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REVIEW

Testosterone supplementation and bone parameters: a systematic review and meta-analysis study

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Abstract

Background The role of testosterone (T) replacement therapy (TRT) in subjects with late onset hypogonadism is still the object of an intense debate.

Methods All observational studies and placebo-controlled or -uncontrolled randomized trials (RCTs) comparing the effect of TRT on different bone parameters were considered.

Results Out of 349 articles, 36 were considered, including 3103 individuals with a mean trial duration of 66.6 weeks. TRT improves areal bone mineral density (aBMD) at the spine and femoral neck levels in observational studies, whereas placebo-controlled RTCs showed a positive effect of TRT only at lumber spine and when trials included only hypogonadal patients at baseline (total testosterone < 12 nM). The effects on aBMD were more evident in subjects with lower T levels at baseline and increased as a function of trial duration and a higher prevalence of diabetic subjects. Either T or estradiol increase at endpoint contributed to aBMD improvement. TRT was associated with a significant reduction of bone resorption markers in observational but not in controlled studies.

Conclusion TRT is able to inhibit bone resorption and increase bone mass, particularly at the lumbar spine level and when the duration is long enough to allow the anabolic effect of T and estrogens on bone metabolism to take place.

Keywords Testosterone · Bone · Bone mineral density · Hypogonadism · Late-onset hypogonadism

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Introduction

Testosterone (T) is essential for bone health during all ages [1], by acting though the androgen receptor expressed on osteoblasts, osteoclasts, osteocytes and marrow stromal cells [2, 3]. Furthermore, these cells express the estrogen receptor, which responds to estradiol (E2) formed from T aromatization through the activity of the CYP19A1 enzyme (aromatase) [4]. During puberty, T together with E2, stimulate periosteal apposition and trabecular bone growth, participate in pubertal growth spurts and acquisition of peak bone mass [1, 5, 6]. As a sum, T action leads to the development of wider bones with a thicker cortex as compared to women, and the final result is a higher peak bone mass and bigger, though not denser, skeletons in men with respect to women [1, 6, 7]. Once the peak bone mass has been achieved, T helps to maintain bone density and strength, by slowing the bone remodeling rate and by maintaining a balance between resorption and formation [1, 6].

Therefore, reduced T levels occurring either before achieving peak bone mass or during adulthood and senescence, might seriously affect bone health, in terms of mass and strength [6]. In addition, it is important to emphasize that other functions of the Leydig cells are important in the testis–bone crosstalk: they produce the peptide hormone Insulin-Like Factor 3 (INSL3) and participate in the activation of vitamin D by converting the inactive cholecalciferol into 25OH-D3 (calcifediol) [6, 8].

As a consequence, the presence of hypogonadism, both in young men (e.g., Klinefelter syndrome) and in aging subjects (late onset hypogonadism—LOH), is associated with lower bone mineral density (BMD) and represents a major risk factor for osteoporosis [6, 8]. Accordingly, current guidelines suggest that hypogonadal patients, patients who need androgen deprivation therapy, and men with a well-documented history of hypogonadism should be screened for osteoporosis by Dual energy X-ray Absorptiometry (DXA) [9]. On the other hand, osteoporotic men should be screened for hypogonadism and eventually treated [9, 10].

According to available guidelines, testosterone replacement therapy (TRT) in the setting of male osteoporosis is particularly recommended in young adult hypogonadal men to prevent bone loss and help acquire peak bone mass [2, 9], and it should be associated with antiresorptive drugs when fracture risk is high [9, 11]. Actually, the effect of TRT alone on bone health in hypogonadal men is still not well defined [6, 12], and no studies with fractures as their primary endpoint have been performed. Indeed, it is assumed that TRT can improve BMD, particularly at the vertebral level and when T levels are very low [6, 9, 13, 14]. The effect is more evident in younger men with organic hypogonadism, as a meta-analysis showed in men with Klinefelter syndrome [15]. In the other groups of patients, mainly older men with functional hypogonadism [11, 16–18], or specific categories of patients, such as those with HIV [19], the benefits of TRT are not well established. The combination of TRT with antiosteoporotic drugs has not been investigated, whereas the combination of TRT with vitamin D and calcium seems more effective in increasing BMD than TRT alone, at least in men with Klinefelter syndrome [20]. In older men, data are limited and heterogeneous in terms of patient selection, definition of hypogonadism and type and duration of TRT.

Therefore, the effect of TRT alone on bone health in hypogonadal men is still not well defined [12, 21]. Here, we aimed at investigating and meta-analyzing the effect of T supplementation on different bone parameters either in observational or placebo controlled and uncontrolled randomized studies.

Methods

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist [see Supplementary file 1].

An extensive Medline, Embase and Cochrane search was performed including the following words ("testosterone"[MeSH Terms] OR "testosterone"[All Fields] OR "testosteron"[All Fields] OR "testosterones"[All Fields] OR "testosterone s"[All Fields]) AND ("bone and bones"[MeSH Terms] OR ("bone"[All Fields] AND "bones"[All Fields]) OR "bone and bones"[All Fields] OR "bone"[All Fields]) AND ("therapeutics"[MeSH Terms] OR "therapeutics"[All Fields] OR "treatments"[All Fields] OR "therapy"[MeSH Subheading] OR "therapy"[All Fields] OR "treatment"[All Fields] OR "treatment s"[All Fields]) AND ("hypogonad"[All Fields] OR "hypogonadal"[All Fields] OR "hypogonadic"[All Fields] OR "hypogonadism"[MeSH Terms] OR "hypogonadism"[All Fields] OR "hypogonadisms"[All Fields]) AND (("testosterone"[MeSH Terms] OR "testosterone"[All Fields] OR "testosteron"[All Fields] OR "testosterones"[All Fields] OR "testosterone s"[All Fields]) AND ("hormone replacement therapy"[MeSH Terms] OR ("hormone"[All Fields] AND "replacement"[All Fields] AND "therapy"[All Fields]) OR "hormone replacement therapy"[All Fields] OR ("replacement"[All Fields] AND "therapy"[All Fields]) OR "replacement therapy"[All Fields])). The search accrued data from January 1, 1969 up to April 15th, 2021. The identification of relevant studies was performed independently by two of the authors (V.G, W.V), and conflicts resolved by the third investigator (A.P). We did not employ search software. We hand-searched

bibliographies of retrieved papers for additional references. The principal source of information was derived from published articles; if data were missing from a publication, an attempt at retrieval was made through clinicaltrial.gov website.

Study selection

We included all observational studies and placebo-controlled or -uncontrolled randomized trials (RCTs) comparing the effect of TRT on different endpoints [22–57] (see also Fig. 1, Table 1 and Supplementary Table 1). Only studies reporting data on areal bone mineral density (aBMD) expressed as g/cm^2 and/or including results on bone remodeling markers were considered (see also Supplementary Table 1). Studies using androgens other than T, as well as studies with concomitant treatment with other hormones and drugs were also excluded, unless there was a clearly defined treatment arm that received only T treatment. Similarly, studies including only patients with genetic causes of male hypogonadism, such as Klinefelter Syndrome, were excluded from the analysis and revised elsewhere [15].

Outcomes

The principal outcome of this analysis was the effect of TRT, as compared with baseline, placebo or control groups, on aBMD at lumbar and femoral levels. Secondary outcomes included several other bone related parameters (Supplementary Table 1). In particular, bone resorption markers analyzed include cross-link, urinary deoxypyridinoline, serum C-terminal telopeptide and serum N-terminal telopeptide. Bone neoformation markers analyzed include bone alkaline phosphatase, propeptide collagen, and osteocalcin.

Quality assessment

The quality of trials was assessed using the Cochrane criteria [58] (see also Supplementary Table 2). In particular, for RCTs, the following criteria were evaluated: how the randomization sequence was generated, how allocation was concealed, whether there were important imbalances at baseline, which groups were blinded (patients, caregivers, data collectors, outcome assessors, data analysts), what the loss to follow-up rate was (in the intervention and the control arm), whether the analyses were by intention to treat, and how missing outcome data were dealt with. In observational studies we evaluated the following criteria: the weaknesses of the designs that have been used (such as noting their potential to ascertain causality), the execution of the studies through a careful assessment of their risk of bias, especially the potential for selection bias and confounding to which all observational studies are susceptible, and the potential for reporting biases, including selective reporting of outcomes For each study, we also assessed how the population was selected, the duration and route of TRT, and the adequacy of study follow-up [58].

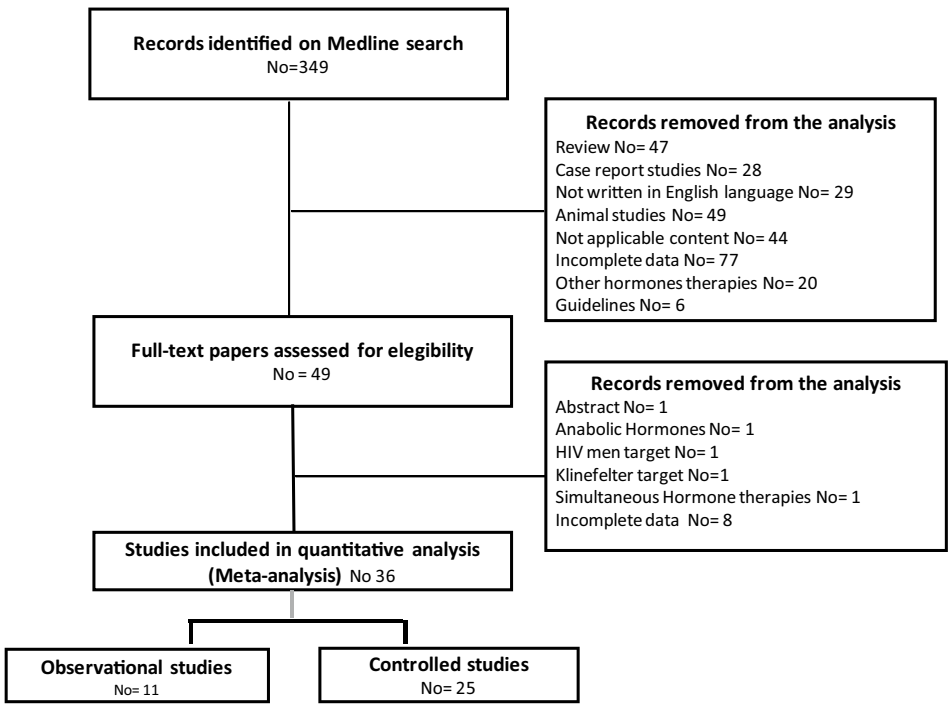

Fig. 1 Trial flow diagram for a systematic review and meta-analysis

Table 1 Characteristics of trials included in the study

Study	No. of treated subjects	No. of untreated subjects	Age (years)	BMI (kg/m^2)	Aetiology of hypogonadism	Study population	Diabetes mellitus prevalence (%)	Total T levels (nmol/L)	TRT regimen	Treatment duration (weeks)	T cutoff for hypogonadism diagnosis	Placebo group	Control group
Morley et al., 1993 [22]	9	7	76.8		LOH	Hypogonadism		9.3	TE 200 mg/2 weeks	12	Bioavailable $T < 70$ ng/dL	0	1
Young et al. 1993 [23]	13	8	30.2	24.7		Healthy subjects	0	20.7	TE 200 mg/week	24	–	0	1
Katznelson et al., 1996 [24]	36	–	53	28.2	Mixed (organic and LOH)	Hypogonadism	0	6.4	TE 100 mg/week	72	10.4 nmol/L	0	0
Hall et al., 1996 [25]	15	15	61.8		Medical treatment (RA)	Mixed[a]		16.1	TE 250 mg/4 weeks	36	–	1	1
Reid et al. 1996 [26]	15	15	61	26.7	Medical treatment (asthma)	Mixed[a]		11.9	T esters (30 proprionate, 60 phenylprionate, 60 isocaproate, 100 decanoate) 250 mg/month	52	–	0	1
Wang et al., 1996 [27]	67			28	Mixed (organic and LOH)	Hypogonadism		4.13	Sublingual T 5 mg 3 times/day	24	8.7 nmol/L	0	0
Snyder et al., 1999 [28]	54	54	73	24.1	LOH	Mixed[a]		12.7	T patch 6 mg/day	144	–	1	1
Snyder et al. 2000 [29]	18	–	51		Mixed (organic and LOH)	Hypogonadism		2.7	T patch 6 mg/day	156	8.7 nmol/L	0	0
Kenny et al., 2001 [30]	34	33	76	26.5	LOH	Mixed[a]		13.5	transdermal T 5 mg/day	52	–	1	1
Howell et al., 2001 [31]	16	19	40.9	25.6	Primary hypogonadism (post CT)	Mixed[a]		13.3	T pacth 2.5–5 mg/d	52	–	1	1

Table 1 (continued)

Study	No. of treated subjects	No. of untreated subjects	Age (years)	BMI (kg/m^2)	Aetiology of hypogonadism	Study population	Diabetes mellitus prevalence (%)	Total T levels (nmol/L)	TRT regimen	Treatment duration (weeks)	T cutoff for hypogonadism diagnosis	Placebo group	Control group
Wang et al., 2001 [32]	76	–	51.1	28.9	Mixed (LOH and organic)	Hypogonadism	0	8.22	T pacth 5 mg/day	24	10.4 nmol/L	0	0
Wang et al., 2001[a] [32]	73	–	51.3	29.3	Mixed (LOH and organic)	Hypogonadism	0	8.22	T gel 50 mg/day	24	10.4 nmol/L	0	0
Wang et al., 2001[b] [32]	78	–	51	28.7	Mixed (LOH and organic)	Hypogonadism	0	8.6	T gel 100 mg/day	24	10.4 nmol/L	0	0
Crawford et al., 2003 [33]	18	16	59.2	26.1	Medical treatment (CS)	Mixed[a]		14.6	TE 200 mg/week	52	–	1	1
Shoubert et al., 2003 [34]	13	–	34.5	25.7	Organic primary and secondary hypogonadism	Hypogonadism	0	2.9	TU 160 mg/day	30	3.6 nmol/L	0	0
Shoubert et al., 2003[a] [34]	14	–	31.9	24.8	Organic primary and secondary hypogonadism	Hypogonadism	0	2.2	TE 250 mg 21 weeks	30	3.6 nmol/L	0	0
Shoubert et al., 2003[b] [34]	15	–	35.8	25.6	Organic primary and secondary hypogonadism	Hypogonadism	0	2.7	pellet 1200 mg	30	3.6 nmol/L	0	0
Amory et al. 2004 [35]	24	24	71	28.2	LOH	Hypogonadism		10.2	TE 200 mg/2 weeks	156	12.1 nmol/L	1	1
Wang et al. 2004 [36]	123	–	51.5	29	Mixed (LOH and organic)	Hypogonadism		8.2	T gel 50–100 mg/day	156	10.4 nmol/L	0	0

Table 1 (continued)

Study	No. of treated subjects	No. of untreated subjects	Age (years)	BMI (kg/m^2)	Aetiology of hypogonadism	Study population	Diabetes mellitus prevalence (%)	Total T levels (nmol/L)	TRT regimen	Treatment duration (weeks)	T cutoff for hypogonadism diagnosis	Placebo group	Control group
Benito et al. 2005 [37]	10	–	54		Secondary hypogonadism	Hypogonadism		3.1	T gel 50–100 mg/day	104	10.4 nmol/L	0	0
Merza et al. 2005 [38]	20	19	61.4		LOH	Hypogonadism		7.96	T patch 5 mg/day	24	10 nmol/L	1	1
Emmelot-Vonk et al., 2008 [39]	113	110	67.2	27.3	LOH	Mixed[a]	0	10.7	TU 80 mg/2 times per day	24	–	1	1
Svartberg et al., 2008 [40]	17	18	69	29.5	LOH	Hypogonadism	8.6	8.29	TU 1000 mg/12 weeks	40	11 nmol/L	1	1
Kenny et al., 2010 [41]	69	62	77.1	26.9	LOH	Mixed[a]	14	13.8	T gel 5 mg/day	52	–	1	1
Aversa et al., 2012 [42]	20	20	57	30.5	Functional hypogonadism	Hypogonadism	35	8.3	TU 1000 mg/12 weeks	144	11 nmol/L	0	1
Bhere et al. 2012 [43]	183	179	62	28.5	LOH	Mixed[a]		10.49	T gel 50 mg/day	24	–	1	1
Deb et al. 2012 [44]	13	–	25.5	22.2	Organic primary and secondary hypogonadism	Hypogonadism		2.56	TE TP 250 mg/2–3 weeks	24	10 nmol/L	0	0
Borst et al., 2014 [45]	14	16	70	29.9	LOH	Hypogonadism		8.82	TE 125 mg/week	52	10.4 nmol/L	1	1
Bouloux et al. 2013 [46]	237	79	58.7	27.3	LOH	Mixed[a]		12.8	TU 80–240/day	52	–	1	1
Lee et al. 2014 [47]	21	–	44	26.7	Secondary hypogonadism (pituitary tumors)	Hypogonadism		5.44	Mixed	242	12 nmol/L	0	0

Table 1 (continued)

Study	No. of treated subjects	No. of untreated subjects	Age (years)	BMI (kg/m^2)	Aetiology of hypogonadism	Study population	Diabetes mellitus prevalence (%)	Total T levels (nmol/L)	TRT regimen	Treatment duration (weeks)	T cutoff for hypogonadism diagnosis	Placebo group	Control group
Rodriguez-Tolra et al. 2013 [48]	50	–	59.14	29	LOH	Mixed[a]		10.19	T gel 50 mg/day	104	–	0	0
Wang et al., 2013 [49]	124	58	62	28.2	LOH	Hypogonadism	0	7.47	TU 20–40/day	104	10.4 nmol/L	1	1
Francomano et al., 2014 [50]	20	20	57.5	31	LOH and MetS	Hypogonadism	30	8.65	TU 1000 mg/12 weeks	260	11 nmol/L	0	1
Tirabassi et al. 2014 [51]	12	–	56		Secondary hypogonadism	Hypogonadism		5.4	TU 1000 mg/12 weeks	80	8 nmol/L	0	0
Dias et al., 2016 [52]	13	9	72	29	LOH	Hypogonadism	0	10.32	T gel 50 mg/day	52	12 nmol/L	1	1
Snyder et al., 2017 [53]	110	101	72.3	31.2	LOH	Hypogonadism	39.3	7.88	T gel 50 mg/day	52	7 nmol/L	1	1
Ng Tang Fui et al., 2018 [54]	48	51	53.4	37.3	LOH	Hypogonadism	22	8.44	TU 1000 mg/12 weeks	56	12 nmol/L	1	1
Barnouin et al., 2021 [55]	42	41	72.9	37	LOH	Hypogonadism		7.75	Androgel 1.62%, 40.5 mg/day	26	10.4 nmol/L	1	1
Colleluori et al., 2021 [57]	49	56[b]	59.7	32.2	LOH	Hypogonadism	46.7	9.3	T cypionate 200 mg/2 weeks	78	10.4 nmol/L	0	
Ng Tang Fui et al., 2021 [56]	92	85	59.6	34.4	LOH	Mixed[a]	20	10.04	TU 1000 mg/12 weeks	108	14 nmol/L	1	1

BMI body mass index, *LOH* late-onset hypogonadism, *T* testosterone, *TRT* testosterone replacement treatment, *RA* reumathoid arthritis, *CT* chemotherapy, *CS* corticosteroids

[a]Studies including both hypogonadal and eugonadal subjects

[b]Non diabetic treated subjects

Statistical analysis

Heterogeneity was assessed using I^2 statistics. Even when a low heterogeneity was detected, a random-effects model was applied, because the validity of tests of heterogeneity can be limited with a small number of component studies. To estimate possible publication or disclosure bias we used funnel plots and the Begg adjusted rank correlation test [58, 59]. However, because these tests have low statistical power when the number of trials is small, undetected bias may still be present. In addition, since in some trials the significance of between group comparisons (p) was not reported, the analysis was performed evaluating the endpoint values of each parameter in different treatment groups, in a non-paired fashion (non-paired analysis). Considering that most of the studies, which did not describe p values, reported non-significant differences across groups, the mean (paired) analysis, which excludes those data is likely to overestimate the effect of treatments. On the other hand, the non-paired analysis is a very conservative approach, which could underestimate treatment effect. Since bone remodeling parameters were evaluated through different approaches and expressed in different ways, the mean difference for each study was divided by the pooled estimate of the SD, to express the effect size for each study in a common metric, namely, the standardized mean difference (SMD). According to Cohen [60], a small treatment-effect size is considered to be about 0.2, a medium effect size to be about 0.5, and a large effect size to be about 0.8. All other data were expressed as weight mean differences. Meta-regression analyses were performed to test the effect of different parameters on aBMD modification. In addition, a multivariate linear regression analysis model, weighting each study for the number of subjects enrolled, was performed to verify in controlled studies the effect of TRT on several parameters (see below). All analyses were performed using Comprehensive Meta-analysis Version 2, Biostat (Englewood, NJ, USA). Multivariate analyses were performed on SPSS (Statistical Package for the Social Sciences; Chicago, USA) for Windows 25.0.

Results

Out of 349 retrieved articles, 36 were included in the study (Fig. 1). In particular, 25 were controlled studies and 11 observational. Among the controlled studies, 19 were placebo-controlled RCTs. The characteristics of the retrieved trials (including parameters on trial quality) and type of outcomes considered are reported in Table 1 and Supplementary Tables 1 and 2. The retrieved studies included 1988 and 1115 individuals in TRT and control groups, respectively; mean trial duration was 66.6 weeks. TRT was administered in different doses, formulations and cohorts (Table 1). The vast majority of the studies included subjects with LOH or a mixed population of organic and functional hypogonadism, whereas only a limited number of the trials enrolled only hypogonadal subjects with an organic origin (Table 1).

The mean age, baseline T and body mass index (BMI) of enrolled patients were 57.2 years, 8.8 nmol/L and 28.5 kg/m^2. Subjects enrolled in controlled studies were older (63.3 ± 10.9 vs. 46.4 ± 10.3 years old; $p < 0.0001$) and had higher T levels (10.9 ± 3.2 vs. 5.4 ± 2.7 nmol/L; $p < 0.0001$), whereas the duration of follow-up (68.9 ± 56.3 vs. 62.5 ± 47.7 weeks; $p = 0.703$), and BMI (29.2 ± 3.6 vs. 27.2 ± 2.2 kg/m^2; $p = 0.09$) was similar in comparison with observational studies. Similar results were observed when placebo-controlled RCTs were compared to observational studies (not shown).

Bone mineral density

Among studies reporting several outcomes, 35 out of 36 included information on aBMD at least in one site, whereas 30 studies analyzed aBMD in more than one section (Supplementary Table 1). When lumbar aBMD was considered, I^2 for controlled and observational studies were 74.43, $p < 0.0001$ and 43.61, $p = 0.036$. Funnel plot and Begg-adjusted rank correlation test suggested no major publication bias in both types of studies (Kendall's τ: 0.10; $p = 0.53$ and -0.17; $p = 0.45$ for controlled and observational studies, respectively). TRT resulted in a significant improvement of aBMD at lumbar and femoral neck level both in controlled and in observational studies (Fig. 2, Supplementary Figs. 1A, B and 2A, B). Similar data were observed when those trials including only subjects with organic problems were excluded from the analysis (aBMD = 0.040[0.026; 0.054], $p < 0.0001$ and 0.041[0.013; 0.069], $p = 0.004$ for observational and controlled studies at endpoint, respectively). Since weighted baseline aBMD was 1.169 ± 0.185 g/cm^2 and 0.870 ± 0.134 g/cm^2 at lumbar and femoral neck, respectively, the relative observed increase of aBMD over controls at the endpoint was 3.08[0.86; 5.22]% and 2.07[0.23; 3.80]% at lumbar at femoral neck.

When aBMD at the lumbar level was considered and the analysis was limited to placebo-controlled RCTs, only a trend toward a significant effect was detected (Fig. 2 and Supplementary Fig. 1C). However, when the latter data were analyzed by including only those studies enrolling hypogonadal subjects (baseline total testosterone < 12 nmol/L), a significant effect of TRT was observed even in placebo-controlled RCTs (Fig. 2 and Supplementary Fig. 1D). Similar data were observed when the analysis was limited to those studies with a mean age of the population above 60 years (not shown). The relative lumbar aBMD increase over placebo at endpoint was 4.40[0.59; 8.20]% in RCTs on hypogonadal subjects. Conversely, no effect of TRT was

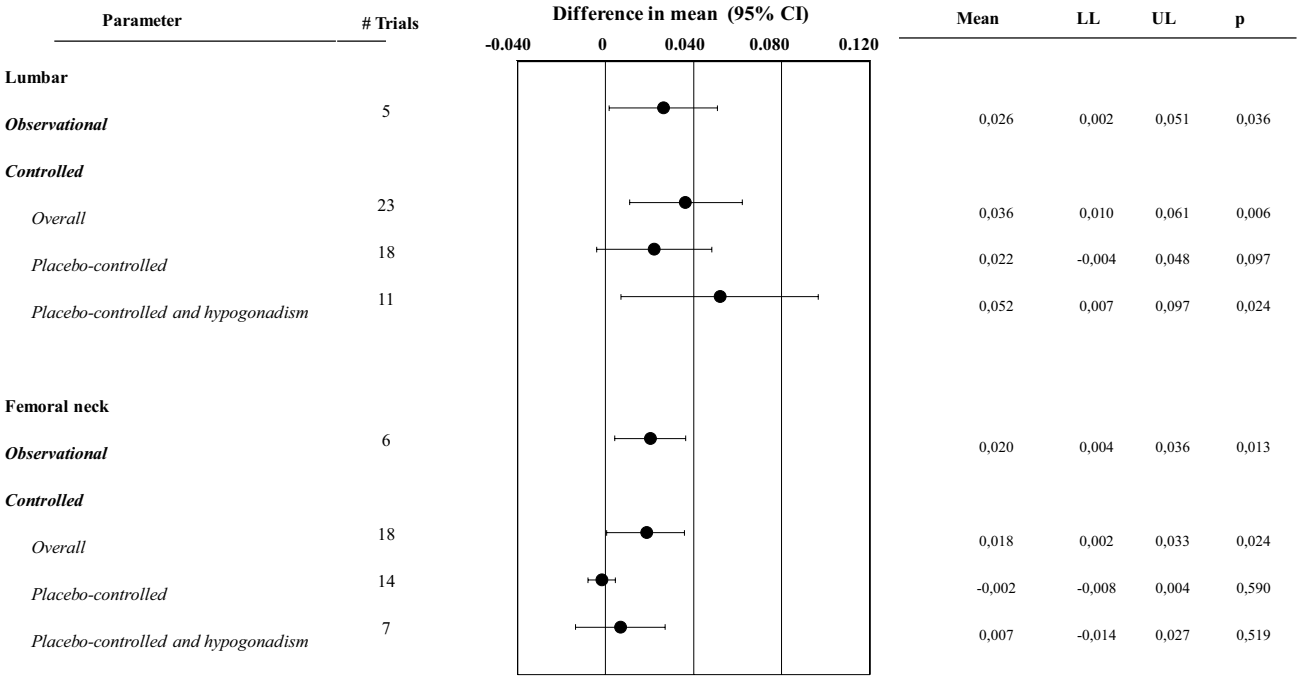

Fig. 2 Overall effects of testosterone replacement therapy at lumbar and femoral neck level in observational and controlled studies. *LL* lower levels, *UP* upper levels

observed at femoral neck when placebo-controlled RCTs were analyzed, even when the data were limited to those studies including only hypogonadal patients (Fig. 2 and Supplementary Fig. 2C, D). When other bone sections were analyzed, TRT resulted in a significant increase in aBMD at femoral trochanter level in observational but not in controlled trials (Table 2 and Supplementary Fig. 3A, B). No further differences were observed in aBMD evaluated at different sites—including femoral wards, hip, radial and total body—when either observational or controlled studies were considered (Table 2 and Supplementary Fig. 3C–L). No further sub-analyses were possible due to the limited data.

In controlled studies, meta-regression analysis showed that the effect of TRT on aBMD at lumbar level was higher with longer duration and when a higher proportion of diabetic subjects was included (Fig. 3A, B). In addition, an

Table 2 Overall effects of testosterone replacement therapy at different bone sections in observational and controlled studies

	Mean differences	Lower level	Upper level	*p*
Hip				
Observational ($n=4$)	0.012	− 0.010	0.033	0.297
Controlled ($n=10$)	0.012	− 0.005	0.029	0.166
Femoral trochanter				
Observational ($n=4$)	0.030	0.018	0.042	0.000
Controlled ($n=6$)	0.015	− 0.006	0.035	0.160
Femoral wards				
Observational ($n=2$)	0.102	− 0.070	0.275	0.246
Controlled ($n=4$)	0.006	− 0.024	0.036	0.703
Radial				
Observational ($n=2$)	0.023	− 0.002	0.049	0.072
Controlled	0.006	− 0.001	0.035	0.160
Total body				
Observational ($n=1$)	− 0.003	− 0.065	0.059	0.925
Controlled ($n=6$)	− 0.005	− 0.010	0.0001	0.081

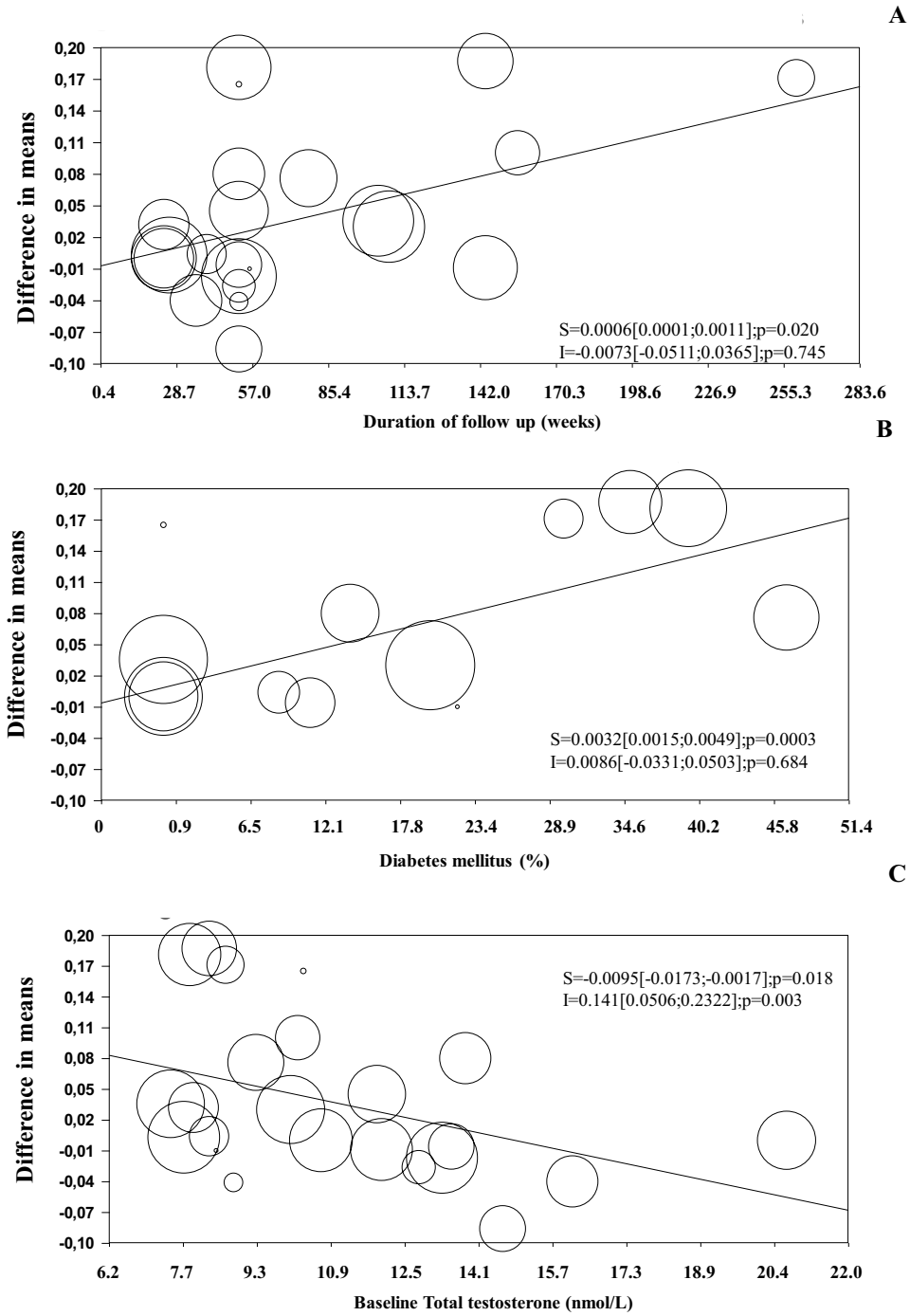

Fig. 3 Effects of testosterone replacement therapy of lumbar bone mineral density as derived from controlled studies according to different parameters: trial duration (**A**), diabetes mellitus (**B**), and baseline total testosterone levels (**C**)

inverse relationship between baseline T levels and lumbar aBMD at follow-up was also observed (Fig. 3C). The association between lumbar aBMD differences at follow-up and Diabetes Mellitus (DM) or T levels at baseline were confirmed after alternative multivariate linear regression analyses, weighting each study for the number of subjects enrolled and adjusting for trial duration, age and BMI ($\beta = 0.817$ and -0.339 for baseline TT and DM, respectively; all $p < 0.0001$). The association between lumbar aBMD differences at the endpoint and DM was confirmed even after the adjustment for baseline TT levels ($\beta = 0.814$, $p < 0.0001$). As expected, TRT resulted in a significant increase of circulating TT and E2 levels at endpoint when compared to controls (Supplementary Fig. 4A, B). Both E2 and TT differences significantly contributed to aBMD modifications at follow-up, even after the adjustment for trial duration, age and BMI ($\beta = 0.312$ and 0.127 for TT and E2, respectively; both $p < 0.0001$).

Remodelling bone markers

Information on bone remodeling markers was available in 20 studies. In particular, among them 12 were controlled and eight were observational studies. TRT was associated with a significant reduction of bone resorption markers in observational but not in controlled studies (Table 3 and Supplementary Fig. 5C, D). Conversely, no modification of bone neoformation markers either in observational or in controlled studies was observed (Table 3 and Supplementary Fig. 5A, B). No difference in both resorption markers in controlled studies were detected even when the data were limited to placebo-controlled RCTs including only hypogonadal patients (not shown).

Discussion

This is the largest meta-analysis evaluating the effects of TRT in male patients on several bone-related outcomes. In addition, for the first time, both controlled and observational studies were analyzed. Our data indicate that TRT improves aBMD either at spine or femoral neck level both in uncontrolled and controlled trials. However, when the analysis was limited to placebo-controlled RCTs, the positive effects of TRT were limited to lumber spine and to those trials including only hypogonadal patients at baseline (TT < 12 nM). Interestingly, the results were more robust in subjects with lower T levels at baseline; in addition, the effect increased as a function of trial duration. Both TT and E2 increase at endpoint independently contributed to aBMD improvement at lumber level. Finally, an original finding of this study is that TRT resulted in a better aBMD increase at lumbar levels in those studies including a larger proportion of diabetic patients.

Table 3 Overall effects of testosterone replacement therapy on bone resorption or neoformation markers at endpoint across observational and controlled trials

	Standarized mean differences	Lower level	Upper level	*p*
Neoformation markers				
Observational ($n=8$)	− 0.402	− 0.853	0.049	0.081
Controlled ($n=12$)	− 0.038	− 0.284	0.208	0.764
Resorption markers				
Observational ($n=8$)	− 1.308	− 2.107	− 0.508	0.000
Controlled ($n=7$)	− 0.094	− 0.275	0.086	0.305

Bone resorption markers analyzed include cross-link, urinary deoxypyridinoline, serum C-terminal telopeptide and serum N-terminal telopeptide. Bone neoformation markers analyzed include bone alkaline phosphatase, propeptide collagen, and osteocalcin

Diabetes mellitus (DM) and osteoporosis are chronic medical conditions commonly affecting aging people [61, 62]. The specific role of DM in the pathogenesis of reduced aBMD, osteoporosis and bone fracture risk is still an object of an intense debate [61, 62]. In 1927, Morrison and Bogan [63] reported, for the first time, a possible association between DM and bone loss. A decreased peak of bone mass-due to insulin and/or insulin-like growth factor defects, causing reduced osteoblast proliferation and poor collagen synthesis—has been considered a crucial factor in type 1 DM (T1DM) [64]. Conversely, the possible association between bone health and type 2 DM (T2DM) is more controversial. Accordingly, normal, reduced or even increased aBMD has been described in T2DM [65–67]. Despite the evidence related to aBMD, several studies have described an increased risk of hip, vertebral and non-vertebral fractures both in T1DM and T2DM [61, 68, 69]. Chronic hyperglycemia and increased advanced glycation end products (AGEs) may support the modification of local bone metabolism, resulting in structural abnormalities, including trabecular bone loss, decreased cortical BMD and increased cortical porosity, which eventually leads to a decreased bone strength [70]. In addition several antidiabetic drugs can contribute to the increased risk of fractures, by increasing the risk of hypoglycemic episodes and falls (e.g., sulfonylureas) [71, 72] or interacting with bone metabolism at several levels, such as thiazolidinediones [71, 73, 74]. The present study suggests that sex hormone alteration should be considered as another important factor in the pathogenesis of bone loss in DM subjects. A large body of evidence has documented that DM, and T2DM in particular, is associated with reduced T levels [75–78]. The pathogenetic mechanisms underlining the latter association are not completely understood and revised elsewhere [76, 77]. Several observational studies have documented that TRT can improve body composition and metabolic profile in T2DM [79, 80]. However, data derived from placebo-controlled RCTs are more conflicting [81]. The present meta-analysis suggests that TRT might improve aBMD at lumbar levels, particularly in the diabetic population. These data are in line to what recently reported by Collelouri et al. [57], in a single arm, open-label clinical trial involving 105 hypogonadal (total $T < 10.4$ nmol/L) with or without T2DM. After 18 month TRT resulted in greater BMD improvement at lumbar in diabetic subjects when compared to non-diabetic counterparts [57]. Whether or not the latter result might reduce the risk of fractures in either a diabetic or general population cannot be determined by the present data and should be investigated in further studies. In addition, it is important to recognize that the role of DM in T-induced aBMD improvement, at least at the

spine level, was confirmed even after the adjustment for T levels, supporting the multifactorial origin of the osteopenia/osteoporosis in the diabetic population.

The association between mild-to-severe T deficiency, reduced BMD and an increased risk of osteopenia/osteoporosis in young adult men with organic hypogonadism is well documented [2, 3]. Conversely, the role of T and TRT on bone homeostasis in aging men with LOH or functional hypogonadism is more conflicting [9, 11]. Data from several population based studies, including the European Male Aging Study (EMAS) [82], the Rancho Bernardo Study [83, 84] and the Tromso Study [85], have reported an inverse association between bioavailable T levels and aBMD. However, data from the EMAS indicated that only individuals with overt hypogonadism (total $T < 8$ nmol/L) have reduced aBMD, when compared to eugonadal subjects [82]. In addition, the same studies have also disclosed a direct relationship between serum E2 levels, particularly the bioavailable fraction, and aBMD, supporting a role for the relative decline of E2, frequently observed in aging men, and bone health [3]. Similarly, Finkelstein et al. [86], in an elegant RCT including 198 healthy men, receiving goserelin acetate to suppresses endogenous gonadal steroid production, showed an association between worse BMD when T levels were below 7 nmol/L. These results have been replicated by the same group [87] and by others authors [88].

The present data are essentially in line with what has been previously reported. The effects of TRT at the endpoint were more defined in patients with a more severe hypogonadism at baseline and confirmed in placebo-controlled RCTs, when only those studies including hypogonadal subjects (total $T < 12$ nmol/L) were considered. Similar results were previously reported when other outcomes, such as sexual function and metabolic profile, were analyzed [89]. Interestingly, the present meta-analysis suggests that both a T or E2 increase at endpoint independently contribute to the increase of aBMD, at least at the lumbar level, supporting a possible role of both sex steroids in bone homeostasis regulation in aging males. Accordingly, these results were confirmed even when only studies considering patients with LOH were investigated. The role of circulating or locally produced estrogens on bone homeostasis is well known and revised elsewhere [5]. T has direct and indirect effects contributing to the maintenance of correct bone homeostasis [3, 8, 9]. In particular, besides the direct effects on osteoblast differentiation and proliferation, T indirectly regulates the activity of osteoclasts by the modulation of the receptor activator of nuclear factor $k - B$ (RANK–ligand). In addition, other indirect effects include the positive action of T on several growth factors and cytokines, such as growth hormone and insulin-like-growth factor 1. Finally, the positive effects of T on muscle mass might positively contribute to bone heath and to a possible fracture risk reduction [8, 9].

Data derived from the present meta-analysis are essentially in line with what was reported by Isidori et al. [90] more than 15 years ago. In fact, TRT resulted in about a 3% and 2% increase in aBMD at lumbar and femoral neck, respectively, when compared to placebo. The data were even more impressive when hypogonadal subjects were considered (up to 8%). Interestingly, antiresorptive drug therapy produces in 12–24 months an effect size ranging from 0.3 to 3.8% using alendronate [91, 92] denosumab [91] or zolendronic acid [93]. Data derived from other available meta-analyses were either supporting [14] or not [16–18], the positive effects of TRT on aBMD. The differences in the study selection and the lack of sub-analysis according to baseline T levels and hypogonadal status can explain, at least partially, the conflicting results [16–18]. In addition, it is important to recognize that the vast majority of the available RCTs have used dual energy absorptiometry (DXA), measuring areal BMD (aBMD). More recently, the quantitative computed tomography (QCT) method, measuring volumetric BMD (vBMD), has been introduced. The latter can distinguish cortical from trabecular structures and predicts fracture risk, independently from aBMD and FRAX score [94]. Using QTC a recent large, placebo-controlled RTC showed that TRT increased vBMD particularly in cortical bone at both tibia and radius, with an effects size ranging from 2.9 to 3.1% after 2 years of treatment [56]. In addition, data from the T-trials study, including 211 patients older than 60 years, found that, after 1 year, TRT resulted in a vBMD increase, particularly at trabecular spine [53]. Data derived from longitudinal studies using QCT have documented that the age-related decline of sex steroids is associated with an accelerated bone loss, particularly at the cortical site [6]. Uncontrolled small studies have shown that TRT can improve both cortical and trabecular bone [24, 37, 91–93, 95]. Hence, available data support the hypothesis that TRT in hypogonadal men can improve both BMD and bone structure.

Another original finding of the present meta-analysis is the evaluation of TRT in observational studies. Overall, the data support what has been derived from RCTs, with an improvement of aBMD allocated with TRT. In addition, a positive effect on bone resorption markers was detected in observational but not in controlled studies. Observational studies included younger and more severe hypogonadal patients; this could explain, at least partly, the observed differences from RCTs with respect to bone resorption markers. However, it is important to recognize that data derived from observational studies should be interpreted with caution, due to the risk of selection bias related to the non-random assignment of T exposure. Accordingly, physicians frequently select to treat healthier individuals, and healthier individuals more often seek medical care for their hypogonadism-related problems. In addition, other limitations include the lack of

information regarding the level of T before and during TRT, as well as the limited data regarding the type of T preparation used and the follow-up performed during treatment.

Other important limitations should be considered for correctly interpreting the data derived from the present study. First, the presence of osteoporosis was not required for trial eligibility. The vast majority of the included studies lasted less than 2 years, whereas conventional osteoporosis studies usually extend over a 3-year period. We here report that the effects of TRT increased as a function of trial duration. Hence, it is possible that TRT effect on bone would be even more evident if conducted for a longer time, in particular when enrolling patients with osteopenia/osteoporosis. Therefore, large placebo-controlled RCTs conducted with a follow-up longer than 2 years are advisable. Significant heterogeneity among studies was detected, which reflects the differences observed in population characteristics and type of T preparation used. Available data were insufficient for investigating the effect of different T preparations on aBMD outcome in hypogonadal subjects or to perform other subgroup analyses to explain reasons for heterogeneity in results. No information regarding the type of DM and the influence of hypoglycemic drugs was available. Considering the limited numbers of available studies, even a few outliers could produce relevant deviations of estimates; for example, the effect of TRT on femoral BMD in RCTs was largely driven by three trials. Finally, several life-style behaviors such as smoking, alcohol consumption, type of diet and level of physical activity can modulate BMD as well as TRT outcomes. Unfortunately, no sufficient information on the latter parameters was available. Similarly, the use of exercise, as well as the concomitant use of phosphodiesterase type 5 inhibitor (PDE5i) and other drugs, can modulate the aromatase activity, [96] resulting in a further possible source of bias.

Although this meta-analysis showed positive effects of TRT on bone health, several aspects should be considered in further studies dealing with osteoporosis in hypogonadal men: estimation of fractures and fracture risk, combined effect of TRT and antiresorptive drugs and vitamin D and calcium supplementation, skeletal muscle mass and strength, type and duration of TRT [6].

Conclusions

Taken together, these data showed that TRT in hypogonadal patients could inhibit bone resorption and increase bone mass. This is particularly evident at the lumbar spine level and when the duration of TRT lasts long enough to allow the anabolic effect of testosterone and estrogens on bone metabolism to take place. However, whether or not TRT is associated with a decreased risk of bone fractures remains to be established. This is particularly relevant in the diabetic population, where there is the need to decrease the risk factors for fragility fractures, because of reduced skeletal strength and increased cortical porosity. Considering that positive effects of TRT are particularly evident in the diabetic population, the present study offers new arguments in support of screening for hypogonadism in diabetes and, if detected, for an appropriate androgen replacement.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any study with human participants or animals performed by any of the authors.

Informed consent Not applicable.

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