Association Between Bone Turnover Markers and Bone Mineral Density in Puberty and Constitutional Delay of Growth and Puberty

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ABSTRACT

The aim of this study was to evaluate associations between the markers of bone formation and resorption and bone mineral density in healthy children throughout puberty and in children with constitutional delay of growth and puberty (CDGP). For this reason, 15 boys with CDGP and 75 other boys in different pubertal stages were included in this study. Although mean serum phosphorus level was higher in stages II, III, IV, V compared to stage I and CDGP, mean bone specific akaline phosphatase (b-AP), parathyroid hormone (PTH), bone mineral density (BMD), bone mineral content (BMC) levels were higher in stages III, IV, V compared to stage I and CDGP. Mean serum calcium (Ca) levels were lower in stages III, IV, V compared to stage I and CDGP. During puberty, urine DPry/Cr levels were not significant. The peak level of b-AP occurred at stage IV. Serum PTH, Ca, b-AP levels, urine Ca/Cr ratio, BMC and BMD measurements significantly changed during puberty in healthy children. While serum Ca levels progressively decreased, serum b-AP, PTH levels, urine Ca/Cr ratio and bone mineralization increased in healthy children with the level of sexual development. The only significant correlation is found between serum PTH levels and bone mineral density ($p < 0.05$*). In our opinion, PTH may be a potent stimulator of skeletal dynamics in boys and may be associated with substantial increases in lumbar spine. We conclude that PTH behaved as a valuable marker in bone mineralization during puberty. Accelerated bone mineralization is reflected by high levels of serum PTH during puberty. All values of the markers of bone formation and bone resorption in children with CDGP were similar to those of prepubertal children. Children with CDGP had prepubertal properties. We suggest that there is a critical age period for accumulation of bone mass according to the results in this study.*

Asociación Entre los Marcadores de Turnover óseo y la Densidad Mineral Ósea en la Pubertad y en el Retraso Constitucional del Crecimiento y la Pubertad

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RESUMEN

El objetivo de este estudio fue evaluar las asociaciones entre los marcadores de formación y resorción ósea y la densidad mineral ósea en niños saludables a lo largo de la pubertad y en niños con retraso constitucional del crecimiento y la pubertad (RCCP). Por esta razón, 15 muchachos con RCCP y otros 75 muchachos en diferentes etapas de la pubertad, fueron incluidos en este estudio. Aunque el nivel medio de fósforo sérico fue más alto en las etapas II, III, IV, V en comparación con la etapa 1 y el RPC, la fosfatasa alcalina especifica ósea media, la hormona paratiroidea (HPT), la densidad mineral ósea (DMO), y los niveles de contenido mineral óseo (CMO) fueron más altos en las etapas III, IV, V en comparación con la etapa I y el RCCP. Los niveles medio de calcio (Ca) en suero fueron más bajos en las etapas II, IV, V en comparación con la etapa I y el RPC. Durante la pubertad, los niveles de orina DPry/Cr no fueron significativos. El nivel pico de b-AP ocurrió en la etapa IV. Los niveles séricos de HPT, Ca, y BAP, el índice Ca/Cr en orina, y las mediciones del CMO y el DMO, cambiaron significativamente durante la pubertad en los niños saludables. Mientras que los niveles de calcio en suero disminuyeron progresivamente, los niveles séricos de BAP y HPT, el índice Ca/Cr en orina, y la mineralización ósea aumentaron en los niños saludables con el nivel de desarrollo sexual. La única

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*correlación significativa se halló entre los niveles de HPT en suero y la densidad mineral ósea (*p < *0.05). En nuestra opinión, la HPT puede ser un potente estimulador de la dinámica del esqueleto en los muchachos y puede asociarse con los aumentos sustanciales de la espina lumbar. Concluimos que la HPT se comportó como un valioso marcador de la mineralización ósea durante la pubertad. La mineralización ósea acelerada es reflejada por los altos niveles séricos de HPT durante la pubertad. Todos los valores de los marcadores de formación y resorción ósea en los niños con RCCP fueron similares a los de los niños en la prepubertad. Los niños con RCCP mostraron características prepubertales. Sugerimos que hay un periodo de edad crítico para la acumulación de masa ósea, de acuerdo con los resultados en este estudio.*

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INTRODUCTION

Bone mass increases normally throughout childhood, reaching peak levels by late adolescence or early adulthood. During puberty, bone growth and bone mass accrual as well as bone turnover markedly increase in both genders (1, 2). Commonly used markers of bone formation are alkaline phosphatase (AP), bone specific alkaline phosphatase (b-AP), osteocalcin and procollagen type-l propeptides, all representing different stages of osteoblast proliferation and differentiation (3). Markers of bone resorption are type-l carboxyterminal telopeptide measured in serum and the urinary marker deoxypyridinoline (DPD) (4).

Constitutional delay of growth and puberty (CDGP) is a common clinical problem potentially affecting up to 3% of adolescent boys. Osteopaenia has been reported in adult men with a history of CDGP in comparison with men who had normal onset of the timing of puberty (5). This finding suggests that the timing of sexual maturation is an important determinant of adult bone mineral density (BMD).

There are few studies in which both bone metabolism markers and bone mass were measured in healthy children throughout puberty and in children with CDGP. The bone turnover markers were not predictive of bone gain (6) or even showed a significant negative relation (7).

The aim of this study was to evaluate associations between the markers of bone formation and resorption and bone mineral density in healthy children throughout puberty and in children with CDGP.

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 (8) was 15. All children were white. Healthy subjects were normally growing children free of chronic disease and had not received prior therapy with any known effect on bone metabolism. In all of them, chronological age and bone age differed by less than a year. The diagnosis of CDGP was based on the following criteria: a) chronological age above 14 years, b) height below the 10th 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 less than 2 years of CA, g) other causes of short stature were excluded by normal blood count and erythrocyte sedimentation rate, electrolyte, creatinine, calcium, inorganic phosphate, alkaline phosphatase, total protein, thyroxine, thyrotropin, antigliadin antibodies, IGF-l, IGFBP-3 and uroanalysis, h) growth velocity (recorded from a minimum period of 6 months) above the 25th percentile, i) family history of CDGP or mid-parental height above the 25th percentile.

Height was 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. Chronological age was determined to the decimal age (9). Skeletal maturation was assessed from radiographs of the left hand and wrist performed prior to measurements of bone density. Bone age was determined with the method described by Greulich and Pyle (10). Subjects were physically active in accordance with their age and their calcium intake per day was similar. Calcium intake was evaluated by a dietician through a standardized questionnaire on food and beverage intake.

Physical examinations were performed by a paediatrician to determine the stages of sexual development. The grading system defined by Tanner (8) was used for classification.

Bone mineral density and bone mineral content BMC was 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 lordosis 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 and urine samples were obtained in the morning following an overnight fast. Serum parathyroid hormone (PTH) levels (ACS:180 Diagnostic Products Corporation, USA) were measured by chemiluminescence enzyme immuno-assay using commercial kits. Serum bone-specific alkaline phospatase (b-AP), calcium (Ca) and inorganic phosphorus (P), urine Ca, creatinine (Cr) (Hitachi 717 autoanalyser, Diasys) and sodium levels (Beckman synchron El-yse autoanalyser, Beckman) were estimated by an autoanalyser. Urine deoxypyridinoline (DPyr) levels were measured by chemiluminescence enzyme immuno-assay using commercial kits (ACS 180, Chiron Diagnostics Corporation, USA) and results were expressed as a ratio of urine DPyr/urine creatinine (DPyr/Cr). For all hormonal analyses, intra- and inter-assay coefficients of variation were less than 6.8 and 9.5%, 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 one-way analysis of variance (ANOVA). A *p*-value of < 0.05 was considered significant.

RESULTS

Mean bone age, weight and height of children with CDGP were similar to those of children with pubertal stage l except for decimal age. Children with CDGP had prepubertal properties.

Descriptive and biochemical characteristics of the study population, grouped according to pubertal stages, were presented in Table 1. Statistical comparisons of results of analyses of groups were shown in Table 2. Although mean serum phosphorus level was higher in stages II, III, IV, V compared to stage l and CDGP; mean b-AP, PTH, BMD and BMC levels were higher in stages III, IV, V compared to stage l and CDGP. Mean serum Ca levels were lower in stages III, IV and V compared to stage l and CDGP (Table 2). During puberty, urine DPry/Cr levels were not significant. Mean Ca intake per day and urinary Na levels were similar in all groups. The peak level of b-AP occurred at stage IV. With one-way ANOVA, statistical results of bone metabolism markers from Stage l to Stage V were given in Table 3. Serum PTH, Ca, b-AP levels, urine Ca/Cr ratio, BMC and BMD measurements significantly changed during puberty in healthy children. While serum Ca levels progressively decreased, serum b-AP, PTH levels, urine Ca/Cr ratio and bone mineralization increased in healthy children with the level of sexual development. But all values were in normal range. Mean Ca, P, b-AP, DPyr/Cr, PTH and BMD levels in all groups were given in Fig. 1.

All values of the markers of bone formation and bone resorption in children with CDGP were similar to those of prepubertal children (Table 2). Children with CDGP had prepubertal properties.

Correlations between bone mineral density and bone turnover markers are shown in Table 4. The only significant correlation is found between serum PTH levels and BMD ($p < 0.05$; Fig. 2).

DISCUSSION

During puberty, bone growth and mineralization as well as bone turnover increase dramatically. Biochemical measurements of bone turnover are helpful in the study of the pathophysiology of skeletal metabolism and growth. However,

Table 1: Descriptive and biochemical characteristics 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
Decimal age (year)	14.2 ± 0.19	13 ± 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 ± 0.4	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
Ca (mg/dL)	10.7 ± 0.6	10.4 ± 0.2	10.2 ± 0.4	9.9 ± 0.5	9.8 ± 0.4	9.8 ± 0.3
P (mg/dL)	4.2 ± 1.3	4.2 ± 0.5	4.6 ± 0.5	4.6 ± 0.7	4.9 ± 0.6	4.6 ± 0.5
b-AP (IU/ml)	383 ± 84	432 ± 161	482 ± 164	524 ± 130	547 ± 168	429 ± 147
PTH (pg/ml)	28 ± 13	25 ± 9.2	30 ± 14	39 ± 16	42 ± 21	55 ± 28
Urine DPyr/Cr (nM/nm Cr)	27.4 ± 8.3	27.9 ± 10.9	28.5 ± 12.07	29.6 ± 8.4	32.2 ± 13.8	32.2 ± 12.2
Urine Na (mmol/L)	73 ± 11	74 ± 12	82 ± 15	60 ± 19	66 ± 14	69 ± 17
Urine Ca/Cr (mg/nm Cr)	0.13 ± 0.08	0.21 ± 0.02	0.13 ± 0.09	0.21 ± 0.02	0.12 ± 0.04	0.14 ± 0.09
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/cm^2)	0.62 ± 0.05	0.60 ± 0.06	0.63 ± 0.06	0.67 ± 0.08	0.69 ± 0.05	0.76 ± 0.12

* CDGP: Constitutional delay of growth and puberty

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
Decimal age	0.001	NS	NS	NS	0.001
		0.01	0.001	0.001	0.001
Bone age (yr)	NS	0.001	0.001	0.001	0.001
		0.01	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	0.001	0.001	0.001
		NS	0.001	0.001	0.001
Ca (mg/dL)	NS	NS	0.01	0.001	0.001
		NS	0.01	0.001	0.001
P (mg/dL)	NS	0.05	0.05	0.01	0.05
		0.05	0.05	0.01	0.05
b-AP (IU/ml)	NS	NS	0.01	0.01	NS
		NS	0.01	0.01	NS
PTH (pg/ml)	NS	NS	0.05	0.05	0.01
		NS	0.01	0.01	0.01
DPyr/Cr	NS	NS	NS	NS	NS
(nM/nmCr)		NS	NS	NS	NS
BMD (gr/cm^2)	NS	NS	0.001	0.001	0.001
		NS	0.001	0.001	0.001
BMC (gr)	NS	NS	0.001	0.001	0.001
		NS	0.001	0.001	0.001

NS: Non significant
* CDGP: Constitutional delay of growth and puberty
**PS: Pubertal stage

Table 3: Statistical results of bone metabolism markers, with one-way ANOVA

	F	p
PTH	5.46	< 0.001
b-AP	2.75	< 0.05
Ca	8.61	< 0.001
P	2.01	> 0.05
DPyr / Cr	0.60	> 0.05
Urine Na	2.23	> 0.05
Urine Ca/Cr	3.32	< 0.01
BMC	17.03	< 0.001
BMD	15.01	< 0.001

Table 4: Correlations between bone mineral density and bone turnover markers

	r	p
PTH	0.279	0.028
b-ALP	0.430	0.686
Ca	-2.220	0.350
P	0.360	0.739
Dpd	0.165	0.121

interpretation of their results is difficult because they depend on age, pubertal stage, growth velocity, mineral accrual, hormonal regulation, nutritional status, circadian variation, method of expression of results of urinary markers, sensitivity and specificity of assay (11).

Mean bone age, weight and height of children with CDGP were similar to those of children with pubertal stage l except for decimal age. Children with CDGP had prepubertal properties. Also, properties of bone turnover markers and bone mineralization in children with CDGP were similar to those in healthy prepubertal children. The rate of bone mineralization depends on pubertal stage. It is well known that CDGP is a risk factor for male osteoporosis (12, 13). There is a critical age for accumulation of bone mass according to our results. Longitudinal studies may elucidate why sufficient mineralization does not take place after puberty starts in CDGP.

Alkaline phosphatase is present in the membrane of osteoblasts and is released into the circulation. Bone specific alkaline phosphatase is a more sensitive diagnostic tool for bone turnover than total AP activity (11). It is known that AP resists degradation caused by freezing and thawing (14). In puberty, b-AP is about 10 times higher than adult values whereas total AP is five times higher (15). Females have their peak serum b-AP levels during puberty about two years earlier than males (16). Bone specific alkaline phosphatase increases until mid-puberty and decreases in late puberty [in girls, after menarche] (3, 16). Our results were also consistent with these previous findings.

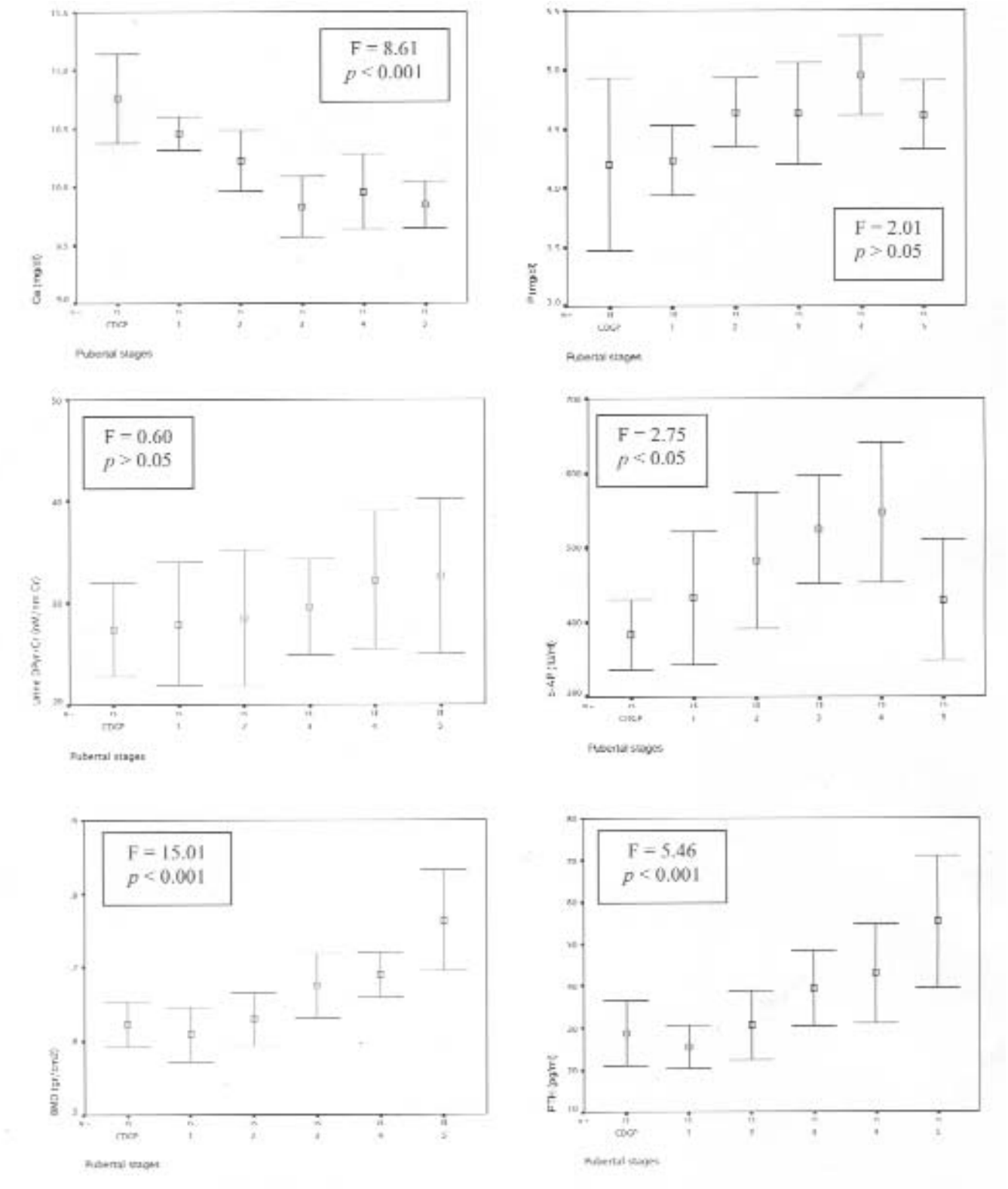

Fig. 1: Mean Ca, P, b-AP, DPyr/Cr, PTH and BMD levels in all groups. Significances were given for puberty with one-way ANOVA.

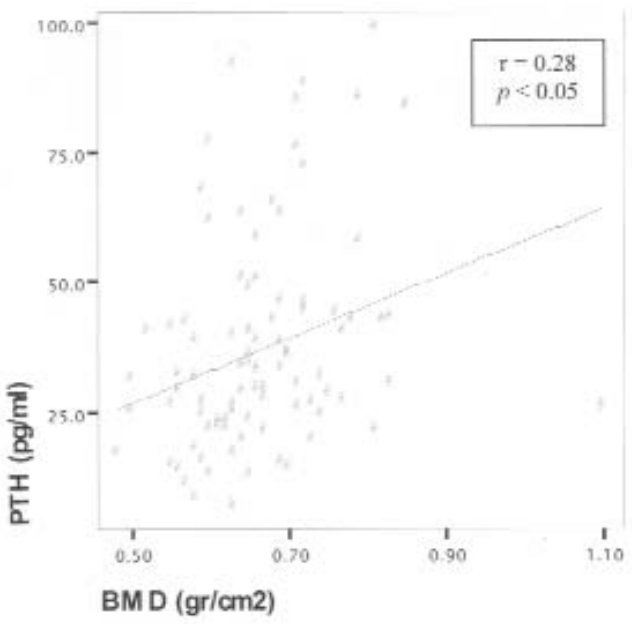

Fig. 2: Correlation between serum PTH levels and bone mineral density

Deoxypyridinoline is generated from hydroxylysine and lysine during post-translational modification of collagen and is released during matrix resorption and excreted in urine (11). Investigators were not able to find a rise in urinary excretion of DPyr in adolescent girls (4, 7). However, an elevation of DPyr/Cr was not found in boys during the pubertal years (4). In our study, changes in DPyr/Cr ratio was not found in healthy boys. Although urinary Cr levels was not given, it may be related to the elevation of creatinine at puberty.

During puberty, there are two phenomena: longitudinal growth and accumulation of bone mineral. The maximum height velocity (HV) was reached at stage IV in boys (4). After HV peaks, and is declining, bone mineralization is still increasing. This was similar to the results of Bailey (17) who showed that the peak velocity in bone mass accumulation occurred 0.7 years after the peak in HV. While van Coeverden *et al* (4) found bone mineral accrual was greatest at stage G4 in boys, our results showed that bone mineralization was greatest at stage V.

Except for PTH, correlations between bone mass and turnover markers were insignificant in pubertal healthy boys. Our results were in contrast with those of van Coeverden *et al* (4) who found that correlations between bone mass and turnover markers were significant in boys. Cadogan *et al* (6) found no correlation between the increase of bone mass and bone turnover markers in a longitudinal study of 37 pubertal girls. On the other hand, Mora *et al* (7) showed significant inverse correlation between b-AP and BMD. We did not evaluate pubertal growth spurt in this study. However, Szulc *et al* (11) thought that the levels of bone markers were better correlated with linear growth than with bone mineral accrual. There is insufficient published data regarding how PTH levels change with bone mineralization during puberty. Kurland *et al* (18) administered parathyroid hormone as a therapy for idiopathic osteoporosis in men and found that PTH-(1-34) was associated with a 13.5% increase in bone mass in the lumbar spine whereas that in the control group did not change. In that study, it was shown that there were no significant changes in serum calcium concentration and 24-h urinary calcium excretion. In the present study, significant correlation was found between BMD and PTH in pubertal healthy boys. Reeve *et al* (19) reported that the mean plasma Ca concentration in osteoporotic patients receiving parathyroid peptide treatment and the AP and urinary excretion of hydroxyproline increased but only AP was statistically significant. Present data suggest that there was Ca retention to supply bone mineral density for the newly formed bone. In our opinion, PTH may be a potent stimulator of skeletal dynamics in boys; may be associated with substantial increases in bone mass in the lumbar spine; and may have widespread use as an efficacious anabolic agent in future. There is no doubt that PTH increased with Tanner stage. Another possibly more likely explanation may be that PTH increased due to the fall in serum Ca. The serum Ca may have dropped due to increased bone formation with growth. Data in this study suggest that bone formation is more important than bone resorption in puberty. As suggested previously, puberty is indeed related to relative increases in bone degradation rates (20). The net result, however, is that bone formation exceeds bone resorption. We concluded that PTH behaves as a marker of bone mineralization during puberty.

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