Association between Anthropometric Hormonal Measurements and Bone Mineral Density in Puberty and Constitutional Delay of Growth and Puberty

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ABSTRACT

The aim of this study is to evaluate the acquisition of bone mineral in healthy children throughout puberty and in children with constitutional delay of growth and puberty (CDGP), and to relate changes in bone mass to age, weight, height, sitting height, body mass index and sex hormones in healthy boys. A total of 90 boys: 15 boys with CDGP and 75 healthy boys in different pubertal stages were examined. The number of children assigned to each Tanner stages was 15. Although bone age, weight and Body Mass Index (BMI) were significantly higher in stages II, III, IV, V compared to stage I and CDGP, mean height and sitting height values were higher in stages III, IV, V compared to stage I and CDGP. Also, serum FSH, LH, oestradiol, total and free testosterone levels progressively increased, although serum sex hormone binding globulin (SHBG) levels decreased, in healthy children with progression of sexual development.

Significant increase was observed for serum oestradiol levels at stage II and above ($p < 0.001$*), for serum total and free testosterone levels at stage III and above (*$p < 0.001$*), for serum FSH and LH levels at stage IV and above (*$p < 0.01$ *and* $p < 0.001$*) respectively.*

Also, it was shown that bone mineral content (BMC) and bone mineral density (BMD) measurements were significantly higher for pubertal stage lll and above groups according to both the CDGP group and stage I group. When BMD and BMC measurements of children with CDGP (0.62 ± 0.05 *gr/cm*^{2} *and* 23.4 ± 2.8 *gr) were compared with bone age, age, BMI and height-matched controls, there was no significant difference between children with CDGP and controls, except for age. Bone mineral density and BMC measurements in children with CDGP were significantly lower than those of age-matched controls (for pubertal stage lll:* $p < 0.05$*, for pubertal stage IV:* $p < 0.01$*).*

The strongest correlation coefficients were found between BMD and height among auxological parameters ($r = 0.63$*,* $p < 0.001$*) and serum oestradiol levels among hormones (*$r = 0.55$*,* $p < 0.001$*).*

The most important findings of this investigation was the determination of body composition and hormonal measurement changes during puberty in boys; oestradiol was the most potent determinant of BMD among pubertal boys. We suggested that there is a critical age period for accumulation of bone mass according to the results. Longitudinal studies will elucidate why sufficient mineralization does take place after puberty starts in CDGP.

Keywords: Bone mineralization, constitutional delay of growth and puberty, gonadotropins, oestradiol level, puberty.

Asociación Entre las Mediciones Hormonales Antropométricas y la Densidad Mineral Ósea en la Pubertad y el Retraso Constitucional del Crecimiento y la Pubertad

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RESUMEN

El objetivo de este estudio es evaluar la adquisición de mineral óseo del hueso en niños saludables a través de la pubertad y en niños varones con retraso constitucional del crecimiento y la pubertad (RCCP), y relacionar los cambios de masa ósea a la edad, el peso, la altura, la altura sentado, el índice de masa corporal, y las hormonas del sexo en niños varones saludables.

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Examinamos un total de 90 niños, 15 niños con RCCP y 75 niños saludables en diferentes etapas de la pubertad. El número de niños asignados a cada etapa de Tanner fue 15. Aunque la edad ósea, el peso y el IMC fueron significativamente más altos en las etapas II, III, IV, V, comparados con la etapa I y el RCCP; la altura promedio y los valores de la altura sentado fueron más altos en las etapas III, IV, V, comparados con la etapa I y el RCCP.

Por otra parte, los niveles séricos de HEF, HL, estradiol y testosterona total y libre, aumentaron progresivamente, aunque los niveles séricos de SHBG disminuyeron en los niños saludables con el avance del desarrollo sexual. Se observó un aumento significativo en los niveles de estradiol sérico en la etapa II y por encima ($p < 0.001$), en los niveles séricos de testosterona libre y total en la etapa II y por encima ($p < 0.001$), y en los niveles séricos de HEF, HL en la etapa IV y por encima ($p < 0.01$ y $p < 0.001$).

Además se observó que las mediciones del contenido mineral óseo (CMO) y la densidad mineral ósea (DMO) fueron significativamente mayores en la etapa III de la pubertad y grupos por encima, de acuerdo tanto con el grupo de RCCP cómo el grupo de la etapa I. Cuando las mediciones de DMO y CMO de niños con RCCP (0.62 ± 0.05 gr/cm2 y 23.4 ± 2.8 gr) fueron comparadas con la edad ósea, la edad, IMC y los controles pareados por altura, no se halló ninguna diferencia significativa entre los niños con RCCP y los controles, excepto la edad. Las mediciones de DMO y CMO en niños con RCCP fueron significativamente más bajas que las de los controles pareados por edad (para la etapa III de la pubertad: $p < 0.05$; para la etapa IV de la pubertad: $p < 0.01$).

Los coeficientes de correlación más fuertes se encontraron entre la DMO y la altura entre los parámetros auxológicos ($r = 0.63$, $p < 0.001$), los niveles séricos de estradiol entre las hormonas ($r = 0.55$, $p < 0.001$).

Los hallazgos más importantes de esta investigación fueron la determinación de la composición corporal y los cambios en la medición hormonal durante la pubertad en los muchachos; el estradiol fue el determinante más potente de la DMO entre los niños en la pubertad. Sugerimos que hay un periodo de edad crítico para la acumulación de masa ósea de acuerdo con nuestros resultados. Los estudios longitudinales esclarecerán por qué se produce suficiente mineralización después de que la pubertad empieza en RCCP.

Palabras claves: Mineralización ósea, retraso constitucional del crecimiento y la pubertad, gonadotropinas, nivel de estradiol, pubertad

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INTRODUCTION

Bone mass increases normally throughout childhood, reaching peak levels by late adolescence or early adulthood. The acquisition of bone mass during childhood and adolescence is the result of the interaction of factors such as genetic heritage, race, hormones, nutrition, lifestyle and physical activity (1). Extensive loss of bone with distruption of trabecular architecture results in osteoporosis, a condition of skeletal fragility that is a major health problem. Osteopenia has been reported in adult men with a history of constitutional delay of growth and puberty (CDGP) in comparison with men who had normal onset of the timing of puberty (2). This finding suggest that the timing of sexual maturation is an important determinant of adult bone mineral density (BMD).

Constitutional delay of growth and puberty is a common clinical problem potentially affecting up to 3% of adolescent boys. However, it is possible that CDGP has an inherent predisposition to osteopenia. The aim of this study is to define the bone mineral density in children with CDGP, to compare the BMD, anthropometry and sex hormones of children at different pubertal stages with those with CDGP. Also, we planned to determine the changes of anthropometric characteristics and hormones according to pubertal stage.

SUBJECTS AND METHODS

We examined a total of 90 boys; CDGP was diagnosed in 15 of them. Other children were healthy boys in different pubertal stages. The number of children assigned to each Tanner stage (3) was 15. These children were identified from among school children by random screening. Healthy subjects were normally growing children free of chronic disease and who had not received prior therapy with any known effect on bone metabolism. In all of them, chronological age and bone age differed by more than one year. The diagnosis of CDGP was made using the following criteria: a) chronological age above 14-years, b) height below the 10^{th} percentile for chronological age (CA); c)Tanner pubertal stage 1; d) normal birthweight and clinical history, and physical examination without evidence of anosmia, micropenis, organic disease, vitamin D deficiency, malnutrition or psychological deprivation; e) no intake of medication with known effect on BMD or growth; f) bone age (BA) at or less than two-years of CA; g) other causes of short stature were

excluded by normal blood count and erythrocyte sedimentation rate, electrolytes, creatinine, calcium, inorganic phosphate, alkaline phosphatase, total protein, thyroxine, thyrotropin, antigliadin antibodies, IGF-l, IGFBP-3 and uroanalysis; h) growth velocity (recorded for a minimum period of six months) above the 25th percentile; i) family history of CDGP or mid-parental height above 25th percentile. The children having all the above criteria were included.

Height and sitting height were measured in centimetres to the nearest 0.1 cm with a wall-mounted stadiometer. Children were weighed in light clothing without shoes, and weight was measured to the nearest 0.1 kg on a standard clinical balance. Measurements were taken twice and the average was used for analyses. Body mass index (BMI) was calculated from height and weight (wt/h^2).

Chronological age (years) was determined as decimal age (measurement date – date of birth) [4]. Skeletal maturation was assessed from radiographs of the left hand and wrist prior to measurements of bone density. Bone age was determined with the method described by Greulich and Pyle (5).

Physical examinations were performed by a paediatrican to determine the stages of sexual development and the grading system defined by Tanner (3) was used for classification. Calcium intake was evaluated by a dietician through a standardized questionnaire on food and beverage intake.

Bone mineral density and bone mineral content (BMC) were measured by dual-energy X-ray absorptiometry [DEXA] (Hologic QDR 4500 Elite densitometer; Hologic, Inc., Waltham, MA, USA) which uses an X-ray tube as the radiation source. During the measurements, the child was supine and the physiological lumbar scoliosis was flattened by elevation of the knees. The system scans the lumbar spine in a rectilinear way. Results for vertebrae L-1 through L-4 were averaged to obtain the patient's total vertebral bone mass. All images were processed by the same investigator. The results were expressed as BMD in grams per cm^2 and BMC in grams.

Venous blood samples and urine samples were obtained in the morning. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone and oestradiol (ACS 180, Chiron Diagnostics Corporation, USA) and sex hormone-binding globulin (SHBG) levels (Diagnostic Products Corporation, USA) were measured by chemiluminescence enzyme immunoassay using commercial kits. Serum free testosterone was evaluated by RIA (Diagnostic Products Corporation, USA). For all hormonal analyses, intra- and interassay coefficients of variation were less than 6.9 and 9.6%, respectively.

Informed consent was obtained from parents, as prescribed by the local human ethics committee. Statistical analysis was conducted with the SPSS/PC programme, version 10.0. Data were presented as the mean ± SEM. Variables were evaluated by Student's unpaired two-tailed *t*-test and Pearson's correlation coefficient. A *p*-value of < 0.05 was considered significant.

RESULTS

Candidates were physically active in accordance with their age and their calcium intake per day was similar according to questionnaires about the child's general physical activity level during the school year and daily calcium intake based on the calcium content of food items. Mean bone age, weight and height of the children with CDGP were similar to those of children with pubertal stage l except for chronological age. Children with CDGP had prepubertal properties.

The anthropometric characteristics and hormonal results of the groups are presented in Table 1. Statistical comparisons of results between groups were shown in Table 2. Although bone age, weight and BMI were significantly higher in stages II, III, IV, V compared to stage l and CDGP; mean height and sitting height values were higher in stages III, IV, V compared to stage I and CDGP.

Also, mean serum FSH, LH, oestradiol, total and free testosterone levels progressively increased, although serum SHBG levels decreased in healthy children with progression of sexual development. Significant increase was observed for serum oestradiol levels at stage II and above, for serum total and free testosterone levels at stage III and above, for serum FSH, LH levels at stage IV and above. However, serum SHBG levels significantly decreased at stage V during puberty (Table 2). Also, it was shown that BMC and BMD measurements were significantly higher at pubertal stage III and above groups according to both CDGP group and stage I group. The children in late puberty had higher values for bone density than those in early puberty.

All parameters of children with CDGP were similar to those of children with pubertal stage I except for chronological age (Table 2). Children with CDGP had prepubertal properties. As seen in Table 2, pubertal stage I for bone age and BMI, pubertal stage l and ll for height and pubertal stages II, III, IV for age were used as bone-age, age, BMI and height-matched controls for comparison. When BMD and BMC measurements of children with CDGP were compared with bone-age, age, BMI and height-matched controls, there was no significant difference between children with CDGP and controls except for age. Bone mineral density and BMC measurements in children with CDGP were significantly lower than those of age-matched controls (for pubertal stage III: $p < 0.05$, for pubertal stage IV: $p < 0.01$).

Correlations between bone mineral density and other parameters are shown in Table 3 in healthy children throughout puberty. The strongest correlation coefficients were found between BMD and height among auxological parameters and serum oestradiol levels.

Table 1: The anthropometric characteristics and hormonal results of the study population, grouped according to pubertal stage

	CDGP	Stage I	Stage II	Stage III	Stage IV	Stage V
n	15	15	15	15	15	15
Chronological age (year)	14.2 ± 0.19	13.0 ± 0.65	13.8 ± 0.86	14.17 ± 0.83	14.17 ± 0.77	15.1 ± 0.42
Bone age (year)	13.1 ± 0.2	13.0 ± 0.4	13.5 ± 0.8	13.8 ± 0.7	13.75 ± 0.6	14.2 ± 0.6
Weight (kg)	34.8 ± 4.99	34.6 ± 4.08	40.13 ± 3.95	43.17 ± 5.81	44.07 ± 4.49	50.77 ± 7.41
Height (cm)	144.8 ± 2.65	143.8 ± 3.28	148.9 ± 5.92	152.7 ± 4.20	153.9 ± 5.67	161 ± 7.48
BMI (kg/ m2)	16.8 ± 2.45	16.8 ± 2.04	18.7 ± 1.69	18.5 ± 2.23	18.7 ± 2.02	19.5 ± 2.17
Sitting height (cm)	73.8 ± 1.81	72.1 ± 2.49	75.4 ± 2.39	76.27 ± 1.93	77.21 ± 3.05	80.33 ± 4.03
FSH (mIU/ml)	2.04 ± 1.08	1.79 ± 1.23	2.35 ± 1.43	3.13 ± 2.57	3.37 ± 1.67	3.51 ± 1.10
LH (mIU/ml)	0.76 + 0.15	1.10 ± 0.9	1.32 ± 0.71	1.55 ± 0.82	1.88 ± 0.93	2.00 ± 1.04
T Testosterone (ng/dl)	40.01 ± 24.6	30.69 ± 15.3	50.70 ± 34.2	201.4 ± 92.1	223.5 ± 94.4	251.8 ± 101
F Testosterone (pg/ml)	0.93 ± 0.3	0.95 ± 0.4	1.14 ± 0.6	4.84 ± 2.1	8.24 ± 6.1	10.9 ± 8.3
Oestradiol (pg/ml)	5.93 ± 1.25	7.52 ± 2.36	18.33 ± 1.39	25.13 ± 16.8	26.85 ± 3.33	60.93 ± 35.8
SHBG (nmol/L)	61.28 ± 22.7	73.76 ± 27.9	62.10 ± 8.34	44.26 ± 14.0	39.8 ± 11.71	28.75 ± 10.2
BMC (gr)	23.4 ± 2.8	22.6 ± 3.5	26.5 ± 5.4	30 ± 6.9	31.5 ± 3.1	41.3 ± 13.2
BMD (gr/cm2)	0.62 ± 0.05	0.60 ± 0.06	0.63 ± 0.06	0.67 ± 0.08	0.69 ± 0.05	0.76 ± 0.12

Table 2. Statistical comparisons of results of analyses of groups

	CDGP-PS* I	CDGP-PS II PS I-PS II	CDGP-PS III PS I-PS III	CDGP-PS IV PS I-PS IV	CDGP-PS V PS I- PS V
chronological age	0.001	ns 0.01	ns 0.001	ns 0.001	0.001 0.001
Bone age (yr)	ns	0.001 0.01	0.001 0.001	0.001 0.001	0.001 0.001
Weight (kg)	ns	0.001 0.001	0.001 0.001	0.001 0.001	0.001 0.001
Height (cm)	ns	ns ns	0.001 0.001	0.001 0.001	0.001 0.001
BMI	ns 0.01	0.01 0.01	0.01 0.01	0.01 0.001	0.001
Sitting height (cm)	ns	ns ns	0.01 0.01	0.001 0.001	0.001 0.001
FSH (mIU/ml)	ns	ns ns	ns ns	0.01 0.01	0.001 0.001
LH (mIU/ml)	ns	ns ns	ns ns	0.01 0.01	0.001 0.001
T Testosterone (ng/dl)	ns	ns ns	0.001 0.001	0.001 0.001	0.001 0.001
F Testosterone (pg/ml)	ns	ns ns	0.001 0.001	0.001 0.001	0.001 0.001
Oestradiol (pg/ml)	ns	0.001 0.001	0.001 0.001	0.001 0.001	0.001 0.001
SHBG (nmol/L)	ns	ns ns	ns ns	ns ns	0.01 0.01
BMD (gr/cm2)	ns	ns ns	0.001 0.001	0.001 0.001	0.001 0.001
BMC (gr)	ns	ns ns	0.001 0.001	0.001 0.001	0.001 0.001

PS: Pubertal stage
ns: Nonsignificant

Table 3: Significant correlations between bone mineral density and other parameters in healthy children throughout puberty

	r	*p*
Height	0.625	0.001
Sitting height	0.578	0.001
Oestradiol	0.548	0.001
Pubertal stage	0.545	0.001
Weight	0.508	0.001
BMI	0.497	0.001
Chronological age	0.478	0.001
LH	0.444	0.001
Bone age	0.405	0.001
Free testosterone	0.320	0.01
Total testosterone	0.289	0.05

DISCUSSION

Although osteoporosis has traditionally been considered a disease of the elderly, there is increasing recognition of the importance of bone mineral acquisition during the growing years as an important preventative factor. Thus, there is interest in BMC and BMD in children. So that intervention programmes can begin at an early age in identifying children, adolescents, and young adults with low BMC and BMD. In this study, bone mineralization was researched in children with CDGP and healthy children during puberty.

When we examined the changes in body mass and bone mineral density of a group of healthy adolescent boys, the results confirmed the dramatic increases in height, weight, sitting height, BMI, BMC, BMD associated with pubertal growth. In the present study, lumbar bone mineral density increased during puberty, similar to the findings of other investigators (6–8). There has been no previous report at which pubertal stage was the bone density significantly higher. Schepper *et al* (7) reported the most important increase in BMD in pubertal stage lV for both genders. In our previous study, it was observed in pubertal stage lll for girls (6). In the present study, the most important increase in lumbar spine BMC and BMD was found in pubertal stage III for boys. lt is during this stage of puberty that the deceleration of the growth spurt occurs and significant increase of sex steroids can be attained.

Like other investigators (7–9), the correlations were significant. The best correlations between BMD and anthropometric measurements were observed for height and sitting height. The same dependency between body height and BMD was shown by Takahashi *et al* (10) and Lu *et al* (8). These data suggest that the association between body size and skeletal status will become less pronounced with increasing age (and so the developmental variables associated with puberty).

Although serum SHBG levels decreased, basal serum FSH, LH, oestradiol, total and free testosterone levels increased during the progression of pubertal development. Here, the interesting result was that oestradiol levels in boys significantly increased earlier than testosterone levels during puberty. It has been shown that the clinical onset of puberty is preceded by an increase in nocturnal pulsatile GnRH secretion for some 2-years, during which a progressive stepwise activation of pituitary and gonadal function occur. In this period of peripubertal maturation, there was a highly significant correlation between the mean nocturnal LH concentration and early morning plasma testosterone concentration (11). Wu *et al* (12) showed that a single measurement of plasma T in the early morning can be used as a simple screening test with high discriminatory power in predicting the onset of puberty. The importance of the timing of blood sampling must be emphasized. In the present study, blood samples were obtained at 09.00 am. Why did oestradiol levels in boys significantly increase earlier than testosterone levels during puberty? The adrenal androgens rise two or more years before gonadotropins and sex steroids. It may be because these adrenal androgens are converted to oestrogen by aromatase. Also, the best correlations between BMD and hormonal measurements were observed for oestrogen levels. Oestrogen exposure will gain relatively greater influence on the accretion of bone and, ultimately, on the obtainment of peak bone mass in boys. In a previous study, it was reported that the best correlation was between bone density and oestradiol levels in girls (6). However, Dhuper *et al* (13) reported that bone density did not correlate with oestradiol levels or any of the other hormones measured except testosterone in girls. In another animal study, there was significant undermineralization of the skeleton in both male and female oestrogen-resistant mice. In contrast, androgen-resistant rats had normal bone mineral density (14). Androgens are obligatory intermediates in oestrogen biosynthesis and are produced by the adrenal glands in both sexes. Similarly, aromatase, the enzyme that catalyzes the conversion of androgen to oestrogen, is present in males as well as females (15).

Females convert the majority and males a minority of androgen to oestrogen. Our result suggested that low concentrations of oestradiol were important for the pubertal bone mineralization in boys. However, it is not known whether oestradiol acts directly on bone or indirectly by stimulating other mediators of bone growth (16).

All parameters of children with CDGP were similar to those of children with pubertal stage I except for chronological age. When BMD and BMC measurements of children with CDGP were compared with bone-age, age, BMI and height-matched controls, there was no significant difference between children with CDGP and controls for bone age, BMI and height. On the other hand, BMD and BMC measurements in children with CDGP were significantly lower than those of age-matched controls. Moreira-Andrés *et al* (17) also found that the mean radial BMD was significantly lower in the group of children with CDGP than in a group with familial short stature whose height and weight were similar to the height and weight of the children with CDGP. The lower BMD findings in the present study may not be exactly explained by differences in body size between the two

groups. Since children with CDGP have similar BMD measurements compared to weight, height and BMI-matched groups, probably, several unrecognized factors affect vertebral mineralization in CDGP. Bone age is retarded in CDGP and the underlying cause for this delay is not well known. Constitutional Delay of Growth and Puberty has insufficient bone mineralization, perhaps as a result of a bone age delay and poor mineralization both of which have the same cause. Some studies indicated that late puberty is associated with reduced bone mineral density and peak bone mass later in life; in these subjects, increases in bone mineral density were reported in response to testosterone therapy (18, 19). The timing and duration of puberty on bone mineralization may be important in CDGP. The issue of bone mineralization should therefore be considered in establishing the optimal timing of hormone replacement in CDGP. Long-term studies are needed to establish whether delayed pubertal onset translates into increased skeletal morbidity as adults. It was found out that the rate of bone mineralization depends especially on pubertal stage. It was well known that CDGP is a risk factor for male osteoporosis (12, 13). The strongest correlation coefficients were found between BMD and serum oestradiol levels among hormones. Interestingly, serum oestradiol levels increased earlier than serum testosterone levels as normal puberty progressed in present study. We suggest that there is a critical age for accumulation of bone mass according to present results. Longitudinal studies will elucidate why sufficient mineralization does not take place after puberty starts in CDGP.

Although both BMC and BMD are reported here, the BMD values should be used with caution since this measurement does not fully adjust for size differences in growing children.

In summary, the most important findings of this investigation was the determination of body composition and hormonal measurement changes during puberty; oestradiol was the most potent determinant of BMD among pubertal boys and children with CDGP had prepubertal properties. Finally, the normative values during puberty presented in this article can be used to assess skeletal status in growing children and can be used as comparative standards for research.

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