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# Long-term effect of testosterone replacement therapy on bone in hypogonadal men with Klinefelter Syndrome

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## Abstract

**Purpose** To assess different aspects of bone damage in untreated adult patients with Klinefelter Syndrome (KS) before and during testosterone replacement therapy (TRT).

**Methods** Fifteen untreated hypogonadal men with KS and 26 control subjects (C) matched for age and BMI were recruited. Sex hormone levels were measured in all subjects. Lumbar spine (LS) and femoral (neck: FN and total hip: TH) bone mineral density (BMD), trabecular bone score (TBS), hip structure analysis (HSA) and fat measures (percentage of fat mass, android/gynoid ratio and visceral adipose tissue) were evaluated by DEXA. In KS patients, blood analysis and DEXA measurements were assessed at baseline and repeated yearly for three years during TRT.

**Results** Fat measures were significantly higher in KS than C ($p < 0.01$). In contrast, mean LS, FN and TH BMD were significantly reduced in KS compared to C ($p < 0.01$), while there was no difference in TBS. HSA revealed a significantly lower cortical thickness and significantly higher buckling ratio in KS compared to C at all femoral sites ($p < 0.01$). In KS patients, TRT significantly increased BMD at LS only, but did not improve TBS and HSA parameters. Fat measures were inversely associated with TBS values, and TRT did not influence this relationship.

**Conclusions** In untreated hypogonadal men with KS, lumbar and femoral BMD was reduced, and femoral bone quality was impaired. Adiposity seemed to have a detrimental effect on lumbar bone microarchitecture, as indirectly evaluated by TBS. However, TRT failed to remedy these negative effects on bone.

**Keywords** Klinefelter syndrome · Bone mineral density · Trabecular bone score · Hip structural analysis

## Introduction

Klinefelter Syndrome (KS) is one of the most common causes of primary hypogonadism and infertility, with a prevalence of one in approximately 600 men. It is commonly defined by the presence of one extra X chromosome in a male phenotype, resulting in a 47,XXY karyotype [1]. The major clinical findings are small and firm testes, gynecomastia, hypogonadism, and elevated levels of follicle-stimulating hormone [2]. The disease has been associated with various endocrine disorders, such as diabetes mellitus and pituitary and thyroid disorders [3], as well as metabolic syndrome [4]. KS is also associated with a decreased bone mass in 25–48% of cases [5] and with osteoporosis in 6–15% of cases [6], but the genesis of the reduced bone mineral density (BMD) is not yet fully understood [6, 7].

Hypogonadism is a well-known cause of secondary osteoporosis. Testosterone replacement therapy (TRT) in men with reduced testosterone levels is recommended to improve or to prevent worsening of lumbar and femoral BMD [8]. However, several studies have reported that TRT in KS men with low testosterone levels and low BMD does not fully reverse the decreased bone mass [9]. Furthermore, a reduced BMD was also found in patients with normal

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testosterone levels, suggesting that bone loss in KS might, at least in part, be independent of hypogonadism [10].

Dual energy X-ray absorptiometry (DEXA) remains the most common technique for diagnosing osteoporosis and estimating fracture risk. However, in recent years the development of additional tools using other DEXA-based techniques has enabled the easy exploration of other skeletal components related to bone strength in addition to BMD. The advanced software needed to calculate these parameters is now offered by most central DEXA densitometers. Among these tools, hip structural analysis (HSA) and trabecular bone score (TBS) are now widely used in clinical practice and provide new insights into several conditions affecting bone [11–13].

HSA measures a number of proximal femur geometry parameters that may be predictive of femoral strength and fracture risk. Notwithstanding the methodological limits of measuring geometry from two-dimensional DEXA scans, HSA could provide additional information on bone properties and strongly correlate with equivalent measurements assessed by 3-dimensional quantitative computed tomography without exposure to high radiation doses [14].

TBS is a tool derived from DEXA lumbar spine (LS) imaging that indirectly reflects the bone micro-architecture [15]. It correlates positively with 3D bone micro-architecture parameters, such as connectivity density and trabecular number, and negatively with trabecular separation [16–18]. Cross-sectional studies indicate that TBS in addition to BMD measurement could be useful in fracture risk assessment [19, 20].

To our knowledge, no studies have explored bone damage in KS patients by using these DEXA-derived tools. Moreover, the effect of TRT on these bone parameters is essentially unknown. We aimed to assess changes in BMD, TBS, and HSA in formerly untreated hypogonadal adult men with KS before and after three years of TRT.

## Materials and methods

### Subjects

Between January 2013 and December 2014, we consecutively enrolled 57 hypogonadal men with KS attending our Centre for Rare Diseases (Section of Medical Pathophysiology and Endocrinology), Department of Experimental Medicine (Sapienza University of Rome). The inclusion criteria were age above 25 years and no previous testosterone treatment. The exclusion criteria were other endocrine diseases possibly affecting bone, present or past malignant diseases, and treatment with drugs known to interfere with bone metabolism. The majority of patients ($n = 30$) were excluded due to previous long-term TRT

treatment, seven patients were excluded because they were younger than 25 years and five were excluded because they had an endocrine disease and/or comorbidity affecting bone metabolism.

This left 15 untreated hypogonadal adult men with KS (mean age $38.46 \pm 9.07$ years) and 26 age-matched control subjects (C) (mean age $39.76 \pm 12.86$ years), who were recruited from healthy volunteers and medical doctors working at our department. All KS patients had the classic 47,XXY karyotype, as verified by chromosome analysis of peripheral blood lymphocytes. Karyotypes were established on 40 metaphases from each patient. No KS patient reported having experienced delayed pubertal development and on physical examination all patients presented regular androgenisation, a marker of complete pubertal development.

Lifestyle habits (smoking, alcohol consumption and level of physical activity) were comparable between KS and controls. No previous fragility fractures were recorded in either group. However, two KS patients and three controls had suffered peripheral traumatic fractures in childhood. Because all enrolled subjects were relatively young and none of them complained of back pain, the X-ray of the spine was not performed, and therefore no information on morphometric vertebral fractures is available.

All KS patients and control subjects received oral and written information concerning the study prior to giving written informed consent. The protocol was approved by the University's Institutional Ethics Committee.

KS patients and healthy subjects were evaluated for height, weight, and BMI. Standing height was determined by a wall-mounted Harpenden Stadiometer (Holtain Limited, Crymych, Dyfed, UK). Body weight was measured to the nearest 0.1 kg using standardised equipment. BMI was calculated as weight in kilograms divided by height in meters squared.

At the baseline, a morning fasting blood sample was taken from all KS patients and healthy subjects for measurement of routine biochemical parameters and sex hormones. Total testosterone (T), estradiol (E2), and sex hormone binding globulin (SHBG) were analysed by chemiluminescent microparticle immunoassay (CMIA, Architect System) (Abbott Laboratories, IL, USA), with limits of detection of 0.28 nmol/L, ≤10 pg/mL, and ≤0.1 nmol/L respectively. Intra-assay and inter-assay coefficients of variation for our laboratory were: 2.1 and 3.6% at 10.08 nmol/L (T); 5 and 7% at 190 and 600 pg/mL (E2); 5.65 and 9.54% at 8.8 nmol/L (SHBG). Normal reference ranges for adulthood were: 10.40–38.20 nmol/L for T; 25–107 pg/mL for E2; and 11.2–78.1 nmol/L for SHBG.

Dual energy X-ray absorptiometry (QDR Discovery Acclaim, Hologic Inc., Waltham, MA, USA) was used to measure LS (L1-L4), femoral neck (FN) and total hip (TH) BMD in the posterior-anterior projection. The coefficients

of variations (CVs) were 1.0% for LS and 1.7% for TH. The percentage (%) of fat mass, android/gynoid ratio, and visceral adipose tissue (VAT) were also assessed by DEXA. TBS was evaluated in the same regions used for LS BMD (L1–L4) using the latest version of TBS iNsight® (version 2.1.2, Med-Imaps, Pessac, France), also validated for men. TBS was calculated as the mean value of the individual measurements for vertebrae L1–L4. The CV for TBS was 1.8% and was unvaried among the measured vertebrae.

The Hologic Hip Structural Analysis (HSA) program was used to measure the structural geometry of the left hip for each scan. Parameters taken into account were: cross-sectional area (CSA, cm$^2$), cross-sectional moment of inertia (CSMI, cm$^4$), section modulus (index of strength of bending, Z, cm$^3$), cortical thickness (Ct, cm), and buckling ratio (index of susceptibility to local cortical buckling under compressive loads, Br). These parameters were evaluated for each site analysed: (1) narrow neck (NN), across the narrowest diameter of the FN, (2) intertrochanteric (IT), along the bisector of the neck-shaft angle, and (3) femoral shaft (FS), 2 cm distal to the midpoint of the lesser trochanter.

The study lasted three years. All KS patients were hypogonadal at the beginning of the study and all of them received TRT during the study (eight patients received intramuscular testosterone undecanoate and seven received transdermal testosterone gel). Serum T level was maintained in the normal reference range for men in all KS patients during the study period. In this group, blood analysis and DEXA measurements were repeated each year during the study.

## Statistics

Data are reported as mean ± 1 SD. Student's *t*-test was used to compare means between KS and control subjects. A linear model was used to test the differences between KS and control subjects at baseline, after controlling for age and BMI. A chi-squared test was used to compare percentages of both KS patients and controls stratified by a TBS cut-off of 1.350.

Given the sample size, the power analysis was assessed selecting a medium level for the effect [21], yielding powers around 0.6 for the linear models and around 0.5 for the chi-squared test.

In order to study the effect of long-term TRT on body composition and bone parameters in KS patients, we used the partial least squares path models (PLS-PM). In fact, since some of the variables studied are both explanatory variables for some characteristics and response variables for others, the standard approach in these situations is to use structural equation models (SEM) or PLS-PM when multiple regression models must be estimated. PLS-PM is a powerful multivariate statistical method that allows the simultaneous assessment of complex cause and effect models between observed variables and a set of latent variables (LVs), estimated as weighted sums of the corresponding manifest variables (reflective approach). This model enables testing of hypotheses on all LV relationships in the same analysis. It is mainly made up of two sub-models: the measurement and the structural model. The first describes the relationships between the manifest variables (the variables that have actually been observed) and the corresponding LVs, while the second describes the relationship between the latent variables. Both the models are explicitly defined by the researcher and are usually depicted using a path diagram. In a PLS-PM the effect that one variable can have on another can be direct (there is a direct link on the path diagram) or mediated (one variable influences another via a third variable). Although similar in appearance to regression models, PLS-PMs differ since the same variable can be dependent in one equation and independent in another. They thus distinguish between exogenous and endogenous more than independent and dependent.

In the PLS-PM, age, BMI, and duration of therapy were considered as exogenous variables and were controlled for in all sub-models. All other variables were endogenous.
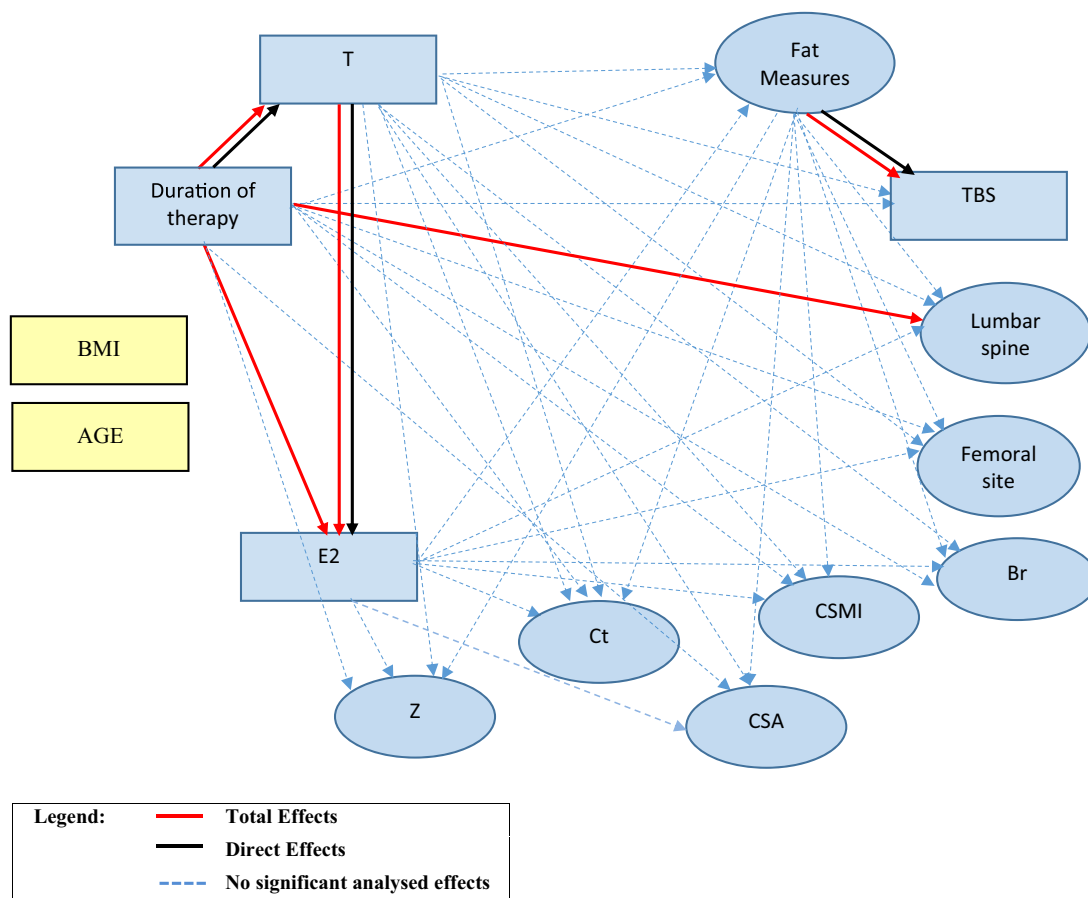
Measured variables were represented using rectangles while LVs were depicted using ovals, as customary in structural equation models (Fig. 1). Internal consistency was ascertained using Cronbach's alpha, with values greater than 0.7 considered acceptable, in line with normal practice. Variations were computed with respect to baseline.

Table 1 reports LVs, measured variables and corresponding Cronbach's alpha values. The subsets of the collected variables are highly correlated. "Fat measures" LVs included variation in % fat mass, A/G ratio and VAT. "Lumbar spine" (LS) LVs included variation in LS T-score and BMD. "Femoral site" LVs included variation in femoral neck (FN) T-score and BMD and in total hip (TH) T-score and BMD. All blocks showed alpha values greater than 0.65, obtained in the femoral site LVs.

The analysis was performed using the PLS-PM package within the statistical framework R v 3.5. The significance of the results was assessed using bootstrap with $B = 5000$ samples.

## Results

Table 2 reports mean values for the demographic, anthropometric, hormonal and densitometric parameters of KS and controls. No differences in mean age (KS = 38.46 ± 9.07 years vs. C = 39.769 ± 12.86 years) and BMI (KS = 25.25 ± 3.27 Kg/m$^2$ vs. C = 24.75 ± 2.67 Kg/m$^2$) were

**Fig. 1** Path diagram of the results obtained using the PLS-PM model, showing the total and direct effects of the duration of therapy on the considered variables. All effects are controlled for age and BMI. Measured variables are represented by rectangles and latent variables by ovals. All effects have been controlled for age and BMI (represented in the external yellow rectangles). Dashed blue lines represent the analysed effects; solid black lines represent significant direct effects; solid red lines represent significant total effects. T total

testosterone, E2 estradiol, TBS trabecular bone score, Fat measures % fat mass, visceral adipose tissue and android/gynoid ratio, Lumbar spine LS T-score, LS BMD Femoral site Femoral Neck (FN T-score, FN BMD) and total hip (TH T-score, TH BMD), CSA cross-sectional area (NN CSA, IT CSA, FS CSA), CSMI cross-sectional moment of inertia (NN CSMI, IT CSMI, FS CSMI), Ct cortical thickness (NN Ct, IT Ct, FS Ct), Br buckling ratio (NN Br, IT Br, FS Br), Z section modulus (NN Z, IT Z, FS Z)

found between the two groups. As expected, T levels were significantly lower in KS patients than controls, whereas there was no statistically significant difference in serum E2 or SHBG. The percentage of fat mass, the VAT and the android/gynoid ratio were significantly higher in KS patients than in controls. Mean LS, FN and TH BMD values were significantly lower in KS patients than controls at all measured sites, while there was no difference in mean TBS. TBS values were also stratified into two categories, normal (TBS ≥ 1.350) and reduced (mildly and markedly reduced, TBS < 1.350) in both KS and control group [22]. Here too, there was no difference in the percentage of subjects with normal and reduced TBS values between KS and controls.

Finally, in relation to HSA parameters, it is noteworthy that Ct was significantly lower and Br significantly higher in KS patients than controls at all femoral sites (Table 2). The

difference between the groups did not change for any of the considered parameters after controlling for age and BMI.

Table 3 reports only the significant direct effects. As expected, the duration of treatment had a significant effect on T ($p < 0.05$) but not on E2 levels; moreover, there was a positive direct effect of T on E2 levels ($p < 0.05$). The only significant effect for "fat measures" LVs was variation in TBS, with higher values associated with lower TBS values ($p < 0.05$). No significant direct effect of "fat measures" was observed on the other LVs.

Table 4 reports only the significant total effects. The duration of treatment showed a total positive effect on both T and E2 levels ($p < 0.05$). The effect on E2 was probably mediated by T, since "duration of therapy" had no significant direct effect on E2 (as shown in Table 3). "Duration of therapy" also showed a significant positive effect on "Lumbar Spine" LVs ($p < 0.05$), thus a longer treatment period was associated with higher LS values. Here too,

**Table 1** Latent variables, measured variables and corresponding Cronbach's alpha

| Latent variables | Measured variables | Alpha[a] |
|---|---|---|
| Fat measures | Variation in % of fat mass | 0.791 |
| | Variation in VAT | |
| | Variation in A/G ratio | |
| Lumbar spine | Variation in LS T-score | 0.997 |
| | Variation in LS BMD | |
| Femoral site | Variation in FN T-score | 0.653 |
| | Variation in FN BMD | |
| | Variation in TH T-score | |
| | Variation in TH BMD | |
| Br | NN Br | 0.745 |
| | IT Br | |
| | FS Br | |
| Ct (cm) | NN Ct | 0.844 |
| | IT Ct | |
| | FS Ct | |
| CSA (cm$^2$) | NN CSA | 0.893 |
| | IT CSA | |
| | FS CSA | |
| Z (cm$^3$) | NN.Z | 0.820 |
| | IT Z | |
| | FS.Z | |
| CSMI (cm$^4$) | NN CSMI | 0.701 |
| | IT CSMI | |
| | FS CSMI | |

*VAT* visceral adipose tissue, *A/G* android/gynoid, *LS* lumbar spine, *FN* femoral neck, *TH* total hip, *Br* buckling ratio, *Ct* cortical thickness, *CSA* cross-sectional area, *Z* section modulus, *CMSI* cross-sectional moment of inertia, *NN* narrow neck, *IT* intertrochanteric, *FS* femoral shaft

[a]Alpha values > 0.7 were considered acceptable in determining the quality of the latent variable

there was a negative effect of "fat measures" LVs on TBS, with higher fat measures associated with lower TBS values ($p < 0.05$).

Figure 1 illustrates the path diagram of the fitted model, highlighting the significant direct and total effects, as previously described.

## Discussion

This study evaluated bone involvement before and after long-term TRT in adult hypogonadal KS patients by using several skeletal and body composition parameters easily derived from DEXA scans. Specifically, the combination of three different methods (BMD, HSA and TBS) to evaluate bone adds novel findings to the literature, showing that not only bone density but also some parameters of bone quality are compromised in men with KS.

In line with previous studies [10, 23], we found that untreated hypogonadal KS patients had significantly lower mean lumbar and femoral BMD than healthy aged-matched men; however, the mean TBS values of the two groups were similar, suggesting that trabecular bone microarchitecture, at this level, may be preserved. Moreover, there was no difference between KS and controls when TBS data were stratified as normal or reduced (using a TBS cut-off value of 1.350), further supporting the hypothesis of preservation of vertebral bone microarchitecture.

It is possible that testosterone deficiency acts differently on the different skeletal components of the trabecular bone, impairing above all BMD while leaving bone structure unchanged.

Interestingly, HSA results showed that Ct was significantly lower and Br significantly higher in KS compared to controls at all femoral sites. These findings demonstrate that in untreated hypogonadal KS men, bone damage may be much more complex than previously realised; not only is BMD reduced, but bone quality is also affected, in particular at the femoral site, where structure and resistance are compromised.

The role of testosterone deficiency in the development of low BMD has not been fully elucidated. Testosterone regulates male bone metabolism both directly, through action of the androgen receptor (AR) on osteoblasts, and indirectly, after aromatisation to oestrogens. Testosterone promotes periosteal bone formation during puberty [24] and reduces bone resorption during adult life [25]. Previous studies reported that hypogonadal patients with KS had a significantly lower BMD at the LS, FN and Ward's triangle [10, 23]. However,ì a reduced bone mass has also been demonstrated in patients with normal circulating testosterone levels [10], and TRT may not fully reverse bone abnormalities in KS men [9] or in XXY mice models [26]. Taken together, these findings seem to suggest that bone loss in KS might at least in part be independent of hypogonadism and that other factors may play a role in the development of bone damage. It is important to underline that it is the age of skeletal growth during which testosterone deficiency develops that should be considered, since some men with KS start pubertal maturation but often they do not complete it on their own. This can lead to differences among the patients, because in some patients there may be effects on both bone modelling and remodelling, while in others there are only effects on bone remodelling. The role of AR CAG polymorphism [27] and of reduced vitamin D levels [23] has been suggested in this respect, but not further confirmed [28]. Estrogen levels have been reported to be increased [29] or normal [30] in these patients, and this might protect against excessive bone loss [31] as

**Table 2** Mean values ± 1 SD of demographic, anthropometric, hormonal and densitometric parameters of patients with Klinefelter Syndrome (KS) and controls (C)

|  | KS | C | $p$ |
|---|---|---|---|
| Mean age, years | 38.46 ± 9.07 | 39.76 ± 12.86 | NS |
| BMI Kg/m$^2$ | 25.25 ± 3.27 | 24.75 ± 2.67 | NS |
| T nmol/L | 7.26 ± 3.13 | 22.28 ± 5.50 | <0.001* |
| E2 pg/mL | 22.93 ± 10.61 | 28.09 ± 9.69 | NS |
| SHBG nmol/L | 56.86 ± 40.76 | 41.27 ± 13.63 | NS |
| % Fat mass | 27.9 ± 4.37 | 19.37 ± 3.80 | <0.001* |
| VAT | 91.54 ± 27.81 | 60.81 ± 17.39 | <0.001* |
| A/G ratio | 0.936 ± 0.141 | 0.79 ± 0.116 | <0.001* |
| T-score LS | −1.647 ± 1.475 | 0.05 ± 01.305 | 0.001* |
| BMD LS | 0.908 ± 0.158 | 1.095 ± 0.144 | 0.001* |
| T-score FN | −1.32 ± 0.627 | −0.304 ± 0.914 | <0.001* |
| BMD FN | 0.746 ± 0.073 | 0.895 ± 0.127 | <0.001* |
| T-score TH | −1.187 ± 0.766 | −0.019 ± 0.894 | <0.001* |
| BMD TH | 0.846 ± 0.103 | 1.031 ± 0.138 | <0.001* |
| TBS | 1.321 ± 0.108 | 1.376 ± 0.079 | NS |
| NN CSA (cm$^2$) | 3.171 ± 0.290 | 3.554 ± 0.527 | 0.007* |
| IT CSA (cm$^2$) | 5.021 ± 0.710 | 5.620 ± 1.038 | NS |
| FS CSA (cm$^2$) | 4.526 ± 0.556 | 5.333 ± 0.699 | 0.001* |
| NN CSMI (cm$^4$) | 3.645 ± 0.599 | 4.018 ± 0.893 | NS |
| IT CSMI (cm$^4$) | 16.498 ± 3.73 | 15.357 ± 4.055 | NS |
| FS CSMI (cm$^4$) | 5.149 ± 1.024 | 4.868 ± 1.012 | NS |
| NN Z (cm$^3$) | 1.775 ± 0.237 | 2.056 ± 0.430 | 0.019* |
| IT Z (cm$^3$) | 4.652 ± 0.918 | 4.754 ± 1.128 | NS |
| FS Z (cm$^3$) | 2.873 ± 0.433 | 3.040 ± 0.460 | NS |
| NN Ct (cm) | 0.169 ± 0.017 | 0.200 ± 0.031 | <0.001* |
| IT Ct (cm) | 0.343 ± 0.062 | 0.440 ± 0.081 | <0.001* |
| FS Ct (cm) | 0.501 ± 0.074 | 0.738 ± 0.166 | <0.001* |
| NN Br | 12.340 ± 2.096 | 10.016 ± 1.715 | <0.001* |
| IT Br | 10.280 ± 1.706 | 7.572 ± 1.646 | <0.001* |
| FS Br | 3.573 ± 0.785 | 2.292 ± 0.614 | <0.001* |

Differences between KS and controls by $T$ test. The same differences remained significant after controlling for age and BMI

*Statistical significance $p < 0.05$

*T* total testosterone, *E2* estradiol, *SHBG* sex hormone binding globulin, *VAT* visceral adipose tissue, *A/G* android/gynoid, *LS* lumbar spine, *FN* femoral neck, *TH* total hip, *TBS* trabecular bone score, *NN* narrow neck, *IT* intertrochanteric, *FS* femoral shaft, *CSA* cross-sectional area, *CSMI* cross-sectional moment of inertia, *Z* section modulus, *Ct* cortical thickness, *Br* buckling ratio

demonstrated in hypogonadal men [32]. It has also been hypothesised that an unfavourable fat/muscle ratio could partly explain poor bone health in KS men [33]; as this finding is already present in young adolescents before the onset of testosterone deficiency and bone mass defects, the authors speculated that it is caused not only by low testosterone levels but also by other mechanisms, probably related to the genetic defect [33].

Taking into account our results, we investigated if TRT could lead to significant changes in bone parameters and body composition in KS men. It has been previously reported that testosterone therapy in hypogonadal men increases both trabecular and cortical BMD of the spine, independently of age and type of hypogonadism [34]. In line with other studies we demonstrated that LS BMD rose significantly with the duration of the testosterone therapy, but femoral BMD did not [35]. Shanbhogue et al. found that KS patients taking long-term TRT had reduced volumetric BMD, trabecular network integrity and estimated bone strength; moreover, they found no significant differences in bone microarchitecture and estimated strength between KS subjects with and without testosterone therapy, suggesting that factors other than hypogonadism may play a role in decreased bone mass [36]. In our study, TRT did not directly modify either TBS or HSA parameters. Over the last 2 decades, HSA has been used in a variety of studies with pharmacologic agents to analyse changes in the structural geometry of the femur that may not be apparent with other imaging techniques, such as DEXA or QCT. The results of these studies are frequently conflicting, which may in part be due to the methodological problems related to these measurements, as recently reported [37, 38]. In fact, the major criticism is that HSA cannot replace 3-D methods because it is two-dimensional, due to the 2-D nature of DEXA scans. Moreover, the use of 2-D data requires a number of assumptions that have not been tested across all populations and treatment groups. Nevertheless, HSA has been shown to correlate very strongly with hip QCT measurement and to predict hip fractures; it has therefore been used in many studies worldwide and has helped to clarify the mechanical implications of age-related change in bone mass.

However, it has been suggested that there is currently insufficient evidence that these tools can be used to assess response to therapy [37, 38]. Considering our results, we believe that other factors should also be taken into account. It should be hypothesised that TRT has no effect on TBS simply because its levels are not sufficiently impaired. In fact, at the baseline, the KS and control groups showed not only comparable mean TBS values but also a similar percentage of subjects with normal or reduced TBS values. However, the small caseload does not allow us to reach definitive conclusions.

In relation to the femoral sites, one possibility is that TRT really is unable to increase bone density and strength in this area, as previously demonstrated [35, 39]. This lack of response could be responsible for an increased fracture risk at this site in the long term, but data in KS patients are currently scanty.

In this study we also found that untreated hypogonadal KS patients have a significantly higher fat mass percentage,

**Table 3** Significant direct effects, mean estimate and corresponding confidence intervals

| Direct path | | | Effect | Mean | Standard error | 95% Confidence interval[a] | |
|---|---|---|---|---|---|---|---|
| Duration of therapy | → | T | 0.482347 | 0.4928 | 0.115 | 0.2431 | 0.69616 |
| T | → | E2 | 0.599760 | 0.5776 | 0.172 | 0.1927 | 0.86549 |
| Fat measures | → | TBS | −0.517548 | −0.5262 | 0.142 | −0.8001 | −0.25802 |

[a]Confidence interval determined using bootstrap with $B = 5000$

*T* total testosterone, *E2* estradiol, *TBS* trabecular bone score

Fat measures: % fat mass, visceral adipose tissue and android/gynoid ratio

**Table 4** Significant total effects, mean estimates and corresponding confidence intervals

| Path | | | Total effect | Mean | Standard error | 95% Confidence interval[a] | |
|---|---|---|---|---|---|---|---|
| Duration of therapy | → | T | 0.48235 | 0.49282 | 0.115 | 0.2431 | 0.6962 |
| Duration of therapy | → | E2 | 0.36229 | 0.36429 | 0.141 | 0.0782 | 0.6248 |
| Duration of therapy | → | Lumbar spine | 0.49267 | 0.50508 | 0.174 | 0.1408 | 0.8131 |
| T | → | E2 | 0.59976 | 0.57756 | 0.172 | 0.1927 | 0.8655 |
| Fat measures | → | TBS | −0.51755 | −0.52622 | 0.142 | −0.8001 | −0.2580 |

[a]Confidence intervals obtained using bootstrap with $B = 5000$ samples

*T* total testosterone, *E2* estradiol, *TBS* trabecular bone score

Fat measures: % fat mass, visceral adipose tissue and android/gynoid ratio

VAT, and android/gynoid ratio than control subjects, as already described by Wong et al [40]. A drop in visceral fat mass during testosterone therapy has been described in hypogonadal obese men [41]. In contrast, we found that testosterone therapy was ineffective in improving body composition indices in KS patients. Moreover, unfavourable fat parameters seem to negatively affect TBS values, a relationship that is not affected by long-term TRT.

TBS is not recommended for use in patients with BMI outside the range 15–37 kg/m$^2$. As the BMIs of our patients were within these limits, our results merit interest, because of the possibility that fat tissue can negatively influence trabecular bone quality in hypogonadal men with KS and that testosterone therapy is ineffective in improving lumbar bone structure. Even if non-invasive imaging techniques such as HR-pQCT and high-resolution magnetic resonance (HR-MRI) are considered the gold standards for investigating both body composition and bone structure, their use in clinical practice is limited by multiple factors, such as cost, time to perform the investigation and, for HR-pQCT, the need for a dedicated scanner. DEXA provides measures substantially equivalent to those of computed tomography or magnetic resonance imaging [42–45] and confers several practical and technical advantages: it is widely available, less costly, requires a short scanning time and delivers a small fraction of the radiation dose. To our knowledge, this is the first study investigating bone involvement in term of bone density and structural parameters in KS men by using DEXA scan and related software. Furthermore, in our study

the TRT duration was longer than in most other studies carried out to date, even if the total number of patients studied is low; this limitation, however, reflects the difficulty in enrolling untreated adult patients with KS, as most of them are treated on first coming to the attention of specialists in this field. An interesting starting point for a future study could be to compare our data with those obtained from a group of hypogonadal 46,XY patients, in order to establish if the reduction in lumbar and femoral BMD and the worsened femoral bone quality found in KS patients is due to hypogonadism itself or to the different genetic profile.

The lack of information on bone turnover markers was another limitation of the study, meaning that the effect of TRT on these indices and their correlation with changes in bone variables could not be explored.

In conclusion, our study demonstrated that in untreated hypogonadal men with KS, both lumbar and femoral BMD are reduced and femoral bone quality is affected, with impaired structure and resistance. Long-term TRT indirectly led to an increase in LS BMD, but had no effects on trabecular bone microarchitecture or femoral strength, as evaluated by TBS and HSA analysis respectively. Moreover, replacement therapy did not modify fat distribution indices as evaluated by the variation in percentage of fat mass, VAT, and android/gynoid ratio; finally, these parameters determine a negative effect on TBS, leading to a compromised LS bone quality.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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