

ORIGINAL ARTICLE



Effect of 12-months testosterone replacement therapy on bone mineral density and markers of bone turnover in testicular cancer survivors – results from a randomized double-blind trial

P. L. Jørgensen^a , M. Kreiberg^a , N. Jørgensen^{b,c} , A. Juul^{b,c,d} , P. S. Oturai^e , C. Dehrendorff^f , J. Lauritsen^a , T. Wagner^a, J. Rosenvilde^a , G. Daugaard^{a,d} , C. R. Medici^a, N. R. Jørgensen^g  and M. Bandak^a 

^aDepartment of Oncology, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark; ^bDepartment of Growth and Reproduction, Copenhagen University hospital - Rigshospitalet, Copenhagen, Denmark; ^cInternational Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Copenhagen University hospital - Rigshospitalet, Copenhagen, Denmark; ^dDepartment Clinical Medicine, University of Copenhagen, Denmark; ^eDepartment of Clinical Physiology, Nuclear Medicine and PET, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark; ^fStatistics and Dataanalysis, Danish Cancer Society Research Center, Danish Cancer Society, Copenhagen, Denmark; ^gDepartment of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

ABSTRACT

Background: Testicular cancer survivors (TCS) are at risk of Leydig cell insufficiency, which is a condition characterized by elevated luteinising hormone (LH) in combination with low levels of testosterone. It has been suggested that this condition is associated with impaired metabolic profile and low bone mineral density (BMD). The primary aim of the randomized double-blind trial NCT02991209 was to evaluate metabolic profile after 12-months testosterone replacement therapy (TRT) in TCS with mild Leydig cell insufficiency. Here we present the secondary outcomes of changes in BMD and markers of bone turnover.

Methodology: In total, 69 TCS with mild Leydig cell insufficiency were randomized 1:1 to 12 months TRT ($n = 35$) (Tostran, gel, 2%, applied transdermally, with a maximum daily dose of 40 mg) or placebo ($n = 34$). BMD and markers of bone turnover were evaluated at baseline, after 6- and 12-months TRT, and 3-months post-treatment. Linear mixed effects models were used to analyse changes in BMD, N-terminal propeptide of type 1 procollagen (P1NP) and C-terminal telopeptide of type I collagen (CTX).

Results: After 12 months treatment, TRT was not associated with a statistically significant difference in BMD compared to placebo; total body BMD: 0.01 g/cm² (95% confidence interval (CI): -0.01 – 0.02), BMD of the lumbar spine: 0.01 g/cm², (95% CI: -0.01–0.03), BMD of the left femoral neck: 0.00, (95% CI: -0.01–0.02). TRT was associated with a small but statistically significant increase in P1NP: 11.65 µg/L (95% CI: 3.96, 19.35), while there was no difference in CTX.

Conclusion: 12 months of TRT did not change BMD, while there was as small and clinically irrelevant increase in P1NP compared to placebo in TCS with mild Leydig cell insufficiency. The findings need validation in a larger cohort.

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Testicular cancer; bone mineral density; markers of bone turnover; testosterone; hypogonadism; osteoporosis; late effects

Introduction

Testicular cancer (TC) is a malignancy with increasing incidence in Western countries [1,2]. It mainly affects young men aged 15–40 [3] with a median age at diagnosis of 34 years [3,4]. In patients with stage I disease, where the tumour is confined to the testicle, treatment includes surgical removal of the tumour-bearing testicle and active surveillance with the option of adjuvant chemotherapy in patients at high risk of relapse [3]. In metastatic disease, cisplatin-based combination chemotherapy or abdominal radiotherapy in patients with seminoma and limited retroperitoneal spread are parts of the treatment regimen [3]. While the cure rates exceed 95%, TC treatment is associated with a range of late

effects [5,6] including testosterone deficiency, as the remaining testicle may not be able to fully compensate for the loss of testosterone production by removal of the tumour-bearing testicle [4,7]. While 5–10% of long-term TC survivors develop testosterone deficiency or initiate testosterone substitution (TRT) [8,9], a substantial proportion of TC survivors are in a compensated state with elevated luteinising hormone (LH) in combination with testosterone low in the normal range (mild Leydig cell insufficiency) [9–11]. Overt testosterone deficiency is associated with low bone mineral density (BMD) [12,13]. In addition, a positive association of LH and LH/T, but not testosterone, with all-cause mortality in otherwise healthy men suggests that mild Leydig cell insufficiency may be a risk factor for death by all causes in men [14]. However, it remains

unknown if TRT improves BMD and markers of bone turnover in patients with mild Leydig cell insufficiency.

The primary aim of this randomized trial was to evaluate if 12-months TRT improves metabolic health in TC survivors with mild Leydig cell insufficiency, which has been presented in a previous publication [7]. Here, we present the secondary outcomes of changes in BMD and markers of bone turnover during 12-months TRT.

Methods

Design of the study and study subjects

This was a single-centre, double-blind, randomised, placebo-controlled trial. The study design, including calculation of sample size, inclusion and exclusion criteria, randomization and masking have been described in detail in two previous publications [7,15]. The main inclusion criterion was presence of mild Leydig cell insufficiency defined as serum concentration of free testosterone below the age-adjusted mean and above the age-adjusted lower limit of normal (-2 standard deviations (SD) in combination with serum LH above the age-adjusted upper limit of normal ($+2$ SD). Due to slower recruitment than anticipated, a protocol amendment was made to allow inclusion of patients with serum free testosterone below the age-adjusted upper limit of normal ($+2$ SD) and above -3 SD from the age-adjusted mean in combination with serum LH above $+1$ SD. Main exclusion criteria were TRT, Diabetes Mellitus, and paternity wish at the time of study inclusion.

Randomization and treatment allocation

Eligible patients were randomized 1:1 to 12-months testosterone or placebo by a web-based randomization tool (<http://www.randomization.com>). Double sealed envelopes were prepared for all patients with a randomization number printed outside.

Experimental treatment and control

Testosterone and placebo were supplied in identical canisters containing 60 gram of gel with a pumping mechanism delivering a fixed amount of gel. The gel was applied transdermally to a maximum daily dose of 40 mg. Compliance was ensured by collecting empty containers and recording kit numbers.

Procedures concerning low serum levels of 25-hydroxyvitamin D at baseline

Patients with serum levels of 25-hydroxyvitamin D <25 nmol/L at baseline initiated daily supplement with 400 mg calcium and 38 microgram vitamin-D3 twice daily, while patients with serum levels of 25-hydroxyvitamin D ≥ 25 nmol/L and <50 nmol/L initiated a daily supplement with 400 mg calcium and 38 microgram of D3 once daily.

Outcomes

Outcomes were assessed at baseline, after 6- and 12-months treatment and 3 months after last treatment (15 months). The primary outcome was change in blood glucose evaluated by oral glucose tolerance test. Secondary outcomes included changes in metabolic profile and body composition evaluated by DXA-scan as well as patient-reported quality of life and sexual function. Here, we present the secondary outcomes of changes in BMD and markers of bone turnover.

Dual-Energy-X-ray Absorptiometry (DXA) was used to assess lumbar BMD (L2–L4), femoral and total body BMD. All patients were scanned on the same scanner (Lunar Prodigy Advance Scanner) (GE Healthcare, Madison, WI, USA), with daily calibration at the Department of Clinical Physiology, Nuclear Medicine and PET, Rigshospitalet, Copenhagen, Denmark [7]. BMD was reported as total BMD (g/cm^2) and T-score.

N-terminal propeptide of type I procollagen (P1NP) and C-terminal telopeptide of type I collagen (CTX) are bone turnover markers, commonly used in the diagnosis and management of bone disorders. CTX reflects bone resorption, while P1NP reflects bone formation [16–18]. The two markers of bone turnover were measured on serum samples collected in the fasting state. Samples were kept at -80°C until analysis. CTX was measured by the IDS-iSYS CTX (CrossLaps®) assay (Immunodiagnostic Systems, plc, Tyne and Wear, UK) and P1NP was measured by the IDS-iSYS intact P1NP assay (Immunodiagnostic Systems) on the iSYS automatic analyser according to the manufacturer's instructions. All samples were measured in one single batch to reduce analytical variation. Both assays are chemiluminescence immunoassays. Assay performance was verified using internal patient pools and external control specimens. The intermediary precisions expressed as coefficients of variation for CTX were $<7.5\%$ (at CTX concentration 191 ng/L) and $<5\%$ (772 ng/L). For P1NP the intermediary precisions were $<5\%$ for both the low (51 $\mu\text{g}/\text{L}$) and high (111 $\mu\text{g}/\text{L}$) internal pool. Lower limit of quantitation (LLOQ) was 33 ng/L for CTX and 2.0 $\mu\text{g}/\text{L}$ for P1NP.

Blood samples were collected and analysed for ionised calcium and 25-hydroxyvitamin D using local standard procedures at the Department of Clinical Biochemistry, Copenhagen University Hospital Rigshospitalet. A detailed flowchart of study procedures is available in the study protocol [7].

Assessment of intervention

Serum concentrations of LH were measured by time-resolved immunofluorometric assay (Delfia; Perkin Elmer, Turku, Finland) with intra-assay and inter-assay coefficient of variation (CV) $<5\%$ and detection limits of 0.05 IU/L. Testosterone concentrations were quantified using an isotope-dilution TurboFlow-LC–MS/MS. In brief, analyses were performed on a Dionex UltiMate 3000 UHPLC system (Thermo Scientific, San Jose, CA, USA) with integrated Transcend TLX TurboFlow sample preparation system

Table 1. Baseline characteristics of 69 included testicular cancer survivors.

	Testosterone (N = 35)	Placebo (N = 34)
Age at study inclusion (median, IQR)	42 (35–47)	44 (37–49)
Time since orchiectomy, years (median, IQR)	5 (3–10)	5 (3–13)
Treatment, No. (%)		
Orchiectomy only	10 (29)	21 (62)
Cisplatin-based chemotherapy	13 (37)	8 (24)
Abdominal radiotherapy	6 (17)	2 (6)
Chemotherapy and radiotherapy	4 (11)	2 (6)
Orchiectomy and radiotherapy to the contralateral testicle due to germ cell neoplasia <i>in situ</i>	2 (6)	1 (3)
Luteinizing hormone, IU/L (median, IQR)	8.0 (5.0–9.0)	7.7 (6.0–9.9)
Free testosterone, pmol/L (median, IQR)	329.7 (291.6–409.3)	315.7 (263.7–382.8)
Total testosterone, nmol/L (median, IQR)	17.6 (15.1–20.6)	15.0 (13.3–18.2)
Body mass index, kg/m ² (median, IQR)	24.8 (23.3–27.6)	27.0 (24.0–28.6)
Smoking, No. (%)		
Never	13 (37)	13 (38)
Current	13 (37)	12 (35)
Former	8 (23)	9 (27)
Alcohol, units per week, No. (%)		
None	6 (17)	6 (18)
< 7	13 (37)	16 (47)
7–14	10 (29)	9 (27)
14–21	3 (9)	3 (9)
> 21	2 (6)	0 (0)
Level of physical activity, No. (%)		
Reading, watching tv, etc	3 (9)	3 (9)
Walking, riding a bicycle, etc	14 (40)	16 (47)
Practising sport at least four times per week	16 (46)	13 (38)
Intense exercise several times per week	1 (3)	2 (6)
PTH, pg/mL (median, IQR)	4.8 (4.2–5.9)	4.8 (3.8–5.0)
TSH uIU/mL (median, IQR)	1.7 (1.2–2.5)	2.4 (1.7–3.1)
Free, ionized calcium, mmol/L (median, IQR)	1.22 (1.19–1.23)	1.20 (1.19–1.22)
Alkaline Phosphatase, U/L (median, IQR)	60.0 (55.0–69.0)	67.5 (54.0–80.0)
25-hydroxy vitamin D, nmol/L (median, IQR)	38.5 (30.0–48.0)	39.5 (28.0–74.0)
Vitamin D supplement at baseline, No. (%)	4 (11)	5 (15)

(Thermo Scientific, San Jose, CA, USA) coupled to a triple quadrupole mass spectrometer (TSQ Quantiva, Thermo Scientific, San Jose, CA, USA). Blood samples from the participants were drawn in the morning. The coefficients of variations (CV's) of the low- and high-level quality control samples were 7.9 and 5.1%. Limits of quantification (LOQ) was 0.1 nmol/l (defined as the lowest concentration resulting in a relative standard deviation (RSD) below 20% [19]. Methods were accredited according by The Danish Accreditation Fund for medical examination according to the standard DS/EN ISO 15189 (www.danak.org). Reference ranges from 30–61 years of age has previously been published with our method [20]. Free testosterone (cFT) was calculated as described by Vermeulen et al. [21] Sex hormone-binding globulin (SHBG) was measured using Access 2 (Beckman Coulter) with intra-assay and inter-assay CVs < 5%. All analyses were performed at Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark.

Statistics

Continuous variables are presented as median with inter-quartile range (IQR), while categorical variables are presented as number and percentages. Linear mixed effects models were used to analyse longitudinal changes in primary and secondary outcomes at 6– 12- and 15-month visits with adjustment for potential baseline differences including difference in TC treatment modalities [22]. Data were collected

and analysed using SPSS Statistics version 28.0.0.0 and R version 3.6.2 by R Core Team (2019) using package nlme.

The study was independently monitored, and quality assured by the Unit for Good Clinical Practice (GCP) at the Copenhagen University Hospital, Bispebjerg, Denmark.

The study was registered at www.clinicaltrial.gov (NCT02991209).

Results

Baseline characteristics

Baseline characteristics of included patients are presented in Table 1. Follow-up time, testosterone and LH levels, lifestyle factors and 25-hydroxy vitamin D levels were comparable between the testosterone group and placebo group. However, more patients in the testosterone group were treated with cisplatin-based chemotherapy and abdominal radiotherapy compared to placebo, (37 vs 24, and 6 vs 2, respectively)

After 12-months treatment, median free testosterone was 542 pmol/L (IQR: 410–715) in the testosterone group compared to 317 pmol/L (IQR: 274–347) in the placebo group (data not shown).

Changes in BMD during the 12-months intervention and 3 months post-treatment are presented in Figure 1, while results from the linear effects model are presented in Table 2. The estimates of mean changes between the groups during and after treatment were minimal with almost all 95%

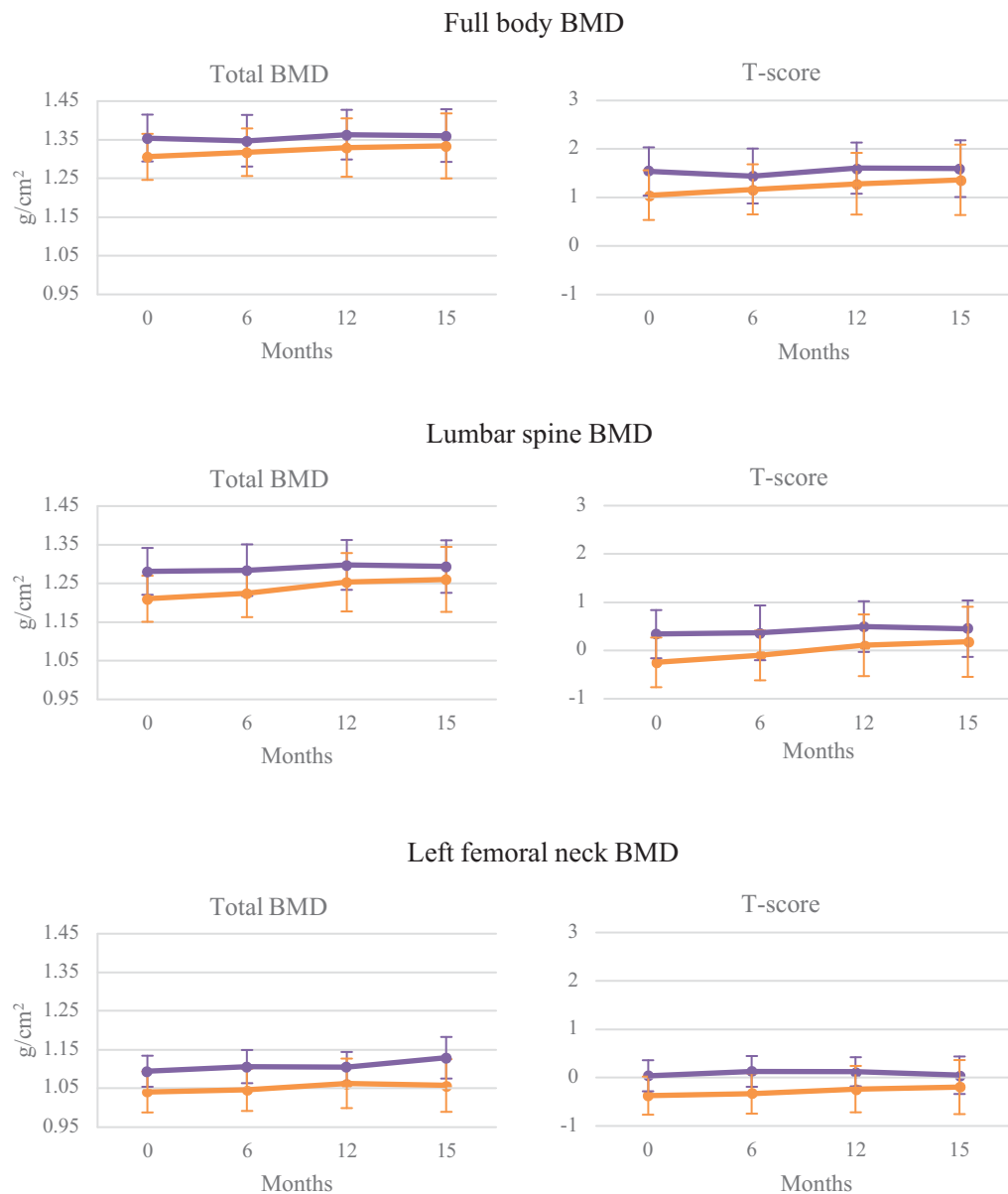


Figure 1. Mean scores with 95% confidence intervals of whole-body bone mass density (BMD), lumbar spine BMD and left femoral neck BMD at baseline, 6 months, 12 months, and 3 months post-treatment (15 months). Purple lines represent mean values for patients treated with placebo and orange lines represent patients treated with testosterone. BMD is presented as total BMD (g/cm²) and T-score.

Table 2. Mean difference in outcomes between patients treated with testosterone and patients treated with placebo during treatment (6 and 12 months) and 3 months post-treatment (15 months). The difference in changes between groups is reported with 95% confidence intervals and was estimated with a linear mixed effects model. CTX-1: C-terminal telopeptides of Type I collagen. PINP: Procollagen Type I N-Terminal Peptide.

	6 months	12 months	15 months
L2-L4 BMD			
Total (g/cm ²)	0.01 (−0.01, 0.02)	0.01 (−0.01, 0.03)	0.00 (−0.02, 0.02)
T-score	0.08 (−0.06, 0.23)	0.07 (−0.10, 0.25)	−0.01 (−0.18, 0.16)
BMD of left femoral neck			
Total (g/cm ²)	−0.02 (−0.07, 0.03)	0.00 (−0.01, 0.02)	0.00 (−0.02, 0.01)
T-score	0.04 (−0.04, 0.12)	0.05 (−0.05, 0.14)	0.12 (−0.01, 0.25)
Total body BMD			
Total (g/cm ²)	0.01 (0.00, 0.03)	0.01 (−0.01, 0.02)	0.00 (−0.01, 0.02)
T-score	0.17 (−0.01, 0.34)	0.08 (−0.08, 0.23)	0.06 (−0.11, 0.22)
CTX-1 (ng/L)	27.94 (−25.09, 80.98)	−0.102 (−57.15, 56.95)	−28.46 (−85.41, 28.48)
P1NP (µg/L)	6.758 (−0.43, 13.95)	11.65 (3.96, 19.35)	7.242 (−0.43, 14.92)

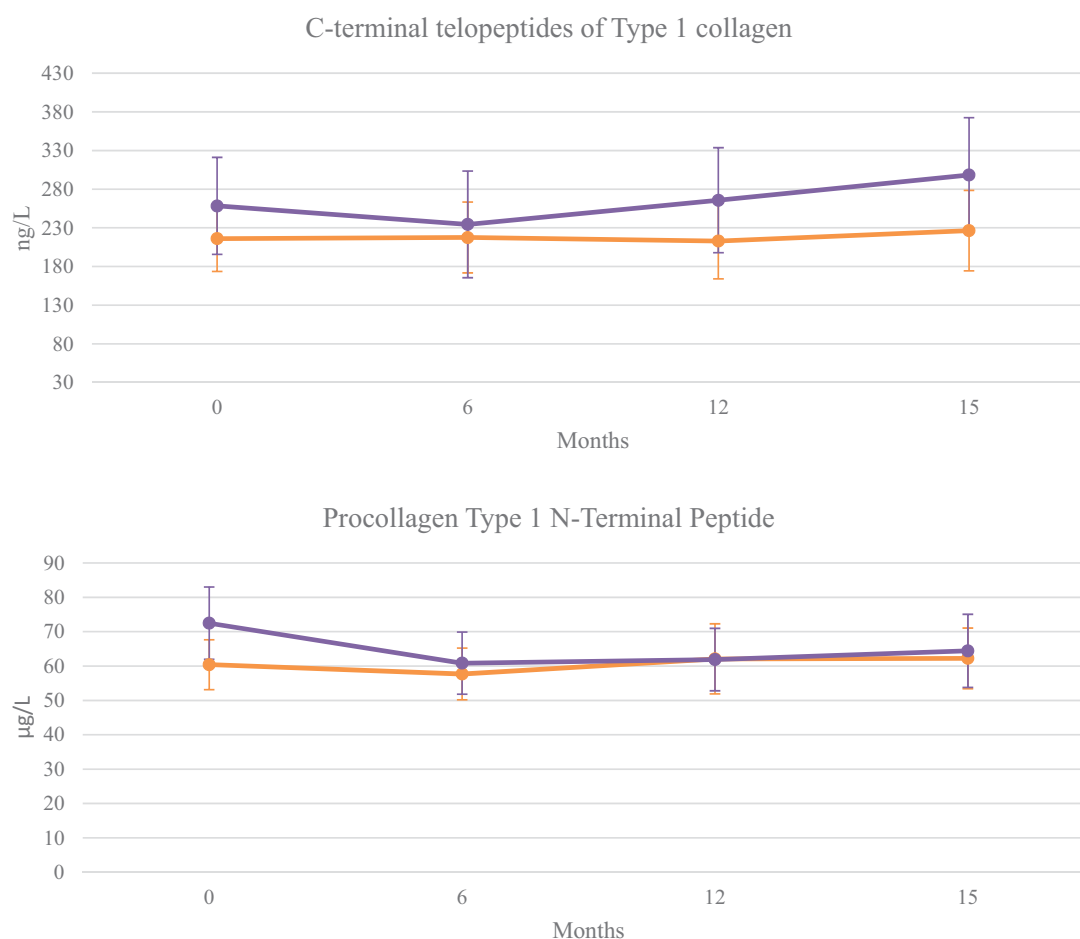


Figure 2. Mean scores with 95% confidence intervals of C-terminal telopeptides of Type 1 collagen (CTX-1) and Procollagen Type 1 N-Terminal Peptide (P1NP) at baseline, 6 months, 12 months, and 3 months post-treatment (15 months). Purple lines represent mean values for patients treated with placebo and orange lines represent patients treated with testosterone.

confidence intervals including zero suggesting no statistical difference between the groups (Table 2).

Changes in markers of bone turnover are presented in Figure 2, while the results of the linear effects model are presented in Table 2: TRT was associated with a small but statistically significant relative increase in P1NP after 12 months treatment (11.65 µg/L; 95% CI: 3.96, 19.35), while no difference was observed in CTX. Compared to patients treated with TRT, mean baseline values of CTX and P1NP were higher among patients treated with placebo, (258 ng/L vs 216 ng/L, and 72 µg/L vs 60 µg/L, respectively) (Figure 2).

Discussion

In this randomized controlled trial of 12-months TRT vs. placebo in TC survivors with mild Leydig cell insufficiency, TRT was not associated with improvement in BMD, while a small but statistically significant relative increase was observed in the marker of bone turnover P1NP.

The current study evaluated changes in BMD over a course of 15 months (as an effect of 12 months TRT). A study of 123 testosterone deficient non-cancerous men reported an increase in BMD after 42 months TRT with an effect already after the first year [23]. In another study, investigating the effect of long-term TRT in 72 non-

cancerous men with testosterone deficiency, a continuous increase in BMD was observed during 16 years of treatment, with the most significant increase in BMD observed during the first year [24]. In a study of 67 testosterone deficient non-cancerous men, TRT was associated with an increase in bone formation markers during 6 months of treatment, however, no change in BMD was observed [25]. The findings of the above-mentioned studies suggest that 12 months of TRT as in the current study might be a too short treatment duration to expect the full effect of TRT on BMD. Changes in markers of bone turnover are expected after a few months of TRT [26], and in the present study, TRT was associated with a small relative increase in P1NP compared to placebo. A change in P1NP of > 25% is considered clinically meaningful [16–18,27]. This is equivalent to at least 15 µg/L in the present trial and, based on this definition, the relative increase in P1NP of 11.65 µg/L observed after 12 months of TRT in the present study was unlikely to be clinically relevant. Both P1NP and CTX were within the normal age-adjusted range [27], and as there was no change in CTX the small relative change in P1NP might be a chance finding. A significant increase in P1NP of > 25% could potentially indicate improvement of BMD with TRT in TCS, although a change in both markers of bone turnover would be expected.

To our knowledge, there is only one other study evaluating the effect of TRT on the skeletal health of TC survivors [28]. This study included 136 young male cancer survivors with borderline low levels of testosterone, of whom 88% were TC survivors. Patients were randomised 1:1 to receive testosterone (Tostran 2% gel) or placebo for 6 months and changes in BMD were a secondary outcome. In line with the present study, no significant difference in BMD between TRT and placebo was observed with a treatment effect on BMD of 0.0 (CI: -0.01 to 0.01, p -value: 0.42). The treatment duration was, however, only 6 months, and based on the two available studies on TRT in TC survivors with borderline low testosterone levels an effect on BMD cannot be completely excluded with a treatment duration >12 months.

The present study has some important limitations: 1) The inclusion criteria were expanded to allow the inclusion of patients with almost normal testosterone levels, although most patients (61/69) had testosterone levels below the age-adjusted mean (data not shown), suggesting evidence of mild Leydig cell insufficiency 2) There was little evidence of impaired BMD at baseline and markers of bone turnover were within the normal age-adjusted range, limiting the room for improvement with TRT. 3) BMD and markers of bone turnover were a secondary endpoint, and interpretation of the present findings should be done with caution, as the study was not statistically powered to evaluate changes in BMD and markers of bone turnover.

Conclusion

TC survivors with mild Leydig cell insufficiency had little evidence of impaired BMD and markers of bone turnover were normal compared to age-matched men. Twelve months TRT was not associated with significant improvement in BMD compared to placebo, while a small and clinically irrelevant increase in the bone marker P1NP was observed. Our findings indicate that low BMD is not an important health problem in TC survivors with mild Leydig cell insufficiency and TRT should not be a standard treatment option among these patients. Our findings should preferably be validated in a larger study with a longer treatment duration, and future studies should further investigate risk factors for impaired BMD in TC survivors.

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Disclosure statement

The authors report there are no competing interests to declare.

ORCID

P. L. Jørgensen <http://orcid.org/0009-0008-9682-7796>
M. Kreiberg <http://orcid.org/0000-0001-5611-5163>
N. Jørgensen <http://orcid.org/0000-0003-4827-0838>

A. Juul <http://orcid.org/0000-0002-0534-4350>
P. S. Oturai <http://orcid.org/0000-0002-9828-9185>
C. Dehlendorff <http://orcid.org/0000-0001-6161-4565>
J. Lauritsen <http://orcid.org/0000-0002-9985-174X>
J. Rosenvilde <http://orcid.org/0000-0002-7293-2339>
G. Daugaard <http://orcid.org/0000-0002-9618-9180>
N. R. Jørgensen <http://orcid.org/0000-0001-9624-5210>
M. Bandak <http://orcid.org/0000-0003-3475-9675>

Data availability statement

According to Danish law individual patient data that underlie the results reported in this paper cannot be shared. Anonymized access to data might be possible, please contact the corresponding author regarding this. Study protocol can be shared upon request.

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