# Bone mineral density and bone markers in hypogonadotropic and hypergonadotropic hypogonadal men after prolonged testosterone treatment

M. De Rosa*, L. Paesano*, V. Nuzzo*, S. Zarrilli*, A. Del Puente**, P. Oriente**, and G. Lupoli*

*Department of Molecular and Clinical Endocrinology and Oncology; **Rheumatologic Unit, School of Medicine, University "Federico II", Naples, Italy

**ABSTRACT. After prolonged treatment (76.4±10 and 70.1±12.3 months, respectively) (mean± SE) with testosterone enanthate (250 mg im every 3 weeks), bone mineral density (BMD) and bone metabolism were evaluated in 12 patients (aged 29.3±1.4 yr) affected by idiopathic hypogonadotropic hypogonadism (IHH), in 8 patients (29.6±2.6 yr) affected by Klinefelter's syndrome (KS), and in 10 healthy men (30.6±1.7 yr) matched according to age and BMI. Spinal BMD in IHH was significantly lower than in controls (0.804±0.04 *vs* 1.080±0.01 g/cm$^2$; $p<0.001$), while there was no difference in neck BMD (0.850±0.01 *vs* 0.948±0.02 g/cm$^2$). Neither spinal (0.978±0.05 g/cm$^2$) nor neck (0.892±0.03 g/cm$^2$) BMD in KS were significantly different from controls. Six IHH and one KS subjects were osteoporotic, while 6 IHH and 2 KS subjects were osteopenic. A significant inverse correlation was found between spinal BMD and age at the treatment onset in IHH ($r$=-0.726, $p$=0.007). In IHH there were significant increases in bone formation (alkaline phosphatase=318.3±33.9 *vs* 205.4±20.0 IU/l; osteocalcin=13.44±1.44 *vs* 8.57±0.94 ng/ml; $p<0.05$) and in bone resorption (urinary cross-linked N-telopeptides of type I collagen=149.1± 32.3 *vs* 47.07±8.4 nmol bone collagen equivalents/mmol creatinine; $p<0.05$) compared to controls, while such differences were not present in KS. Our results outline the importance of BMD evaluation in all hypogonadal males. Nevertheless, bone loss is a minor characteristic of KS, while it is a distinctive feature of IHH. Therefore, early diagnosis and age-related replacement therapy coupled with a specific treatment for osteoporosis could be useful in preventing future severe bone loss and associated skeletal morbidity.**

**(J. Endocrinol. Invest. *24:* 246-252, 2001)**



## INTRODUCTION

Osteoporosis is a well known complication of male hypogonadism (1), particularly of Klinefelter's syndrome (KS), idiopathic hypogonadotropic hypogonadism (IHH), hyperprolactinemia, hemochromatosis and primary testicular failure (2).

Testosterone (T) is certainly the major determinant of bone mass in men. In fact, the presence of androgen receptors on normal human osteoblast-like bone cells (3, 4) has been shown; it has also been demonstrated that T determines a direct stimulation of osteoblast proliferation and the differentiation of bone cells *in vitro* (5). Moreover, in hypogonadal males, T therapy increases the relative osteoid volume, the total osteoid surface, the linear extent of bone formation and bone mineralization (6).

The effects of T deficiency on bone mass are also related to contemporaneous estrogen (E) deficiency, probably due to the reduced amount of T available for peripheral conversion (7, 8). In male hypogonadal osteoporosis, low serum T levels may account for reduced bone formation while low serum E levels for an increase in bone resorption (9).

The aim of this study was to evaluate the bone mass and metabolism in both hypogonadotropic and hypergonadotropic hypogonadal males after prolonged T therapy.

*Key-words:* Bone mineral density, bone turnover, bone markers, osteoporosis, osteopenia, hypogonadism, Klinefelter's syndrome, testosterone therapy.

*Correspondence:* Dr. Michele De Rosa, Via Belsito 19, 80123 Napoli, Italy.

*E-mail:* miderosa@unina.it

Accepted September 4, 2000.

## PATIENTS AND METHODS

### *Patients*

Twenty male patients were studied. They were selected from a pool of patients who had been in our Clinical Hospital Department over the previous 10 years. Twelve patients (aged 23-37 yr) were affected by IHH diagnosed by a history of absence of any pubertal development beyond 18 yr of age, basal T levels <1 ng/ml, normal pituitary RMN, reduced testicular volume to prepubertal levels, normal other pituitary hormones and low serum levels of gonadotropins (Table 1). None of them had anosmia, but 2 showed various degrees of hyposmia.

Eight patients (aged 19-41 yr) were affected by KS diagnosed on the basis of increased gonadotropins, low T serum levels and a 47,XXY karyotype (Table 1).

All patients had been treated with T enanthate, 250 mg administered im every 3 weeks for a variable period (Table 1). The IHH patients did not use gonadotropin treatment because they did not want to induce a pregnancy in their spouses. T levels had been within the normal adult range for at least 3 years. All patients were free of other significant medical illnesses and none used medications known to affect pituitary or gonadal function or bone metabolism. None of them was an alcohol consumer. All patients had a normal calcium intake (800-1200 mg/day) and normal physical activity. Twelve of them (7 IHH and 5 KS) smoked cigarettes, with a range of consumption between 5 and 10 per day.

As controls we used 10 healthy males (aged 22-40 yr) matched according to age and BMI. Their spouses had conceived in the 6-month period preceding this study. They were selected on the basis of the following criteria: normal pubertal development, no disorders or use of medications known to affect bone and mineral metabolism, normal serum calcium (Ca), phosphate (P), creatinine , alkaline phosphatase (ALP) and T concentrations (Table 1). None of the patients or controls had a history of bone fracture.

All subjects gave their informed consent to be enrolled in the study.

### *Sex hormones and biochemical measurements*

To test the effectiveness of T replacement, blood samples were taken every day for 3 days during the second week after the injection.

Serum levels of FSH [normal range (NR)=0.7-11.1 mIU/ml], LH (NR=0.8-7.6 mIU/ml), PRL (NR=2.5-17 ng/ml), E2 (NR=20-56 pg/ml), total T (NR=2.7-17.3 ng/ml), DHEAS (NR=80-560 µg/dL), intact PTH (NR=12-72 pg/ml) were determined by chemiluminescent immunometric assays using commercial available kits for the IMMULITE Automated Analyzer [Diagnostic Products Corporation (DPC), Medical System, Genova, Italy]. Serum levels of $\Delta_4$ androstenedione ($\Delta_4$ A) (Active Androstenedione Diagnostic Systems Laboratories, Inc.) (NR=1.0-3.5 ng/ml), 17 OH-progesterone (17 OH-P) (Active 17 α-OH-progesterone DSL-5000, Diagnostic Systems Laboratories, Inc.) (NR=0.6-3.3 ng/ml), CT (Double Antibody Calcitonin, DPC, Medical System, Genova) (NR=0-50 pg/ml) and osteocalcin (OC) (Osteocalcina Myria, TechnoGenetics-Bouty) (NR=5-18 ng/ml) were measured by radioimmunoassay using commercial available kits. Intra- and interassay coefficients of variation were, respectively, for FSH 5.4% and 8.1%, for LH 4.8% and 10.6%, for PRL 6.8% and 9.6%, for E2 15% and 16%, for T 13% and 16%, for DHEAS 7.6% and 15%, for PTH 5.4% and 5.0%, for $\Delta_4$ A 3.2% and 4.1%, for 17 OH-P 9.3% and 9.7%, for CT 8% and 10%, for OC 4.0% and 5.1%.

Serum Ca (Calcium Biolab, Italy) (NR=8.8-10.2 mg/dl), P (Inorganic phosphorus determination, Seradyn, Italy) (NR=2.5-4.8 mg/dl) and ALP (Alkaline Phosphatase Biolab, Italy) (NR=98-275 IU/l) were tested with a colorimetric method.

Urinary cross-linked N-telopeptides of type I collagen (Ntx) levels were measured by enzyme-linked immunosorbent assay (Osteomark, Ostex International, Seattle) and corrected because of the concurrent excretion of creatinine, determined by routine colorimetric methods [NR=23-110 nmol bone collagen equivalents (BCE)/mmol creatinine (Cr)]; the intra- and interassay coefficients of variation were 5% and 7%, respectively.

Table 1 - *Clinical features (mean±SE) of 12 patients affected by idiopathic hypogonadotropic hypogonadism (IHH), 8 patients affected by Klinefelter's syndrome (KS) and 10 normal healthy males (NC).*

| | Age (yr) | Height (cm) | Weight (kg) | BMI (kg/m$^2$) | Treatment (months) | Treatment (start age) |
|---|---|---|---|---|---|---|
| IHH | 29.3±1.4 | 173.7±1.3 | 77.2±2.9 | 25.7±1.2 | 76.4±10.0 | 21.2±0.7 |
| KS | 29.6±2.6 | 180.1±1.2 | 82.3±3.3 | 25.3±0.9 | 70.1±12.3 | 21.6±2.2 |
| NC | 30.6±1.7 | 173.4±1.7 | 73.7±2.0 | 24.5±0.6 | - | - |

*Bone mineral density*

Radiographs were made of the hand and wrist of all patients and controls to assess bone age to be compared with chronological age. They all showed to have fused epiphyses. The method of Tanner and Whitehouse was used.

BMD, at lumbar spine (L1-L4) and right proximal femur (neck) levels, was assessed by dual-energy X-ray absorptiometry (DEXA), using a Hologic QDR 1000 densitometer (Hologic Inc., Waltham, Mass., USA). Data were expressed in $gcm^2$.

For the evaluation of the BMD data, the international reference pooled sample age-sex matched provided by the densitometer manufacturer (10) was used as young adult reference range because it did not differ significantly from the data obtained from a local sample in a previous study performed when the machine was being set (unpublished data). Individual values of vertebral and femoral BMD in our patients and controls were also expressed as Z-scores and T-scores, calculated on the basis of this reference range. In line with previous reports, patients were considered osteopenic when the T-score was between –1 and –2.5 standard deviation (SD) and were considered osteoporotic when the T-score was lower than –2.5 SD. Ninety-five% of the confidence interval (95% CI) of the Z-score was compared with the Z-score mean value of subjects of the same sex and age. The difference is considered statistically significant if the 95% CI does not include zero.

All scans were analysed by the same operator, who was blind with respect to the pathological (or not) status of subjects.

*Statistical analysis*

The Newman-Keuls test was used to compare BMD, hormonal patterns and biochemical markers in patients and control subjects. Linear regression analysis was used to determine the correlation between BMD and characteristics of hormonal treatment. The significance level was set at $p<0.05$. In particular, the analysis was performed by comparing the control group (10 subjects) first with the IHH group (12 patients) and then with the KS group (8 patients). Lastly, the KS group was compared with the IHH group.

## RESULTS

*Hormones and biochemical measurements*

Serum FSH and LH levels in IHH were lower than normal adult range and significantly lower than in KS and controls ($p<0.001$); on the contrary, gonadotropins levels in KS were above the normal range and significantly higher than controls (for FSH $p<0.001$, for LH $p<0.01$) (Table 2). Although all the patients had received a substitutive treatment and serum T levels were maintained within the normal range, both groups of patients showed T to be significantly lower than controls ($p<0.001$) (Table 2). PRL, E2, 17 OH-P, $\Delta_4$ A, DHEAS were within the normal range and there was no significant difference between the two groups of patients and the controls (Table 2).

In the IHH group, serum ALP, OC and urinary Ntx levels were significantly higher than controls ($p<0.05$). However, no significant difference in such levels was found either between the two groups of hypogonadal patients or between the KS subjects and the controls (Table 3). Serum levels of intact PTH, CT, Ca and P in the patients did not differ from those in the controls (Table 3).

*Bone mineral density*

Lumbar spine BMD in IHH was significantly lower than controls ($p<0.001$), while femoral neck in IHH and both sites BMD in KS did not significantly differ from controls. Moreover, IHH showed a lumbar BMD significantly lower than KS ($p<0.05$), while no significant difference was found in femoral neck BMD between hypo- and hypergonadotropic hypogonadal patients (Table 4, Fig. 1).

In addition, in order to compare our data with the international pooled sample, we calculated for each patient the 95% CI of the BMD Z-score. Data sho-

Table 2 - *Hormonal patterns (mean±SE) in 12 patients affected by idiopathic hypogonadotropic hypogonadism (IHH), 8 patients affected by Klinefelter's syndrome (KS) and 10 normal healthy males (NC).*

| | FSH (mIU/ml) | LH (mIU/ml) | PRL (ng/ml) | T before therapy (ng/ml) | T (ng/ml) | E2 (pg/ml) | 17 OH-P (ng/ml) | $\Delta_4$ A (ng/ml) | DHEAS (µg/dl) |
|---|---|---|---|---|---|---|---|---|---|
| IHH | 0.3±0.05*° | 0.45±0.07*° | 6.7±0.5 | 0.6±0.1*° | 4.0±0.1* | 28.6±4.0 | 1.4±0.14 | 2.5±0.24 | 261±27.6 |
| KS | 28.7±3.3* | 18.0±4.8** | 8.5±0.4 | 2.6±0.4** | 4.0±0.2* | 33.6±3.1 | 2.1±0.32 | 2.4±0.29 | 246±18.9 |
| NC | 4.7±0.5 | 3.3±0.3 | 7.9±0.6 | - | 6.3±0.4 | 28.0±3.7 | 1.4±0.17 | 2.1±0.25 | 253.4±32 |

*$p<0.001$ *vs* NC; **$p<0.01$ *vs* NC; °$p<0.001$ *vs* KS. 17 OH-P: 17 OH-progesterone; $\Delta_4$ A: $\Delta_4$ androstenedione.

Table 3 - *Markers of bone metabolism (mean±SE) in 12 patients affected by idiopathic hypogonadotropic hypogonadism (IHH), 8 patients affected by Klinefelter's syndrome (KS) and 10 normal healthy males (NC).*

| | Ca (mg/dl) | P (mg/dl) | ALP (IU/l) | PTH (pg/ml) | CT (pg/ml) | OC (ng/ml) | NTx (nmol BCE/mmol Cr) |
|---|---|---|---|---|---|---|---|
| IHH | 9.6±0.1 | 3.9±0.1 | 318.3±33.9* | 47.4±3.6 | 31.0±2.7 | 13.44±1.44* | 149.1±32.3* |
| KS | 9.7±0.1 | 3.6±0.1 | 243.2±27.1 | 43.5±5.0 | 29.8±2.8 | 10.82±1.25 | 80.9±25.7 |
| NC | 9.8±0.1 | 3.8±0.1 | 205.4±20.0 | 46.4±4.1 | 27.8±2.7 | 8.57±0.94 | 47.07±8.4 |

*$p<0.05$ *vs* NC. ALP: alkaline phosphatase; Ca: calcium; NTx: cross-linked N-telopeptides of type I collagen; OC: osteocalcium; P: phosphate.

wed that mean lumbar and femoral BMD in hypogonadotropic hypogonadal patients were significantly lower in comparison with the reference population matched according to age and sex. Neck BMD in hypergonadotropic hypogonadal patients was not significantly different from the reference mean value, while lumbar BMD was slightly lower (Table 5).

Evaluating T-scores, osteoporosis and/or osteopenia were diagnosed in all IHH subjects and in 3 out of 8 KS subjects, whereas 5 KS patients had normal BMD. In particular, at the lumbar spine level 6 IHH and 1 KS patients were osteoporotic, while 6 IHH and 2 KS subjects were osteopenic. At the femoral level, no IHH or KS patient was osteoporotic, while 3 IHH and 2 KS patients were osteopenic.

A significant inverse correlation was found only in IHH patients between L1-L4 BMD and the age at T replacement treatment onset ($r$=-0.726; $p$=0.007) (Fig. 2).

## DISCUSSION

Severe osteopenia showed in patients with isolated GnRH deficiency and hormonal treatment did not normalize BMD, despite the fact that serum T levels were maintained within the male reference range for at least 2 years (11). Probably bone improvement is related to the age at the treatment onset (12), thus confirming that there is a critical period of skeletal response to sex hormones (13). Adequate bone development and formation during puberty might be impaired so that subsequent androgen therapy may not be able to restore normal bone mass (14). Moreover, the currently used androgen replacement therapy does not mimic the physiological serum concentration and secretory pattern of T production in normal men (15). Even though serum T levels are within the normal range, they are not able to maintain normal bone metabolism (13). Behre *et al.*, on the contrary, demonstrated that in hypogonadal men, before treatment, a significant association of BMD with T levels and age was present, while there was no relation between age at treatment onset and the increase in BMD during T treatment (16). Guo *et al.* did not find a correlation between the BMD and the duration of treatment, probably because the response of bone formation to T is dependent on dose, length of course and serum E levels (13). In particular, no significant association was detected between the duration of a therapy lasting more than 1 year and BMD. These observations have led us to hypothesize that prolonged androgen treatment maintains the BMD achieved during the initial period of the therapy, without any further significant increase (16).

In our IHH patients, BMD is lower than in controls and age-matched reference range, above all at lumbar spine level, in spite of prolonged T treatment for a mean of 76.4 months and serum T levels being in the normal range. On the contrary, after the same therapy for a mean of 70.1 months, KS patients had a BMD not significantly different from

Table 4 - *Bone mineral density (BMD, mean±SE) in 12 patients affected by idiopathic hypogonadotropic hypogonadism (IHH), 8 patients affected by Klinefelter's syndrome (KS) and 10 normal healthy males (NC).*

| | BMD L1-L4 ($g/cm^2$) | Z-score | T-score | BMD neck ($g/cm^2$) | Z-score | T-score |
|---|---|---|---|---|---|---|
| IHH | 0.804±0.04*,** | -2.59±0.34 | -2.60±0.35 | 0.850±0.01 | -0.81±0.11 | -0.77±0.11 |
| KS | 0.978±0.05 | -0.97±0.41 | -1.02±0.42 | 0.892±0.03 | -0.41±0.37 | -0.39±0.31 |
| NC | 1.080±0.01 | -0.06±0.09 | -0.07±0.09 | 0.948±0.02 | +0.14±0.25 | +0.12±0.22 |

*$p<0.001$ *vs* controls; **$p<0.05$ *vs* KS. BMD L1-L4: BMD at lumbar spine; BMD neck: BMD at femoral neck.

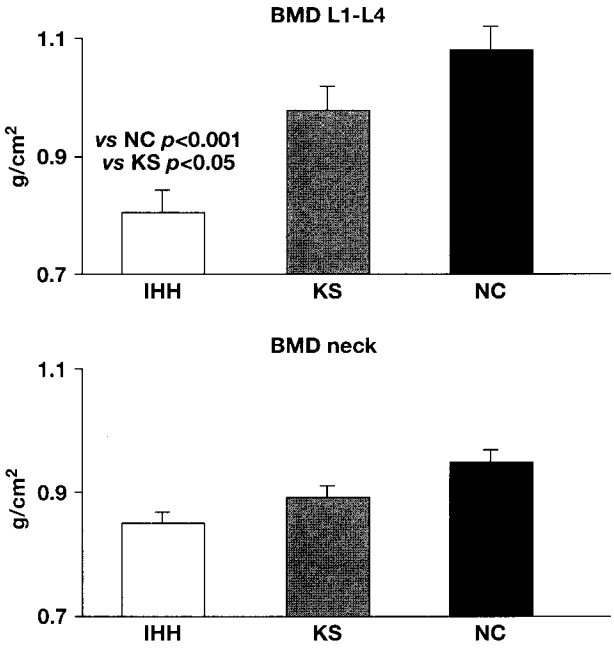


Fig. 1 - *Comparison between mean bone mineral density (g/cm²) at lumbar spine (BMD L1-L4) and at femoral neck (BMD neck) in 12 patients affected by idiopathic hypogonadotropic hypogonadism (IHH), 8 patients affected by Klinefelter's syndrome (KS), both after prolonged testosterone treatment, and 10 normal controls (NC).*

controls. As regards bone metabolism, the biochemical markers (ALP, OC and Ntx) are high in the IHH suggesting that T treatment stimulates bone formation, but it is not able to suppress increased bone resorption; thus all the IHH patients are affected by osteoporosis or osteopenia despite hormonal therapy. It has been reported that T replacement significantly reduces bone remodelling and suppresses bone resorption (17) and it increases bone mineralization and density (18), although the increase seems to be only a feature of patients with open epiphyses (19).

In our patients the diagnosis was made after pubertal development, when skeletal maturation had already taken place. Androgen replacement was probably started too late and therefore it was not successful in restoring normal bone mass. Moreover, a significant inverse correlation has been found between lumbar BMD of IHH patients and the age at treatment onset. Probably, hormonal therapy before or during puberty is more successful in preventing bone loss. BMD increases during gonadal steroid replacement, particularly in men who are skeletally immature, but despite these increases, bone density does not reach the normal levels (19). In our opinion, T treatment cannot restore the pubertal muscular and skeletal growth, but it can only modify the post-pubertal bone remodelling. In fact, bone growth is complete by the mid-twenties and peak bone density is reached by the third decade in men with a history of constitutionally delayed puberty. In these conditions, their BMD could not improve or even normalize over time, suggesting that the timing of puberty is an important determinant of bone mass peak (20). It is unknown whether, in hypogonadal men, early administration of androgens would lead to normal bone density and to a reduction in the risk of subsequent osteoporosis (18). It was also demonstrated that androgen replacement, before puberty, leads to increase both trabecular and cortical bone while, after puberty, only cortical bone (21), suggesting a skeletal site-specific effect. Our results suggest that the hormonal therapy is not able to restore bone mass in IHH patients, therefore a specific therapy for osteoporosis could be useful in association with T replacement.

As regards KS patients, BMD and bone metabolism did not show any significant difference with controls, according to Luisetto *et al.* Probably, KS patients are not affected by osteoporosis because their hypogonadism is only minor (22). In fact, it has been reported that KS subjects with normal T levels have normal bone structure, while KS subjects with low T levels have variable degrees of osteoporosis (23). Horowitz *et al.* found a lower mean forearm mineral density and no significant difference was found between those patients who had been pre-

Table 5 - *Bone mineral density Z-scores (mean±SE) and 95% of their confidence interval (95% CI) in 12 patients affected by idiopathic hypogonadotropic hypogonadism, and 8 patients affected by Klinefelter's syndrome.*

| | Idiopathic hypogonadotropic hypogonadism | | Klinefelter's syndrome | |
|---|---|---|---|---|
| | Z-score | 95% CI | Z-score | 95% CI |
| Lumbar spine | -2.59±0.34 | -3.30/-1.88* | -0.97±0.41 | -1.85/-0.09* |
| Femoral neck | -0.81±0.11 | -1.04/-0.58* | -0.41±0.37 | -1.20/+0.38 |

*significantly different from the international reference mean value.

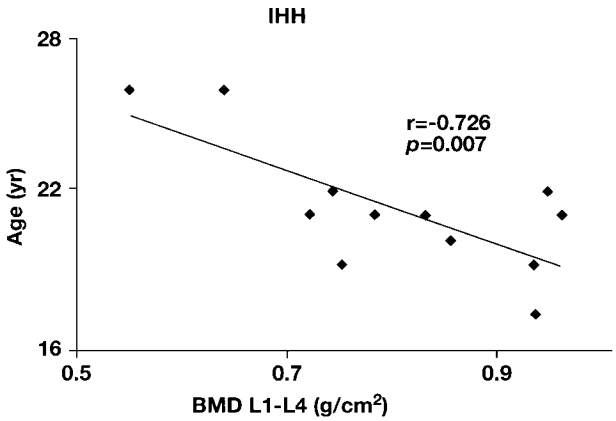


Fig. 2 - *Correlation between bone mineral density at lumbar spine (BMD L1-L4) and age at the beginning of prolonged testosterone treatment in 12 patients affected by idiopathic hypogonadotropic hypogonadism (IHH).*

viously treated with T and those who had not; thus demonstrating that decreased BMD occurs only in about 25% of KS patients (9). It has also been observed that, in patients with a left proximal femur BMD which is markedly lower than in normal controls, sufficient T replacement does not restore the decreased bone mass (14). Nevertheless, T replacement significantly increases BMD at all skeletal sites (24). In our KS patients, the BMD and the bone markers did not significantly differ from controls, suggesting that bone loss affects only some patients and also that it is usually a minor occurrence that can probably be reversed by androgen therapy.

Differences in bone metabolism and mass between the two kinds of male hypogonadism may be related to the pubertal development of these patients. In fact, the timing of puberty in KS patients is less impaired than in IHH, resulting in a major and better bone growth.

In conclusion, T replacement therapy should be started during puberty to obtain a normal skeletal development, above all in IHH patients. However, also in boys with delayed puberty an earlier beginning of T treatment could be useful. In fact, T treatment at low doses in relation to age could increase bone formation as it does in normal subjects.

Our results outline the importance of an evaluation of bone density in all hypogonadal males, because osteoporosis is a clear feature of hypogonadism: an early diagnosis of hypogonadism and replacement therapy are necessary to increase their peak bone density and prevent severe bone loss and associated skeletal morbidity. Nevertheless, the therapeutic approach to low bone mass should not be limited to testosterone treatment: regular clinical checks and a specific treatment for osteoporosis might be necessary, besides hormonal therapy.

## REFERENCES


1. Seeman E., Melton L.J., O'Fallon W.M., Riggs B.L. Risk factors for spinal osteoporosis in men. Am. J. Med. 1983, *75:* 977-983.
2. Jackson J.A., Kleerekoper M. Osteoporosis in men: diagnosis, pathophysiology, and prevention. Medicine 1990, *69:* 137-152.
3. Colvard D.S., Erikson E.F., Keeting P.E., Wilson E.M., Lubahn D.B., French F.S., Riggs B.L., Spelsberg T.C. Identification of androgen receptors in normal human osteoblast-like cells. Proc. Natl. Acad. Sci. USA 1989, *86:* 854-857.
4. Orwoll E.S., Stribrska L., Ramsey E.E., Keenan E.J. Androgen receptors in osteoblast-like cell lines. Calcif. Tissue Int. 1991, *49:* 183-187.
5. Kasperk C.H., Wergedal J.E., Farkey J.R., Linkhart T.A., Turner R.T., Baylink D.J. Androgens directly stimulate proliferation of bone cells in vitro. Endocrinology 1989, *124:* 1576-1578.
6. Baran D.T., Bergfeld M.A., Teitelbaum S.L., Avioli L.V. Effect of testosterone therapy on bone formation in an osteoporotic hypogonadal male. Calcif. Tissue Res. 1978, *26:* 103-106.
7. Crilly R.G., Francis R.M., Nordin B.E.C. Steroid hormones, ageing and bone. J. Clin. Endocrinol. Metab. 1989, *10:* 115-139.
8. Smith E.P., Boyd J., Frank G.R., Takahashi H., Cohen R.M., Specker B., Williams T.C., Lubahn D.B., Korach K.S. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N. Engl. J. Med. 1994, *331:* 1056-1061.
9. Horowitz M., Wishart J.M., O'Loughlin P.D., Morris H.A., Need A.G., Nordin B.E.C. Osteoporosis and Klinefelter's syndrome. Clin. Endocrinol. (Oxf.) 1992, *36:* 113-118.
10. Favus M.J. Bone density reference data. In: Favus M.J. (Ed.), Primer on the metabolic bone diseases and disorders of mineral metabolism, ed 2. Raven Press, New York, 1993, p. 426.
11. Finkelstein J.S., Klibanski A., Neer R.M., Greenspan S.L., Rosenthal D.I., Crowley W.F. Osteoporosis in men with idiopathic hypogonadotropic hypogonadism. Ann. Intern. Med. 1987, *106:* 354-361.

12. Canale D., Vignali E., Golia F., Martino E., Pinchera A., Marcocci C.
Effects of hormonal replacement treatment on bone mineral density and metabolism in hypogonadal patients.
Mol. Cell. Endocrinol. 2000, *161:* 47-51.

13. Guo C.Y., Jones T.H., Eastell R.
Treatment of isolated hypogonadotropic hypogonadism effect on bone mineral density and bone turnover.
J. Clin. Endocrinol. Metab. 1997, *82:* 658-665.

14. Wong H.W., Pun K.K., Wang C.
Loss of bone mass in patients with Klinefelter's syndrome despite sufficient testosterone replacement.
Osteoporos. Int. 1993, *3:* 3-7.

15. Cantrill J.A., Denis P., Large D.M., Neuman M., Anderson D.C.
Which testosterone replacement therapy.
Clin. Endocrinol. (Oxf.) 1984, *21:* 97-107.

16. Behre H.M., Kliesch S., Leifke E., Link T.M., Nieschlag E.
Long-term effect of testosterone therapy on bone mineral density in hypogonadal men.
J. Clin. Endocrinol. Metab. 1997, *82:* 2386-2390.

17. Katznelson L., Finkelstein J.S., Schoenfeld D.A., Rosenthal D.I., Anderson E.J., Klibanski A.
Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism.
J. Clin. Endocrinol. Metab. 1996, *81:* 4358-4365.

18. Scane A.C., Francis R.M.
Risk factors for osteoporosis in men.
Clin. Endocrinol. (Oxf.) 1993, *38:* 15-16.

19. Finkelstein J.S., Klibanski A., Neer R.M., Doppelt S.H., Rosenthal D.I., Segre G.V., Crowley W.F.
Increases in bone density during treatment of men with idiopathic hypogonadotropic hypogonadism.
J. Clin. Endocrinol. Metab. 1989, *69:* 776-783.

20. Finkelstein J.S., Klibanski A., Neer R.M.
A longitudinal evaluation of bone mineral density in adult men with histories of delayed puberty.
J. Clin. Endocrinol. Metab. 1996, *81:* 1152-1155.

21. Bardin C.W., Swerdloff R.S., Santen R.J.
Androgens: risks and benefits.
J. Clin. Endocrinol. Metab. 1991, *73:* 4-7.

22. Luisetto G., Mastrogiacomo I., Bonanni G., Pozzan G., Botteon S., Tizian L., Galuppo P.
Bone mass and mineral metabolism in Klinefelter's syndrome.
Osteoporos. Int. 1995, *5:* 455-461.

23. Foresta C., Ruzza G., Mioni R., Meneghello A., Baccichetti C.
Testosterone and bone loss in Klinefelter's syndrome.
Horm. Metab. Res. 1983, *15:* 56-57.

24. Choi H.R., Lim S.K., Lee M.S.
Site-specific effect of testosterone on bone mineral density in male hypogonadism.
J. Korean Med. Sci. 1995, *10:* 431-435.