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Original Article

Bone Mass and Mineral Metabolism in Klinefelter's Syndrome

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Abstract. A reduced bone mineral density (BMD) is frequently observed in hypogonadal males; however, very little is known on bone and mineral metabolism in Klinefelter's syndrome (KS). In this study 32 XXY KS patients and 24 healthy age-matched male controls were examined. Serum total and free testosterone (TT and FT) were significantly lower in patients than in controls (TT in KS, 15.1 ± 7.8 nmol/l; controls, 30.4 ± 9.1; $p < 0.001$. FT in KS, 81.8 ± 24.9 pmol/l; controls, 135.7 ± 16.4; $p < 0.001$). 17β-Estradiol was slightly higher in KS patients (KS, 49.0 ± 27.1 pg/ml; controls, 39.3 ± 16.4 pg/ml), but the difference was not significant. BMD, measured at the spine (L2–4) and at the proximal epiphysis of the left femur, was similar in patients and in the control group (spine: KS, 1.016 ± 0.142; controls, 1.085 ± 0.144 g/cm^2; p = not significant. Femoral neck: KS, 0.926 ± 0.149; controls, 0.926 ± 0.122 g/cm^2; p = not significant). Bone GLA protein (BGP) was significantly higher in the KS group (12.7 ± 4.8 vs 8.9 ± 5.2 ng/ml; $p < 0.02$), while serum calcium, serum phosphate, calciotrophic hormones and the fasting urinary hydroxyproline/creatinine ratio (OHP/Creat) were similar in the two groups. A positive relationship between FT and both spine and femoral BMD was found in KS patients. Furthermore, OHP/Creat ratio was inversely related to BMD at the femur, and positively related to BGP in KS patients, but not in normal subjects. These findings suggest that (1) KS patients have normal bone mass, most probably because the hypogonadism is moderate; and (2) patients with lower bone mass appear to have higher bone turnover.

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Introduction

Osteoporosis in males is less frequent than in females. Riggs and Melton [1] reported that the incidence of spinal fractures in men is about 6-fold lower than in women. The reason for this is that the peak bone mass is higher and the rate of bone loss is slower in men than in women, who show an increased rate of bone loss at menopause [2–6]. It is questionable whether the bone loss in males might be due to the age-dependent fall in gonadal function [7,8]; however, hypogonadism surely represents one of the major risk factors for osteoporosis in men. Seeman et al. [9], studying a cohort of 105 men with atraumatic spinal fractures, pointed out that the incidence of hypogonadism was particularly high immediately after corticosteroid treatment. Testosterone replacement therapy increases bone mass in hypogonadal osteopenic subjects [10–12], and appears to be more effective in patients who have skeletal immaturity [11].

Data on the incidence of osteoporosis in Klinefelter's syndrome (KS) are rather limited and heterogeneous, at least regarding the methods of measurement of bone mass: radiomorphometric [13,14], histomorphometric [15,16] and densitometric [17–19] methods have been used to assess bone density in several studies. Moreover, in a large number of studies, patients with KS were considered together with patients with other types of hypogonadism, and only in a few reports were KS patients considered separately. In this study we report the vertebral and femoral bone density, as well as some

biochemical and hormonal data on sexual function and mineral metabolism, in a group of 32 patients with KS.

Materials and Methods

Patients

Forty-two patients aged from 17 to 39 years (mean ± SD: 25.8 ± 6.1 years), referred to the Department of Endocrinology or Pediatrics of the University of Padua for either infertility or cryptorchidism, were diagnosed as suffering from KS on the basis of the presence of a 47 XXY karyotype. Ten patients treated with testosterone replacement therapy for between 3 months and 8 years were excluded from the study. Consequently, 32 patients (age range: 18–34 years; mean ± SD: 25.4 ± 5.1 years), who had never been treated with testosterone, were studied. In addition, 24 healthy males, aged from 19 to 44 years (25.5 ± 6.1 years) and recruited from the medical and technical staff of the hospital, were examined as a control group. Anthropometric characteristics of the controls were similar to those of the KS group (Table 1). Control subjects did not show any physical or sexual abnormalities, namely testicular volume was normal for age. In the control group karyotype was not assessed. All patients and controls were free from chronic diseases, and had not been taking drugs affecting bone mass or mineral metabolism. Informed consent to the study was obtained from all the subjects.

Sex Hormones

Gonadotrophins were assayed by immunoradiometric assay (IRMA; MAIA-Clone, Serono Diagnostics, Italy); intra-assay coefficient of variation (CV) was 7% for both LH and FSH, inter-assay CV was 4% for LH and 3% for FSH. Total testosterone (TT) was measured by radioimmunoassay (RIA; Testosterone-MAIA, Biodata-Ares Serono, Italy); intra-assay and inter-assay CV were 5% and 6.1%, respectively. Free testosterone (FT) was measured by RIA (Coat-a-Count free testosterone, Diagnostic Products Corporation, Los Angeles, CA, USA); intra-assay and inter-assay CV were 3.2% and 3.4%, respectively. Estradiol was assayed by RIA (Estradiolo-MAIA, Biodata-Ares Serono, Italy); intra-assay and inter-assay CV were 4.3% and 8.5%, respectively. Androstenedione was measured by RIA (Androstenedione, Medical System s.p.a., Italy); intra-assay and inter-assay CV were 4.7% and 7.6%, respectively. Sex hormone binding globulin (SHBG) was measured by IRMA (Orion Diagnostics, Finland); intra-assay and inter-assay CV were 2.2% and 9%, respectively. Prolactin was assayed by IRMA (Prolactin-MAIA Clone, Serono Diagnostics, Italy); intra-assay and inter-assay CV were 3.2% and 6%, respectively. Progesterone was measured by RIA (Progesterone-MAIA, Serono Diagnostics, Italy); intra-assay and inter-assay CV were 7% and 8%, respectively.

Bone Mineral Density

Bone mineral density (BMD) was assessed at the lumbar spine (L2–4) and proximal femur by dual-energy X-ray absorptiometry (DXA), using a Hologic QDR 1000 densitometer (Boston, MA, USA). For spine measurements, the patient was placed in the supine position over the table with the lower limbs raised 45° to flatten the physiological lumbar lordosis. Then, the proximal epiphysis of the left femur was measured: the legs were extended on the table and the left foot medially rotated 33° for better visualisation of the femoral neck. Long-term (1 year) CV in vitro was 0.43%; short-term (1 week) CV in vivo was 1.2% for the spine and 2.6% for the femoral neck. Individual values of vertebral and femoral BMD in KS patients were also expressed as *Z*-scores. Since no variation of BMD with age was observed in the control subjects, probably due to the rather narrow age range, average BMD and standard deviation (SD) of the control group were used to calculate *Z*-scores according to the following formula:

$$Z\text{-score} = (\text{BMD of KS patients} - \text{average BMD of control group})/\text{SD of control group}$$

Biochemical Measurements

Serum calcium (Ca) was measured by a cresolphthalein colorimetric method (kit calcium, code 434101, Diagnostics Clinicals Pasteur, Milan, Italy). Serum phosphate (P) was assayed by an ultraviolet colorimetric method (kit phosphorus, code 452651, Diagnostics Clinicals Pasteur, Milan, Italy). Creatinine (Creat) was assessed by a modified colorimetric Jaffé's method (Bioelectron Instruments, Milan, Italy). Fasting urinary hydroxyproline (OHP) was measured by the Hypronosticon kit (Organon Teknika, Boxtel, Holland), after an extraction procedure with a cationic exchange resin followed by hydrolysis at 100 °C for 16 h. Parathyroid hormone (PTH) was assayed by RIA using a commercial kit from Incstar (Stillwater, MN, USA) which employs an antiserum directed against the mid-region (44–68) of the molecule; intra-assay and inter-assay CV were 5% and 8%, respectively. Calcitonin (CT) was measured by RIA, using a commercial kit from Nichols Institute Diagnostics (San Juan Capistrano, CA, USA); intra-assay and inter-assay CV were 5% and 8%, respectively. Bone GLA protein (BGP) was measured by RIA (CIS, Gif sur Yvette, France), using bovine osteocalcin as standard; intra-assay and inter-assay CV were 6% and 11.5%, respectively. 1,25-Dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] was assayed by a radioreceptor method, using a kit from Incstar, after chromatographic

purification of the sample by Sephadex C-18 columns (Incstar). Recovery was always higher than 70%, the binding capacity of the system ranged from 23% to 27%; intra-assay and inter-assay CV were 8% and 11.5%, respectively. 25-Hydroxyvitamin D_3 ($25OHD_3$) was measured by RIA, using a kit from Incstar; intra-assay and inter-assay CV were 7% and 12%. respectively. Cyclic AMP (cAMP) was measured by a RIA from Amersham (UK) in serum and urine; intra-assay and inter-assay CV were 9.5% and 15%, respectively. The estimation of nephrogenous cAMP was calculated by subtracting the plasma cAMP (nmol/dl) from the total urinary cAMP excretion (nmol/dl FG).

Statistical Analysis

The average values of the two groups were compared using Student's *t*-test for non-paired data. The correlation between two variables was assessed by linear regression analysis. The significance level was set at $p < 0.05$ (two-tailed) for all tests.

Results

Results are summarized in Table 1.

Sex Hormones

LH and FSH levels were significantly higher ($p < 0.001$) in patients with KS than in controls. In the KS group, TT and FT were significantly lower than in controls ($p < 0.001$), TT and FT serum levels being below the lower limit of the reference range in 10 (31%) and 29 (91%) patients, respectively. Androstenedione was lower in KS patients than in controls ($p < 0.05$). Levels of prolactin, progesterone and SHBG did not differ significantly between the two groups. Estradiol was slightly higher in KS patients than in controls (49.0 ± 27.1 vs 39.3 ± 16.4 pg/ml), but the difference was not significant ($p = 0.1$).

Table 1. Anthropometric characteristics, hormonal assays, biochemical measurements and bone density in patients with Klinefelter's syndrome and in controls

	Klinefelter's syndrome		Controls		*p* value
	Range	Mean±SD	Range	Mean±SD	
Age (years)	(18–34)	25.4±5.1	(19–44)	25.5±6.1	NS
Height (cm)	(165–192)	179.8±6.3	(161–193)	177.5±7.1	NS
Weight (kg)	(55–105)	75.2±14.1	(58–88)	72.5±8.8	NS
BMI	(18–32.3)	23.2±3.7	(18.9–27.7)	23.0±2.3	
Total testosterone (nmol/l)	(2.6–40.5)	15.1±7.8	(12.5–51.89)	30.4±9.1	<0.001
Free testosterone (pmol/l)	(17.7–140.2)	81.8±24.9	(110.7–170.0)	135.7±16.4	<0.001
Androstenedione (ng/ml)	(0.9–5.5)	2.4±0.9	(1.7–5.7)	3.0±1.2	<0.05
Estradiol (pg/ml)	(13.3–131)	49.0±27.1	(4.1–66.4)	39.3±16.4	NS
Progesterone (ng/ml)	(0.1–1.0)	0.4±0.2	(0.02–1.3)	0.3±0.3	NS
LH (mIU/ml)	(3.9–23.5)	13.9±4.9	(1.0–8.4)	2.9±1.4	<0.001
FSH (MIU/ml)	(13.4–75.8)	34.5±15.1	(1.4–11.0)	3.9±2.0	<0.001
Prolactin (ng/ml)	(3–37.1)	10.0±6.6	(2.0–22.2)	8.5±4.3	NS
SHBG (nmol/l)	(8.4–55.1)	25.0±9.6	(8.8–74.4)	26.4±14.1	NS
Bone mineral density					
L2–4 (g/cm^2)	(0.766–1.400)	1.016±0.142	(0.863–1.407)	1.085±0.144	NS
L2–4 *Z*-score	(−2.21–2.19)	−0.480±1.000			NS
Neck (g/cm^2)	(0.616–1.252)	0.926±0.149	(0.773–1.238)	0.926±0.122	NS
Neck *Z*-score	(−2.54–2.65)	0.002±1.234			NS
Total (g/cm^2)	(0.703–1.242)	0.981±0.128	(0.843–1.418)	1.014±0.129	NS
Total femur *Z*-score	(−2.41–1.77)	−0.244±1.018			NS
Ward's triangle (g/cm^2)	(0.488–1.193)	0.787±0.171	(0.537–1.143)	0.771±0.145	NS
Ward's triangle *Z*-score	(−1.95–2.91)	0.108±1.200			NS
Serum Ca (mmol/l)	(2.2–2.62)	2.4±0.1	(2.15–2.65)	2.4±0.2	NS
Serum P (mmol/l)	(0.97–1.81)	1.4±0.2	(1.26–1.819)	1.5±0.2	NS
BGP (ng/ml)	(1.7–26.3)	12.7±4.8	(1.1–18.3)	8.9±5.2	<0.02
Fasting urinary OHP/Creat ratio (mg/g Creat)	(17.5–54.0)	29.2±10.0	(13.6–52.2)	27.2±10.5	NS
Calcitonin (pg/ml)	(5–22)	9.7±3.3	(6–28.3)	10.5±5.9	NS
PTH (mid-region 44–68) (pmol/l)	(31–64)	40.9±8.4	(29–65)	44.7±8.7	NS
$1,25(OH)_2D_3$ (pg/ml)	(32.1–91.0)	51.9±13.4	(37.0–62.0)	52.4±6.8	NS
$25OHD_3$ (ng/ml)	(5.2–36.2)	12.7±5.5	(6.9–23.3)	14.9±4.8	NS
Nephrogenous cAMP (nmol/100 ml FG)	(0.1–8.5)	2.9±2.2	(0.1–5.2)	2.9±1.4	NS

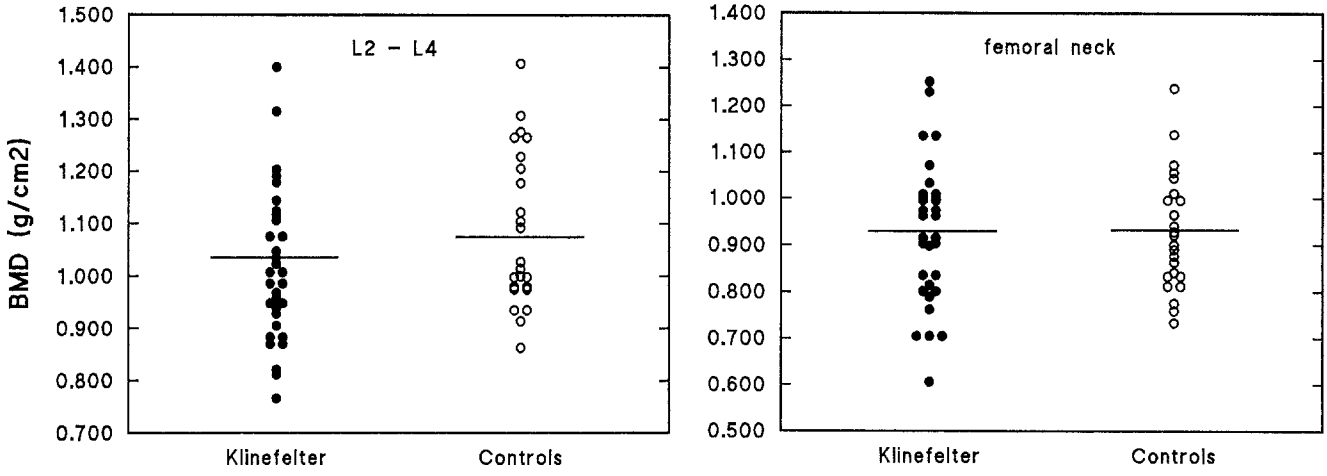

Fig 1. Individual densitometric values at the lumbar spine and femoral neck in patients with Klinefelter's syndrome (*filled circles*) and in controls (*open circles*).

Table 2. Densitometric parameters in patients with Klinefelter's syndrome with serum testosterone levels lower or higher than 12 nmol/l (this value represents the lower limit of the control range)

Bone mineral density (g/cm^2)	Serum testosterone level		*P*
	<12 nmol/l (n=10)	>12 nmol/l (n=22)	
L2–4	0.978±0.176	1.033±0.128	NS
Neck	0.943±0.166	0.918±0.147	NS
Total	1.063±0.282	0.971±0.127	NS
Ward's triangle	0.813±0.190	0.775±0.170	NS

Bone Mineral Density

There was no significant difference in vertebral and femoral BMD between KS patients and controls (Fig. 1). At the spine, only 4 patients (12.5%) showed densitometric values below the lower limits of the reference range. Similarly, only 2–6 patients (6%–19%) showed BMD below the control range in the different measurement areas of the femur. No difference between the two groups was found either when BMD was expressed as a *Z*-score (Table 1). BMD of KS patients with testosterone serum levels below the control range was similar to that of patients with higher testosterone and of the controls (Table 2).

Biochemical Measurements

Serum calcium and phosphate did not significantly differ between the two groups. CT, PTH, $1,25(OH)_2D_3$, $25OHD_3$, nephrogenous cAMP and fasting urinary OHP/Creat ratio were similar in KS patients and in controls. KS patients showed a significantly higher level of BGP than controls (p <0.02), despite the wide scatter of the values.

Relationship Between Biochemical and Densitometric Parameters

There was an inverse relationship between femoral BMD and fasting urinary OHP/Creat ratio in KS patients, but not in controls (neck: r = 0.41, p <0.02; total femur: r = 0.5, p <0.01; Ward's triangle: r = 0.43, p <0.025) (Fig. 2). A significant positive correlation between both vertebral (r = 0.43, p = 0.01) and femoral (r = 0.40, p <0.03) BMD and FT was found in KS patients, but not in controls (Fig. 3). OHP/Creat ratio and BGP were significantly related in KS patients (r = 0.47, p <0.01), but not in controls (Fig. 4). TT, as well as estradiol, androstenedione, BGP and calciotrophic hormones, were not significantly related to BMD at any site, either in patients with KS or in normal subjects.

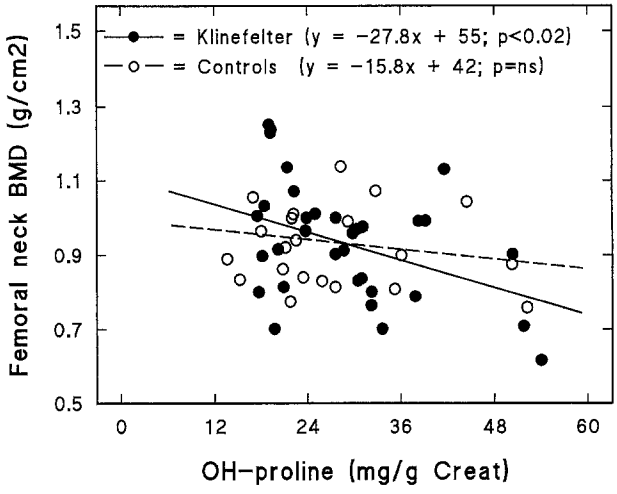

Fig 2. Relationship between fasting urinary OHP/Creat ratio and femoral neck BMD in patients with Klinefelter's syndrome (*filled circles*) and in controls (*open circles*).

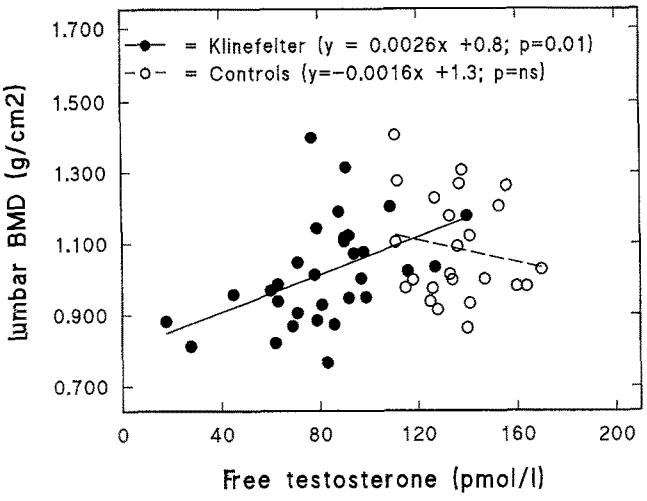

Fig 3. Relationship between free testosterone serum levels and BMD in patients with Klinefelter's syndrome (*filled circles*) and in controls (*open circles*).

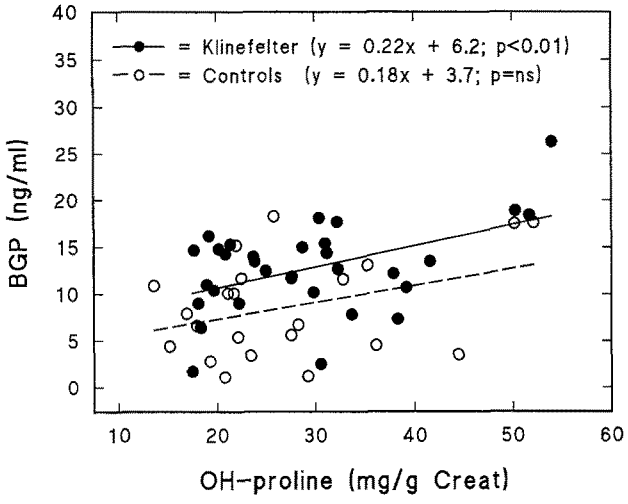

Fig 4. Relationship between BGP and fasting urinary OHP/Creat ratio in patients with Klinefelter's syndrome (*filled circles*) and in controls (*open circles*).

Discussion

In KS patients, BMD was comparable with that of normal subjects in all the areas measured. These findings are in contrast to those reported by others. In 1977 Smith and Walker [13], using a semiquantitative radiological method which compared the density of the midpoint of the third metacarpal with that of a standard aluminium step wedge, found that 75% of 29 patients with KS were below the 50th percentile and 31% below the 5th percentile. Foresta et al. [14], using a radiomorphometric index, found significantly reduced values in KS patients with testosterone serum levels lower than 200 ng/dl, but normal values in those with higher testosterone serum levels. Delmas and Meunier [15], using histomorphometric analysis, found a picture of osteoporosis, with a marked reduction of bone trabecular volume, in 5 patients with KS and low back pain. More recently, Horowitz et al. [17], using single-photon absorptiometry in 22 KS patients, found forearm BMD lower than in controls. Kubler et al. [18] found a low forearm BMD in 21 untreated KS patients and in 11 who started testosterone replacement therapy after 20 years of age. Wong et al. [19] found that 14 testosterone-treated KS patients had a femoral BMD lower than controls, but failed to find any difference in total body and lumbar spine BMD between the two groups of subjects.

The different results that we found might be due to the different measurement site. In most previous studies BMD was calculated on sites where cortical bone was prevalent, and only in Wong's study were different skeletal areas, including spine and femur, examined. Meier et al. [20] reported a trabecular age-related bone loss greater than the cortical loss in healthy men; however, histomorphometric measurements showed that both cortical and trabecular bone are similarly affected in hypogonadal patients [15,16]. The hypothesis of a stronger effect of testosterone deficiency on cortical than on trabecular bone seems to be unlikely in our series, because a significant relationship between bone density and FT was found in all measurement sites.

It is likely that the different behaviour of BMD is due to the different testosterone serum levels found in the various series examined. Despite the fact that BMD was significantly related to FT in KS patients, and serum levels of FT were below the lower limit of the control range in 91% of KS patients, BMD was similar in the two groups. The lack of difference in BMD between the two groups might be due to the fact that our patients had testosterone serum levels lower than normals but enough to provide for a good bone mass. In a previous study [21] we showed that KS patients had both testosterone levels and BMD values significantly higher than those observed in patients with hypogonadotrophic hypogonadism. In the whole case series there was a significant relationship between testosterone and BMD, which suggests a positive effect of the hormone on bone so we cannot exclude the possibility that osteoporosis can occur in KS patients if testosterone serum levels are very low. In the present study, testosterone serum levels were, on average, higher than those reported in other published series. On the basis of statistical analysis (Student's *t*-test for non-paired data), such a difference appears to be significant when our results are compared with those of Foresta et al. [14], Horowitz et al. [17] and Kubler et al. [18], but not with those of Smith and Walker [13] and Wong et al. [19]. The difference in testosterone serum levels is probably due to different recruitment criteria. In some cases the hypogonadism was the main reason for checking genetic or hormonal defects [15,18,19]. Hypogonadism and even low back pain were the main features in the 5 cases presented by Delmas and Meunier [15]. Our patients, in contrast, were recruited mainly for infertility, which does not presume hypogonadism. Furthermore, our patients were, on average, younger than those in the other

series. Some authors have shown an inverse relationship between testosterone serum levels and age [18]. The same authors failed to find any correlation between BMD and age, but did report that more than 45% of the patients older than 30 years showed BMD values below the 5th percentile. This percentage fell to 4.5% when considering patients younger than 30 years. In our series all the patients were younger than 40 years, and 69% were younger than 30 years, so we can not exclude osteopenia in more advanced years.

Two other mechanisms, involving estrogens and calcitonin (CT), should be mentioned to explain the lack of reduction of BMD in KS patients. In KS patients, high estrogen serum levels could be found [22,23] due to the enhanced LH-induced peripheral aromatisation of androgens to estrogens. In our series, estradiol was slightly higher than in controls, but the difference was not significant. CT reduces bone resorption [24], and CT deficiency may play a role in the pathogenesis of osteoporosis [25–27]. Although CT serum levels have been reported to be lower in KS patients than in normals [28], we failed to find any significant difference between the two groups.

In our study BGP was significantly higher in KS patients than in normals. This finding is in contrast to that of Horowitz et al. [17] who found BGP serum levels significantly lower and an OHP/Creat ratio significantly higher than in normals. Such a difference could be due to the different series studied. BGP is known to be increased in children, peaking during the adolescent growth spurt [29–31]. Our patients were younger than those of Horowitz's series (mean age: 25.4 vs 37 years), and delayed puberty in Klinefelter's syndrome has been reported [32]. However, we can not emphasize this point because the onset of puberty was not considered in our patients; furthermore, no correlation between BGP and either age or testosterone was found in KS group. The high BGP serum levels could be evidence of increased bone turnover. Although the OHP/Creat ratio was not significantly different from that of controls, 56% of KS patients showed an OHP/Creat ratio higher than the average value found in the control group. Furthermore, the OHP/Creat ratio was positively related to BGP, and negatively to femoral bone density, suggesting that patients with lower bone mass also had a higher bone turnover.

In conclusion, patients with KS show spine and femoral BMD similar to those found in normal subjects, even though we can not exclude the presence of moderate or severe osteopenia in older patients or in those with higher bone turnover.

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