Clinical Endocrinology (2003) **58**, 691–695

Bone mineral density outcomes following long-term treatment with subcutaneous testosterone pellet implants in male hypogonadism

Margaret R. Zacharin*†, Joseph Pua* and Shankar Kanumakala*

**Department of Endocrinology & Diabetes, Royal Children's Hospital, Melbourne, Australia and*
†Department of Endocrinology, St Vincent's Hospital, Melbourne, Australia

(Received 10 April 2002; returned for revision 18 September 2002; finally revised 14 October 2002; accepted 19 November 2002)

Summary

BACKGROUND Osteoporosis is a common complication of untreated male hypogonadism. Even mild hypogonadism due to suboptimal testosterone replacement may result in decreased bone mineralization and osteoporosis.

OBJECTIVE To assess bone mineral density in hypogonadal men following long-term long-acting subcutaneous testosterone pellet implants as replacement therapy.

PATIENTS A cross-sectional study of 37 patients with primary or secondary hypogonadism receiving long-term (mean 6·6 years) subcutaneous testosterone pellet implants as replacement therapy.

MEASUREMENTS Bone mineral density was measured in all patients using dual energy X-ray absorptiometry. Serum testosterone 3–4 months after insertion of pellets was measured in all patients to assess adequacy of replacement therapy.

RESULTS Mean areal bone mineral density were 1·02 (SD 0·14) g/cm^2 with a mean Z score of –0·64 (SD 1·3) and 0·87 (SD 0·13) g/cm^2 with a mean Z score of –0·72 (SD 1·2) at lumbar spine and neck of femur, respectively. Mean serum testosterone 3–4 months after pellets insertion was 15·45 nmol/l (SD 4·2 nmol/l). There was no significant correlation between bone mineral density and patient's age at start or duration of testosterone therapy.

CONCLUSIONS Bone mineral density in long-term regularly treated hypogonadal men was not different from the age-matched reference range for normal men. Long-acting subcutaneous testosterone pellet implants as replacement therapy in male hypogonadism are safe, acceptable to the patient, result in adequate bone mass accumulation and maintenance of normal bone mineral density. By provision of sustained physiological levels of testosterone they may contribute to increased androgen effect at the receptor level.

Bone mass in adulthood is determined by peak bone accumulation achieved during growth and puberty and subsequent rate of bone loss (Glastre *et al*., 1990; Lu *et al*., 1992). A progressive increase in areal bone density but not volume, occurs throughout childhood, with a marked increase in bone mineralization during puberty (Rubin *et al*., 1993). Increases in serum androgens during puberty allow for skeletal maturation and attainment of peak bone mass, and the persistence of normal testosterone (T) secretion in adulthood is important for maintenance of bone density (Katznelson, 1998). In patients with hypogonadism due to T deficiency, decreased bone mass accumulation with heightened bone turnover lead to premature osteopaenia or osteoporosis (Medras *et al*., 2000; Snyder *et al*., 2000). In addition, patients with inadequately treated or untreated hypogonadism continue to have an increased rate of bone loss (Daniell *et al*., 2000).

A variety of T preparations such as oral, intramuscular, sublingual, *trans*-dermal and *trans*-scrotal patches have been used for replacement treatment of male hypogonadism (Arisaka *et al*., 1995; Leifke *et al*., 1998; Behre *et al*., 1999; Francis, 1999; Snyder *et al*., 2000; De Rosa *et al*., 2001). The choice of T preparation used, the duration of treatment and the different techniques used for measurement of bone mineral density (BMD) may explain the varying effects of T replacement therapy on BMD seen in previous studies.

There are limited published clinical data regarding the long-term use of subcutaneous T pellet implants and their efficacy in maintainance of bone mass and prevention of bone loss in hypogonadal men. The primary objective of this study was to quantify the adult bone mass in a group of hypogonadal men, all of whom received subcutaneous pellet implants as T replacement therapy. We also aimed to assess if there was any association between

Correspondence: Margaret Zacharin Department of Endocrinology & Diabetes, Royal Children's Hospital, Parkville 3052, Australia.
Tel.: +61-3-9345 5951; Fax: +61-3-9347 7763;
E-mail: margaret.zacharin@rch.org.au

BMD and clinical parameters such as patient's age at start or duration of subcutaneous T implant treatment.

Patients and methods

All male patients with primary or secondary hypogonadism attending the Endocrine Clinic at St Vincent's Hospital, Melbourne and who received subcutaneous T implants every 6 months were invited to participate in this study. Twenty-one patients with primary hypogonadism (12 with anorchia, seven with Klinefelter's syndrome and two with Noonan's syndrome) and 16 patients with secondary hypogonadism (eight with Kallman's syndrome and eight with hypopituitarism) participated in the study. Patient characteristics are summarized in Table 1. Only those patients who had hypopituitarism were receiving any medications other than testosterone. This group had all received complete pituitary hormone replacement and all had been treated with GH until bone age > 15·5 years. The oldest hypopituitary patient was 30 years at the time of this study and had finished GH at 18 years as it is not available for adult use in Australia. In particular, no patient had received anticonvulsant treatment, which might interfere with vitamin D metabolism. Each patient received 600–800 mg (8–10 mg/kg) of subcutaneous T pellet implants every 6 months. All patients had been diagnosed with hypogonadism either at the time of presentation with delayed puberty (Kallman's, Klinefelter's) or concurrently with their primary diagnosis (hypopituitarism, Noonan's, anorchia, Klinefelter's). Those in whom absence of puberty was anticipated were commenced on oral androgen at age 13–13·5 years. Boys presenting with pubertal delay started at an older age (15–18 years). All had received oral T (as testosterone undecanoate) and had later introduction of intramuscular T for further pubertal progress, with increasing doses over 2–3 years, until adult doses were reached. They were then changed to subcutaneous T pellet implants. The implants were inserted using a trochar and cannula, into the buttock using a previously described method (Handelsman *et al*., 1997; Zacharin & Warne, 1997). Serum T was analysed in all patients 3–4 months after the insertion of the implant using a chemiluminascent immunoassay for the Centaur automated analyser (Bayer Diagnostics, Victoria, Australia).

All participating patients had BMD measured at the time of clinic attendance. The same operator performed all BMD measurements at lumbar spine and neck of femur, using dual energy X-ray absorptiometry (DEXA), Hologic QDR 2000 densitometer (Picker Australia Pty Ltd) with 1% precision at spine and 2% precison at hip. The patient values were compared with an age- and gender-matched white population (reference ranges provided by the Hologic QDR 2000 and 4500 operators' manual).

Statistical analyses

The BMD and Z score values between patients with hypogonadism and reference values established for normal men were compared using Student's *t*-test. Significant differences between the means of different patient groups could not be determined due to the limited number of patients in each group and low statistical power for ANOVA. Regression analysis was performed to determine if there was any association between BMD and clinical parameters such as age of start and duration of subcutaneous T pellet implant therapy. Pearson's correlation coefficient was used to determine any association between BMD and patient's height and weight.

Results

All patients tolerated the subcutaneous T implants well without any adverse events. The mean T level measured 3–4 months after the implant insertion was 15·45 nmol/l (SD 4·2 nmol/l), which is within the normal range (9·0–35·0 nmol/l) for an adult male.

The BMD results of all patients are summarized in Table 2. The mean age of the patients at the time of measuring their BMD was 25·4 years. They had received T pellet implants for a mean 6·6 years (range 4–10 years). Mean areal BMD at lumbar spine was 1·02 g/cm^2, which was an average 93·6% of normal adult reference range. Mean areal BMD at neck of femur was 0·87 g/cm^2, which was an average 91·2% of normal adult reference range. These measurements correspond to a mean Z score of –0·64 and –0·72 at lumbar spine and neck of femur, respectively. Patients' BMD measurements at either lumbar spine ($P = 0·06$)

Table 1 Summary of patient clinical parameters by diagnostic groups

Clinical parameters	Primary hypogonadsim ($n = 21$)	Secondary hypogonadism ($n = 16$)	All patients ($n = 37$)
Age (years) at time of BMD	26·0 (7·0)	24·8 (4·3)	25·4 (5·9)
Weight (kg)	66·3 (10·8)	54·6 (6·9)	63·4 (9·8)
Height (cm)	177·2 (11·9)	174·5 (7·4)	176·0 (10·2)
Age at start of treatment (years)	18·6 (5·5)	17·6 (2·8)	18·2 (4·9)
Duration of treatment (years)	7·1 (2·2)	6·4 (2·6)	6·6 (2·4)

All data expressed as mean (SD).

Table 2 Summary of bone mineral density measurements by patient diagnostic groups

BMD measurements	Primary hypogonadism ($n = 21$)	Secondary hypogonadism ($n = 16$)	All patients ($n = 37$)
At lumbar spine			
BMD (g/cm^2)	1·01 (0·03)	1·03 (0·16)	1·02 (0·14)
Z score	−0·73 (0·28)	−0·50 (1·37)	−0·64 (0·13)
T score	−0·77 (0·27)	−0·53 (1·40)	−0·67 (1·50)
% Normal Adult reference	92·70 (2·80)	94·80 (13·80)	93·59 (13·0)
At neck of femur			
BMD (g/cm^2)	0·88 (0·14)	0·86 (0·12)	0·87 (0·13)
Z score	−0·66 (1·25)	−0·81 (1·18)	−0·72 (1·20)
T score	−0·91 (1·29)	−1·12 (1·25)	−1·0 (1·21)
% Normal Adult reference	92·20 (13·95)	89·80 (13·40)	91·20 (13·56)

All data expressed as mean (2SD).

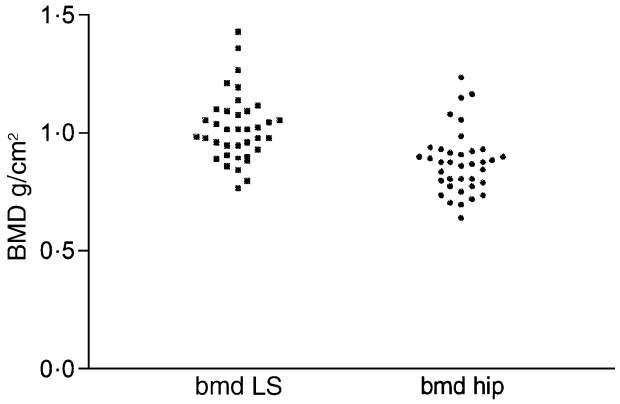

Fig. 1 Individual bone mineral density measurements of lumbar spine and neck of femur in all patients (g/cm^2).

or neck of femur ($P = 0·24$) were not statistically significant from the normal adult male reference range using a one-sample *t*-test.

Individual BMD values at lumbar spine and neck of femur for all patients are represented in Fig. 1. Individual values between patients with primary and secondary hypogonadism were not statistically different using the two-sample *t*-test. When analysed within each group, the mean BMD (0·86 g/cm^2 and 0·75 g/cm^2 at lumbar spine and neck of femur, respectively) of two patients with Noonan's syndrome who were significantly short differed from the other patients with primary hypogonadism ($P < 0·001$). The BMD measurements after adjustment for height were within the normal population reference range.

The regression analysis showed no significant correlation between BMD and the patient's age at start or duration of subcutaneous T pellet implant therapy in our sample of long-term treated patients. However, there was an association, as expected, between the patient's height and BMD ($P = 0·01$ at lumbar spine and $P = 0·03$ at neck of femur) and between weight and BMD ($P = 0·05$ at neck of femur).

Discussion

The beneficial effect of adequate T replacement therapy in hypogonadal men is well established, in relation to changes in bone mass and biochemical markers of bone activity, independent of age and type of hypogonadism (Katznelson *et al*., 1996; Leifke *et al*., 1998; Francis, 1999; Snyder *et al*., 2000). This study provides evidence that regular long-term treatment with subcutaneous T pellet implants provides normal levels of serum T and results in normal bone mass accumulation.

T replacement therapy is said to be more effective during puberty and in those whose epiphyses have not fused (Eastell *et al*., 1998; Francis, 1999). A 5-year study of 42 patients with Klinefelter's syndrome where 250 mg intramuscular T injections were administered to two groups, started before or after age 20, reported a significant increase in BMD in the younger age group (Kubler *et al*., 1992). In another study with 22 hypogonadal men, a positive correlation between BMD and duration of treatment has been reported (Canale *et al*., 2000). In contrast, our study demonstrated that regular and ongoing long-term adequate T replacement therapy helped all young men to attain normal BMD, regardless of age at start or duration of treatment.

A variety of dosing schedules and a variety of T preparations have been used in the past. Some investigators have reported poor outcomes in BMD following oral or intramuscular T. Twelve hypogonadal men had significantly lower spinal BMD following a mean 6·3 years treatment with 3-weekly intramuscular T enanthate 250 mg as compared to age- and BMI-matched controls (De Rosa *et al*., 2001). In another study, 14 hypogonadal patients failed to reverse their low BMD after apparently adequate long-term T replacement therapy using either oral or high-dose

intramuscular T for a mean 5·5 years (Wong *et al*., 1993). In another study, 19 of 26 hypogonadal men (73%) had their total bone mineral content below −1 SD as compared to age-matched controls after regular treatment with intramuscular T injections (Medras *et al*., 2000). The pharmacokinetics of intramuscular T are far from ideal and it is necessary to give T injections every 2 weeks to provide sustained normal levels of T with this type of treatment (Sokol *et al*., 1982; Behre *et al*., 1998). Even during that time there is a 20% reduction of SHBG levels, with wide and unphysiological fluctuations in T levels (Plymate *et al*., 1983). The pharmacokinetics of subcutaneous T implants have been clearly described, with a sustained and constant plasma T level from 24 h after administration over a mean of 24 weeks (Handelsman *et al*., 1990; Behre *et al*., 1998) and maintenance of normal levels of SHBG. We measured normal serum T levels in all our patients 3–4 months following the pellet insertions, because a normal random T level assayed at this time implies normal integrated values for the whole 24-week period. This is also reported elsewhere (Zacharin & Warne, 1997; Kelleher *et al*., 2001). We suggest that sustained T levels, without marked peaks/troughs found with intramuscular injections or marked portal-peripheral gradient found with oral preparations, may contribute to the increased biological androgen effect in our sample of patients.

The BMD Z scores of −0·64 and −0·72 at lumbar spine and neck of femur, respectively, for our patients is below the mean of the population reference group. There are a number of reasons for a BMD within the young age-matched population range (Z score) but below the population mean (T score) Our patients were young adults (mean age 24·3 years), many of whom had late onset of puberty due to timing of diagnosis. T substitution is only commenced for hypopituitary patients where there is a definite lack of gonadotrophins, manifested by delay in onset of puberty. Therefore, this population had not necessarily achieved peak bone mass at the time of investigation. Delayed puberty has also been described as a cause for decreased peak bone mass (Finkelstein *et al*., 1992). Adequacy of previous T treatment prior to subcutaneous T implant therapy might have affected the attainment of peak bone mass, given that optimal administration intervals for intramuscular delivery of T should be 2 weeks (Sokol *et al*., 1982), although this schedule is seldom adhered to. However, our patients had adequate T replacement during subcutaneous T pellet implants, as shown by normal measured T concentrations at 4 months.

All standard reference ranges for BMD measurements in adults are based on age and gender but not on patient size. Thus adult patients with extreme small size or short stature can have low cross-sectional areal BMD unless adjusted for body size (Prentice *et al*., 1994). In our study, two patients with Noonan's syndrome had low BMD both at lumbar spine and neck of femur, which normalized after adjusting for body size. Similarly, the seven patients with Klinefelter's syndrome had a slightly higher BMD (mean BMD at lumbar spine and neck of femur were 1·09 and 0·97 g/cm^2), but they were also tall by a mean 4 cm. However, they were not significantly different when analysed separately. Thus it is always essential to evaluate BMD in relation to body size, especially with extreme short or tall stature.

It is also possible that the Klinefelter subgroup of patients also had less severe androgen deficiency, as half of these patients had normal pubertal onset with subsequent pubertal arrest. This might have permitted a relatively greater BMD.

There was a slightly lower areal BMD at neck of femur in the group of patients with secondary hypogonadism, although still within the adult normal range. Other investigators have reported decreased total bone mineral content in this group of hypogonadal men as compared to age-matched controls (Medras *et al*., 2000). Three of our patients in this group (two patients with Kallman's syndrome and one patient with hypopituitarism) were less than 22 years of age at the time of BMD measurement and therefore may not have reached peak BMD. It is possible that these values may further increase with time. Longitudinal data will be obtained for all patients.

This study, like many other studies in this area, suffers from the lack of a control group. The ethical issue of radiation exposure where adequate adult normal population data already exist, makes it difficult to establish a control group. This study also provides no baseline BMD data at the start of subcutaneous T implant therapy. As patients in our series were young and still growing when started on subcutaneous T implants, they had certainly not reached their peak bone mass. Baseline BMD studies would have yielded widely varying and uninterpretable information. A recent study of BMD and bone turnover with the use of transdermal T in hypogonadal men (Wang *et al*., 2001) provides a model for future studies in this area.

In summary, regular and adequate long-term subcutaneous T pellet implants in hypogonadal men result in adult BMD within the normal range expected for an age/gender-matched population. In addition to being safe and with an acceptable mode of delivery, subcutaneous T pellet implants are efficient and provide sustained physiological levels of T, with no changes in SHBG, and may thus contribute to increased androgenic effect at the receptor level.

Acknowledgements

Shankar Kanumakala was the Novo-Nordisk UK Fellow at Royal Children's Hospital, Melbourne for 2001–02.

References

Arisaka, O., Arisaka, M., Nakayama, Y., Fujiwara, S. & Yabuta, K. (1995) Effect of testosterone on bone mineral density and bone metabolism in adolescent male hypogonadism. *Metabolism: Clinical and Experimental*, **44**, 419–423.

Behre, H.M., Oberpenning, F. & Nieschlag, E. (1998) Comparative pharmacokinetics of testosterone preparations: application of computer analysis and simulation. In: *Testosterone-Action, Deficiency, Substitution* (eds Nieschlag, E. & Behre, H.M.), pp. 115–135. Springer, Berlin.

Behre, H.M., von Eckardstein, S., Kliesch, S. & Nieschlag, E. (1999) Long-term substitution therapy of hypogonadal men with trans-scrotal testosterone over 7–10 years. *Clinical Endocrinology*, **50**, 629–635.

Canale, D., Vignali, E., Golia, F., Martino, E., Pinchera, A. & Marcocci, C. (2000) Effects of hormonal replacement treatment on bone mineral density and metabolism in hypogonadal patients. *Molecular and Cellular Endocrinology*, **161**, 47–51.

Daniell, H.W., Dunn, S.R., Ferguson, D.W., Lomas, G., Niazi, Z. & Stratte, P.T. (2000) Progressive osteoporosis during androgen deprivation therapy for prostate cancer. *Journal of Urology*, **163**, 181–186.

De Rosa, M., Paesano, L., Nuzzo, V., Zarrilli, S., Del Puente, A., Oriente, P. & Lupoli, G. (2001) Bone mineral density and bone markers in hypogonadotrophic and hypergonadotrophic hypogonadal men after prolonged testosterone treatment. *Journal of Endocrinological Investigations*, **24**, 246–252.

Eastell, R., Boyle, I.T., Compston, J., Cooper, C., Fogelman, I., Francis, R.M., Hosking, D.J., Purdie, D.W., Ralston, S., Reeve, J., Reid, D.M., Russell, R.G.G. & Stevenson, J.C. (1998) Management of male osteoporosis: report of the UK Consensus Group. *Quarterly Journal of Medicine*, **91**, 71–92.

Finkelstein, J.S., Neer, R.M., Biller, B.M., Crawford, J.D. & Klibanski, A. (1992) Osteopenia in men with a history of delayed puberty. *New England Journal of Medicine*, **326**, 600–604.

Francis, R.M. (1999) Effects of testosterone on osteoporosis in men. *Clinical Endocrinology*, **50**, 411–414.

Glastre, C., Braillon, P., David, L., Cochat, P., Meunier, P.J. & Delmas, P.D. (1990) Measurement of bone mineral content of the lumbar spine by dual energy X-ray absorptiometry in normal children: correlations with growth parameters. *Journal of Clinical Endocrinology and Metabolism*, **70**, 1330–1333.

Handelsman, D.J., Conway, A.J. & Boylan, L.M. (1990) Pharmacokinetics and pharmacodynamics of testosterone pellets in man. *Journal of Clinical Endocrinology and Metabolism*, **71**, 216–221.

Handelsman, D., Mackey, M.A., Howe, C., Turner, L. & Conway, A. (1997) An analysis of testosterone implants for androgen replacement therapy. *Clinical Endocrinology*, **47**, 311–316.

Katznelson, L. (1998) Therapeutic role of androgens in the treatment of osteoporosis in men. *Baillieres Clinical Endocrinology and Metabolism*, **12**, 453–470.

Katznelson, L., Finkelstein, J., Schoenfeld, D., Rosenthal, I., Anderson, E. & Klibanski, A. (1996) Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. *Journal of Clinical Endocrinology and Metabolism*, **81**, 4358–4365.

Kelleher, S., Conway, A.J. & Handelsman, D.J. (2001) Influence of implantation site and track geometry on the extrusion rate and pharmacology of testosterone implants. *Clinical Endocrinology*, **55**, 531–536.

Kubler, A., Schluz, G., Cordes, U., Beyer, J. & Krause, U. (1992) The influence of testosterone substitution on bone mineral density in patients with Klinefelter's syndrome. *Experimental and Clinical Endocrinology*, **100**, 129–132.

Leifke, E., Korner, H.C., Link, T., Behre, H., Peters, P. & Nieschlag, E. (1998) Effects of testosterone replacement therapy on cortical and trabecular bone mineral density, vertebral body area and paraspinal muscle area in hypogonadal men. *European Journal of Endocrinology*, **138**, 51–58.

Lu, P.W., Briody, J.N., Ogle, G.D., Morley, K., Humphries, R., Allen, J., Howman-Giles, R., Sillence, D. & Cowell, G. (1992) Bone mineral density of total body, spine, and femoral neck in children and young adults: a cross-sectional and longitudinal study. *Journal of Bone and Mineral Research*, **9**, 1451–1458.

Medras, M., Jankowska, E.A. & Rogucka, E. (2000) Effects of long-term testosterone substitutive therapy on bone mineral content in men with hypergonadotrophic hypogonadism. *Andrologia*, **33**, 47–52.

Plymate, S., Leonard, J., Paulsen, C., Fariss, B. & Karpas, A. (1983) Sex hormone binding changes with androgen treatment. *Journal of Clinical Endocrinology and Metabolism*, **57**, 645–648.

Prentice, A., Parsons, T.J. & Cole, T.J. (1994) Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the identification of bone mineral determinants. *American Journal of Clinical Nutrition*, **60**, 837–842.

Rubin, K., Schirduan, V., Gendreau, P., Sarfarazi, M., Mendolaa, R. & Dalsky, G. (1993) Predictors of axial and peripheral bone mineral density in healthy children and adolescents, with special attention to the role of puberty. *Journal of Pediatrics*, **123**, 863–870.

Snyder, P.J., Peachey, H., Berlin, J.A., Hannoush, P., Haddad, G., Dlewati, A., Santanna, J., Loh, L., Lenrow, D.A., Holmes, J.H., Kapoor, S.C., Atkinson, L.E. & Strom, B.L. (2000) Effects of Testosterone replacement in hypogonadal men. *Journal of Clinical Endocrinology and Metabolism*, **85**, 2670–2677.

Sokol, R.Z., Palacrois, A., Campfield, L., Saul, C. & Swerdloff, R.S. (1982) Comparison of the kinetics of injectable testosterone in eugonadal and hypogonadal men. *Fertility and Sterility*, **37**, 425–430.

Wang, C., Swerdloff, R.S., Iranmanesh, A., Dobs, A., Snyder, P.J., Cunningham, G., Matsumoto, A.M., Weber, T. & Berman, N. (2001) Effects of transdermal testosterone gel on bone turnover markers and bone mineral density in hypogonadal men. *Clinical Endocrinology*, **54**, 739–750.

Wong, F.H.W., Pun, K.K. & Wang, C. (1993) Loss of bone mass in patients with Klinefelter's syndrome despite sufficient testosterone replacement. *Osteoporosis International*, **3**, 3–7.

Zacharin, M.R. & Warne, G.L. (1997) Treatment of hypogonadal adolescent boys with long acting subcutaneous testosterone pellets. *Archives of Diseases in Childhood*, **76**, 495–499.