Osteoporosis Int (1993) 3:3–7

Osteoporosis International

Original Article

Loss of Bone Mass in Patients with Klinefelter's Syndrome Despite Sufficient Testosterone Replacement

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Abstract. To determine whether testosterone replacement therapy reverses the detrimental effects of hypogonadism on bone density, we measured the total body, lumbar spine and proximal femur bone mineral density (BMD) by dual-energy X-ray absorptiometry in 14 patients with Klinefelter's syndrome on long-term testosterone replacement therapy and compared the results with 14 age- and sex-matched normal controls. Seven of the patients were receiving oral testosterone undecanoate thrice daily (240 mg/day) and the others were having intramuscular testosterone enanthate injections once every 3 weeks (250 mg/injection). Their serum testosterone levels were maintained within the normal limits (10–40 nmol/l). We showed that patients on testosterone replacement had decreased amount of bone density in the left femoral neck when compared with the controls ($p<0.01$). Similar decreases were also observed in the left Ward's triangle ($p<0.01$) and in the left trochanter ($p<0.05$). There were no significant differences in the total body and the lumbar spine measurements in these two groups of subjects. No correlation was found between the BMD values of femur and the duration of testosterone treatment in the patients with Klinefelter's syndrome. The type of testosterone treatment was also not associated with significant differences in BMD. In conclusion, sufficient testosterone replacement with currently available methods does not reverse the decrease in bone mass associated with hypogonadism in patients with Klinefelter's syndrome.

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Keywords: Bone mineral density; Klinefelter's syndrome; Testosterone replacement therapy

Introduction

Klinefelter's syndrome is the most common developmental defect of the testis, occurring in approximately 1 in 500 men [1]. The disorder is characterised by small firm testes, varying degrees of impaired sexual maturation, azoospermia, gynaecomastia, and elevated gonadotrophins [2]. The underlying defect is the presence of an extra X chromosome in a male leading to the common karyotype 47,XXY (the classic form) or 46,XY/47,XXY (the mosaic form) [3]. Loss of bone tissue has been demonstrated to be associated with hypogonadism in males [4]. Patients with Klinefelter's syndrome were found to have lower whole bone density (mass per unit volume) than normal age-matched controls [5]. The bone loss seemed to be correlated with the deficiency of androgen [6]. Recent studies have shown that testosterone therapy increases the relative osteoid volume, the total osteoid surface, the linear extent of bone formation and bone mineralisation in the hypogonadal male [7]. Similar studies on patients with Klinefelter's syndrome have not been reported.

Testosterone replacement is essential therapy for androgen deficiency in patients with Klinefelter's syndrome. Treatment with the currently commonly available methods of androgen replacement (either intramuscular injection of testosterone or oral replacement with testosterone undecanoate) does not mimic the physiological serum concentration and secretory pattern of testosterone production in normal men [8]. Androgen replacement therapy has been shown to increase bone density in men with hypogonadotrophic

hypogonadism. The effects were more marked in men with open epiphyses [9]. The effects of androgen replacement therapy on bone structure in patients with Klinefelter's syndrome have not been studied. In the present study, we measured the total body and regional bone density by dual-energy X-ray absorptiometry in patients with Klinefelter's syndrome on long-term testosterone replacement therapy and in eugonadal age-matched controls to determine whether testosterone replacement therapy reversed the detrimental effects of hypogonadism on bone density.

Materials and Methods

Test Subjects

Fourteen patients with Klinefelter's syndrome ranging in age from 26 to 45 years (mean ± SE, 32.6 ± 1.3) were studied. The diagnosis was based on clinical features, elevated gonadotrophins with low or low-normal testosterone levels and karyotype showing XXY or mosaicism. Testosterone therapy was initiated at the age of 20–38 (26.5 ± 0.5) years. At the time of the study, the patients had already been on testosterone for 24–149 (65.8 ± 11.5) months. Seven of them were receiving oral testosterone undecanoate thrice daily (240 mg/day), while the other 7 were having intramuscular testosterone enanthate injections once every 3 weeks (250 mg/injection).

Fourteen age-matched healthy male volunteers were included as controls in the study. Their body weights were comparable to those of the age-matched patients (Student's paired *t*-test, patients: controls = 66.0 ± 8.4 kg: 64.3 ± 8.3 kg; $p>0.05$). All patients and controls were free from endocrine diseases including diabetes, Cushing's disease and osteomalacia, and had not been taking drugs that affect bone mass, such as anti-epilepsy drugs and glucocorticoids. Written informed consent was obtained from all subjects and controls and the study was approved by the Ethics Committee, University of Hong Kong.

Serum Testosterone Assay

Samples were taken from 7 subjects with serum testosterone levels quantified serially at 0, 2, 4, 6 and 8 h after administration of oral testosterone undecanoate, and 7 other subjects at 0, 1, 7, 14 and 21 days after intramuscular testosterone enanthate injections. The serum was then separated by centrifugation and subsequently stored at −20 °C until analysed.

Serum testosterone level was measured by radioimmunoassay using kits from the WHO Matched Reagent Programme (Special Programme Research in Human Reproduction, World Health Organization, Geneva, Switzerland) as previously described [10]. The intra- and interassay coefficients of variation were 6% and 12% respectively.

Bone Density Determination

Regional and total body bone mineral density (BMD) were measured by the Norland XR-26 bone densitometer (Norland Corp., WI) which is based on the principle of dual-energy X-ray absorptiometry (DXA). The detailed procedures of the measurement have been discussed elsewhere [11,12]. Bone density measurement was done on each subject (both patients and controls) for the total body, lumbar spine (L2–4) and left neck of femur. The duration for a total body measurement was about 20 min, while the durations of spine and femur scans were 9 and 12 min respectively. The precision of BMD measurements, as documented by repeated in vitro measurements on a dedicated step phantom by Kotzi et al. [13], was found to be 99% (coefficient of variation around 1%). Repeated in vivo measurement of total body and lumbar spine BMD valves on 3 individuals resulted in precisions of 98.5% (range 97.8%–99.1% for 5 consecutive measurements) and 99.0% (range 98.5%–99.2% for 5 consecutive measurements) respectively.

Dietary Calcium Assessment

Daily calcium intake was assessed in the study subjects. A series of questions were asked about the frequency with which the subject consumed specific food items each day, each week, each month or each year. Then the subject was asked about the portion sizes that were usually served. Portions were assessed with models of various sizes, and each subject chose the model closest to the portion size that he usually consumed. This method of food frequency interview technique has been validated by Muller et al. [14] and used by the authors in the study of calcium intake [15].

Statistical Analysis

The results of bone mass measurement were expressed as BMD in units of grams per square centimetre. Total body and regional BMD values of patients with Klinefelter's syndrome were compared with those of age-matched normal controls using the Student's *t*-test (SPSSPC plus package, version 3.0, Toronto, Ontario). Linear regression was used to determine the correlation of BMD as well as daily calcium intake with age in this group of subjects.

Results

Serum Testosterone Levels

The mean testosterone levels (normal range in our laboratory 10–40 nmol/l) for patients on oral testosterone undecanoate were (n = 7): 0 h, 14.8 ± 1.5 nmol/l (mean ± SE); 2 h, 19.6 ± 3.2 nmol/l; 4 h, 24.2 ± 5.3

nmol/l; 6 h, 23.0 ± 6.7 nmol/l; 8h, 15.8 ± 2.9 nmol/l. The mean testosterone levels for patients on testosterone enanthate injection once every 3 weeks were (n = 7): day 0, 12.0 ± 1.7 nmol/l; day 1, 26.7 ± 8.2 nmol/l; day 7, 28.9 ± 3.7 nmol/l; day 14, 18.4 ± 0.7 nmol/l; day 21, 14.3 ± 1.1 nmol/l.

Daily Calcium Intakes

The daily calcium intake, as estimated by dietary frequency recall was found to be 661.8 ± 26.6 mg/day in patients with Klinefelter's syndrome and 540.5 ± 8.9 mg/day in the normal controls. The latter finding is comparable to the daily calcium intake for normal healthy males between 20 and 40 years of age estimated recently by the authors (520.1 ± 19.8 mg/day) [15]. Insignificant linear correlation was found between the daily calcium intake and age among the patients and controls ($p>0.1$).

Age-Related Change of BMD in Patients with Klinefelter's Syndrome and Controls

Figure 1 shows the age-related change of femoral BMD values in both patients with Klinefelter's syndrome and controls. The BMD of patients was found to decline gradually with age, with the slopes for Ward's triangle, trochanter and femoral neck at −0.01078, −0.00939 and −0.00725 respectively. Similar declines were also observed in the slopes for the control subjects, which were −0.01981, −0.01085 and −0.01409 respectively. Significant linear correlations were obtained between age and the BMD of all three regions (patients: femoral neck $r = -0.38$ $p<0.05$, trochanter $r = 0.53$ $p<0.005$, Ward's triangle $r = 0.44$ $p<0.01$; controls: femoral neck $r = -0.42$ $p<0.05$, trochanter $r = -0.47$ $p<0.005$, Ward's triangle $r = -0.50$ $p<0.01$).

Higher BMDs of the femur were observed in the controls than in patients with Klinefelter's syndrome, and the difference tended to decrease in older ages. No correlation was obtained between BMD values of femur and the duration of testosterone replacement in this group of subjects ($r = -0.2054$ $p>0.1$). The type of testosterone replacement also has no significant effect on the resultant BMD values of the patients ($p>0.1$).

Comparison with Age-Matched Controls

Figure 2 shows the comparison of mean BMDs of the femur between the controls and patients with Klinefelter's syndrome. Patients on testosterone replacement had significantly decreased BMD values in the femoral neck (mean ± SE 0.835 ± 0.025 g/cm^2) when compared with the age-matched controls (0.923 ± 0.032 g/cm^2, $p<0.05$). Similar decreases were also observed in Ward's triangle (patients:controls = 0.767 ± 0.034:0.932 ± 0.045, $p<0.005$) and the trochanter

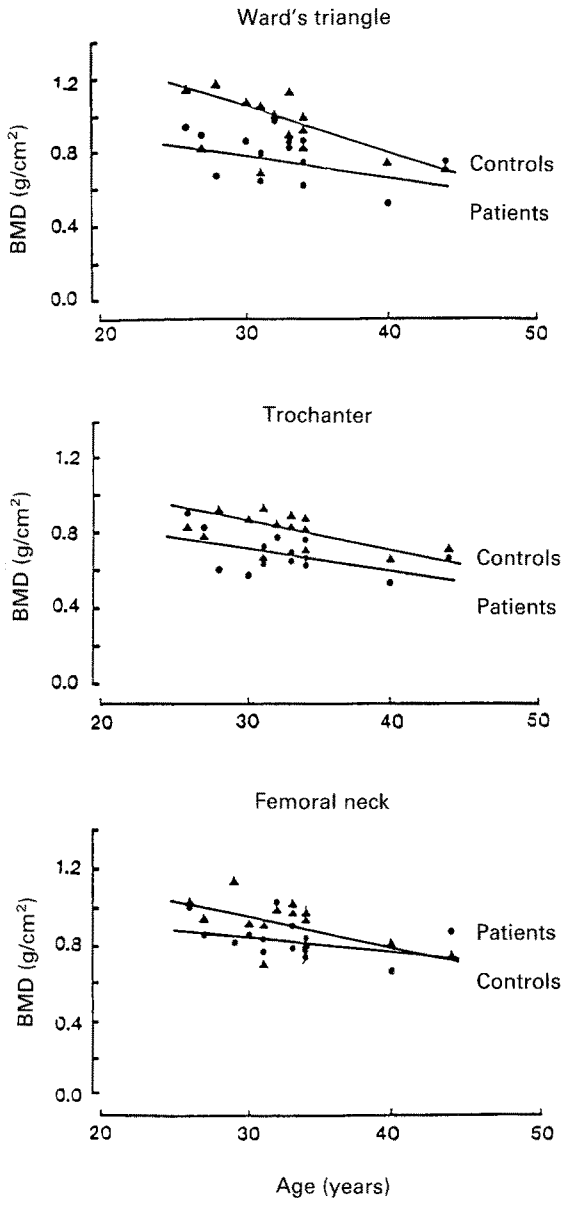

Fig. 1. Age-related change in bone mineral density (BMD) of Ward's triangle, femoral neck and trochanter in patients with Klinefelter's syndrome (*circles*) and normal controls (*triangles*).

(0.695 ± 0.027:0.815 ± 0.025, $p<0.005$). The other regions of bone were not significantly different between this group of subjects and normal controls (Table 1).

Discussion

Premature osteoporosis has been reported in men with hypogonadism associated with hyperprolactinaemia [16], anorexia nervosa [17] and Klinefelter's syndrome [6,7,18,19]. Although testosterone therapy has been shown to be associated with increases in bone formation in men with primary hypogonadism [8,16], the effects of androgen replacement on bone mineral density in patients with Klinefelter's syndrome have not been

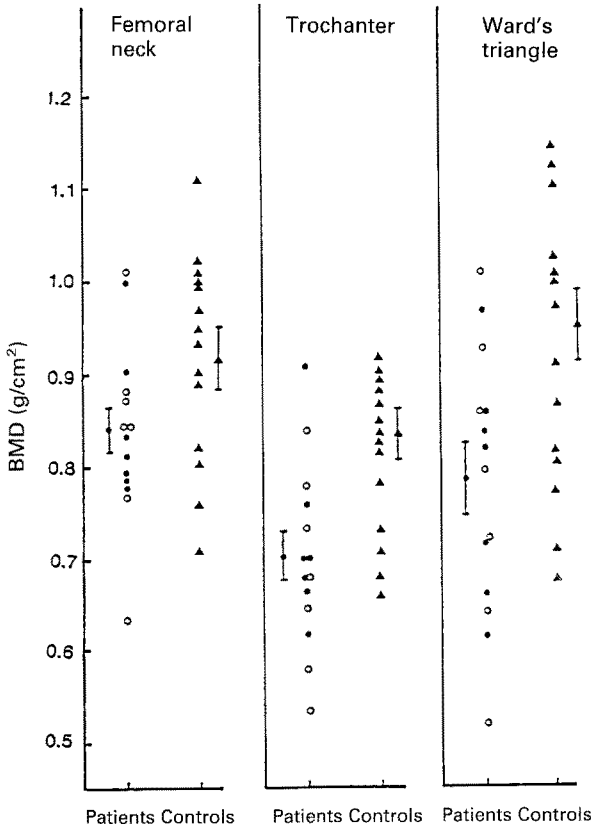

Fig. 2. Age-matched comparison of left femoral BMDs in patients with Klinefelter's syndrome (*filled circles*, oral testosterone undecanoate; *open circles*, testosterone enanthate injection) and normal controls (*triangles*). The bar represents the mean ± SE of each group. There was no significant difference between the BMDs of the group of patients on oral versus parenteral testosterone replacement.

Table 1. Comparison of total body and regional BMD values between patients with Klinefelter's syndrome and age-matched controls

Body regions	Patients	Controls	Significance
Total body	0.75±0.01[a]	0.76±0.02	NS[b]
Head	1.39±0.07	1.37±0.09	NS
Truck	0.43±0.02	0.44±0.02	NS
Left arm	0.72±0.03	0.74±0.02	NS
Right arm	0.73±0.04	0.73±0.01	NS
Pelvis	0.79±0.04	0.84±0.03	NS
Legs	0.88±0.02	0.93±0.02	NS
Lumbar spine (L2–4)	0.93±0.05	0.99±0.03	NS

[a] NS, not significant.
[b] Mean±SE.

studied. When studying 29 men with Klinefelter's syndrome, Smith and Walker [5] were unable to show a correlation between serum testosterone levels and bone density. Foresta et al., however, reported that the patients with Klinefelter's syndrome who had normal testosterone levels, had normal bone structure as did the controls, while in hypotestosteronaemic Klinefelter patients varying degrees of osteoporosis had been observed [6].

Our results indicate that despite the higher daily calcium intakes observed in patients with Klinefelter's syndrome, they exhibited significantly more bone loss in the femoral neck, trochanter and Ward's triangle than did age-matched controls. Despite sufficient testosterone replacement for 24–149 months as evidenced by the clinical response and the serum testosterone profiles after testosterone replacement, the bone density in patients is still significantly lower than in normal controls. This may be related to the fact that the currently and commonly available parenteral and oral testosterone replacement does not provide physiological testosterone concentrations and normal secretion patterns. The use of a long-acting form of the hormone, giving a constant level of testosterone, may be of value. Another possibility is that these patients with Klinefelter's syndrome were diagnosed after pubertal development when epiphyseal bone closure and skeletal maturation had taken place. Androgen replacement was instituted late and after puberty. It may be that inadequate bone development and formation at a critical stage of development, i.e. puberty, may be impaired in these patients so that subsequent therapy with androgen replacement may not be sufficient to restore normal bone mass. Evidence to support this hypothesis is based on the finding of Finkelstein et al. [9] that hypogonadal men with open epiphyses responded better to androgen replacement in terms of increasing trabecular bone density. Their study also showed that androgen replacement was unable to restore bone density to normal. A third possibility is that even longer periods of testosterone replacement may eventually restore the bone density and mass to normal. This is unlikely because the duration of treatment in the present study has no relationship with the bone density.

The fact that with the exception of the femur no region showed significant differences in BMD, may imply that loss of bone occurs mainly in trabecular bone rather than cortical bone. In hypogonadal women, a greater decrease in trabecular than cortical bone density has been observed [20–23]. In men, some investigators have reported that a greater loss of trabecular than cortical bone occurred with age [24], whereas others have not detected such difference [23]. Finkelstein et al. have observed an equally severe osteopenia in both cortical and trabecular bone in patients with idiopathic hypogonadotrophic hypogonadism [9]. The reason for the unequal losses of trabecular and cortical bone in some situations but not in others is still unclear.

In the present study, we also observed a gradual decline of BMD with age in both the patients with Klinefelter's syndrome and normal controls. This can be due to age-related bone loss or, more likely, to the cohort effect of birth year on the estimation of age-related bone loss. In a recent study of age-related bone mineral content in 449 Caucasian women by Recker et al., the increase in average height of 0.079 m among those who were born after the 1960s was found to account for 49.1% of the bone loss over the age span of 20–86 years in cross-sectional study [25]. This indicates

that nearly half of the age-related bone loss is actually due to the fact that people born earlier in this century had smaller skeletons. The recent increase of body size among newborns and adults may be a result of improvements in nutrition and health through environmental, social and medical advances [26].

In conclusion, the BMDs of femur are markedly decreased in patients with Klinefelter's syndrome compared with age-matched normal healthy controls. This finding underscores the fact that sufficient testosterone replacement with currently available methods, as monitored by hormonal assays, does not reverse the decrease in bone mass associated with Klinefelter's syndrome. Treatment with androgens at an earlier age at diagnosis and more physiological methods of androgen replacement may be necessary to prevent osteopenia and future risk of fractures.

Acknowledgements. This study is supported by the UPGC Strategic Research Grant 'Formulation of Strategies and Guidelines for Prevention and Treatment of Osteoporosis in Hong Kong' awarded by the University-Polytechnic Grant Committee.

This paper was presented as abstract at the workshop Conference on Androgen Therapy: Biological and Clinical Consequences, Marco Island, Florida, 17–20 January 1990.

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Received for publication 7 October 1991
Accepted in revised form 18 June 1992