the present cohort.

Heredity may account for 70% of peak bone mass and explain part of the ethnic variations in BMD, calcium, and vitamin D metabolism.[26] According to the literature, Afro-descendants have serum concentrations lower than 25 (OH) D3, resulting from resistance to sunlight, typical of melanoderma, but a higher metabolization of 25 (OH)D to 1.25 (OH) D2, allowing a more efficient intestinal and renal calcium absorption. Therefore, these individuals have bones that are more resistant to osteoporotic fractures, due to the thicker cortical bone.[27] Assessments with DXA also demonstrated that bone mass acquisition is also greater in blacks aged 9 to 26 years, when compared to Asians and Caucasians.[28] However, contrary to literature data, the authors observed that Afro-descendants had similar 25(OH)D values, but significantly lower spine and total body Z scores than whites, which could be a result of the large miscegenation of the Brazilian population, especially in the state of São Paulo, where there are many Europeans and Asians. The present results are similar to those of Ribeiro et al, who, although they used phalangeal ultra sonometry to assess bone mass, observed a reduction in Afro-descendants from 6 to 11 years of age, from Paraná, where the miscegenation with Asians and Europeans is also great.[18]

Weight, height, body composition, and ethnicity also contribute to differences in BMD and fracture risk.[18] In the present study, Afro-descendants were thinner and had lower spine and body z scores. Although areal DXA overestimates bone BMD of tall individuals, and interpretation should be cautious during growth,[29] height interference was minimized in the present results, because all used rhGH, mean height was similar between groups, and the final height had already been reached. Furthermore, as there was no difference between serum calcium, phosphorus, bone markers, calcium intake, or frequency of physical activity, the authors also ruled out these influences on Z-score results. According to the 'National Nutrition Examination Survey', North Americans of African descent, in addition to having higher bone mass than Asians and whites, had more overweight and obesity, which diverges from the present study's cohort.[30] Weight is positively correlated with BMD, because of the pressure exerted on bones, stimulating

osteoblastic activity and inhibiting osteoclastogenesis. Lean mass is most associated with bone mineral gain,[2] and therefore, osteogenic physical exercises (such as tennis, soccer, basketball, judo, and volleyball) are indicated in puberty, when sex steroids and GH constitute major regulators of bone mineral gain. They would be particularly useful for Brazilians of African descent.

The authors would like to emphasize that as this is a cross-sectional study, it was not possible for us to monitor the evolution of bone mass and osteo metabolic profile since the beginning of treatment with somatropin. The International Society of Densitometry recommends evaluation by DXA in pediatrics for patients at high risk of fracture, to guide therapy.[30] However, in order to monitor the evolution of peak bone mass, the authors suggest that rhGH users perform an initial assessment of the osteo metabolic profile and DXA in the prepubertal phase. If they are normal, annually repeat the osteo metabolic profile, and DXA in the transitional phase. However, in those at risk or with low bone mass, the authors suggest a biannual assessment of the osteo metabolic profile, regular osteogenic exercise, and DXA also at the beginning of puberty and in transition. The adequacy of calcium in the diet, its supplementation, and that of cholecalciferol should be done in all, when necessary.

The present research has some limitations. The first is related to the sample size, which may explain the lack of correlation between weight and spine z score, but could demonstrate ethnic differences in relation to bone mass. The second is that the assessment of calcium intake was estimated by the dietary inquiry. However, the adolescents were always accompanied by their parents or guardians, who helped in collecting this information. The third refers to the fact that the authors did not assess the daily intake of proteins, macronutrients, and socioeconomic status, which could influence body weight. However, this was not the main objective of the study. It is also noteworthy that the allocated population was predominantly from the State of São Paulo, requiring caution in extrapolating the results. Finally, the authors would like to highlight that most of the studies comparing ethnic differences related to bone mass in Brazil were performed with phalangeal ultra-sonometry in healthy children and adolescents. As far as we know, this is the first one performed with DXA that makes this comparison in teenagers, who had been treated with rhGH since childhood. Although robust LS and TB reference z scores have been generated for black and non-black controls in several countries other than Brazil, there is great miscegenation in the studied country. Therefore, the authors suggest further studies with DXA be performed in somatropin users, including healthy Brazilian controls.

The present results alert to the insufficient consumption of calcium, hypovitaminosis D, and physical inactivity in most Brazilian adolescents in transition, who were treated with rhGH. Therefore, periodic assessment of lifestyle, osteo metabolic profile, and DXA are important for optimizing peak bone mass. Afro-descendants may constitute a vulnerable group to the risk of low bone mass, requiring special strategies to increase bone mineral gain. More studies are needed to assess ethnic differences in bone mass in Brazilians treated with somatropin in a transition phase, including healthy controls.

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Conflicts of interest

The authors declare no conflicts of interest.

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