The Journal of Clinical Endocrinology & Metabolism, 2021, Vol. 106, No. 8, e3143–e3158
doi:10.1210/clinem/dgab149
Clinical Research Article

Clinical Research Article

Effect of Testosterone Treatment on Bone Microarchitecture and Bone Mineral Density in Men: A 2-Year RCT

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Abbreviations: aBMD, areal bone mineral density; BMD, bone mineral density;BV/TV, bone volume/tissue volume; CT, computed tomography; CTX, C-terminal type I collagen telopeptide; CV, coefficient of variation; DXA, dual-energy x-ray absorptiometry; HA, hydroxyapatite; HR-pQCT, high resolution–peripheral quantitative computed tomography; LCMS/MS, liquid chromatography–tandem mass spectrometry; MAD, mean adjusted difference; P1NP, procollagen type 1 N-terminal propeptide; QCT, quantitative computed tomography; RCT, randomized controlled trial; T4Bone, a planned substudy of the T4DM trial; T4DM, Testosterone for Diabetes Mellitus trial; vBMD, volumetric bone mineral density.

Received: 20 December 2020; Editorial Decision: 27 February 2021; First Published Online: 8 March 2021; Corrected and Typeset: 26 June 2021.

Abstract

Context: Testosterone treatment increases bone mineral density (BMD) in hypogonadal men. Effects on bone microarchitecture, a determinant of fracture risk, are unknown.

Objective: We aimed to determine the effect of testosterone treatment on bone microarchitecture using high resolution–peripheral quantitative computed tomography (HR-pQCT).

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Methods: Men ≥ 50 years of age were recruited from 6 Australian centers and were randomized to receive injectable testosterone undecanoate or placebo over 2 years on the background of a community-based lifestyle program. The primary endpoint was cortical volumetric BMD (vBMD) at the distal tibia, measured using HR-pQCT in 177 men (1 center). Secondary endpoints included other HR-pQCT parameters and bone remodeling markers. Areal BMD (aBMD) was measured by dual-energy x-ray absorptiometry (DXA) in 601 men (5 centers). Using a linear mixed model for repeated measures, the mean adjusted differences (95% CI) at 12 and 24 months between groups are reported as treatment effect.

Results: Over 24 months, testosterone treatment, versus placebo, increased tibial cortical vBMD, 9.33 mg hydroxyapatite (HA)/cm^3) (3.96, 14.71), $P < 0.001$ or 3.1% (1.2, 5.0); radial cortical vBMD, 8.96 mg HA/cm^3 (3.30, 14.62), $P = 0.005$ or 2.9% (1.0, 4.9); total tibial vBMD, 4.16 mg HA/cm^3 (2.14, 6.19), $P < 0.001$ or 1.3% (0.6, 1.9); and total radial vBMD, 4.42 mg HA/cm^3 (1.67, 7.16), $P = 0.002$ or 1.8% (0.4, 2.0). Testosterone also significantly increased cortical area and thickness at both sites. Effects on trabecular architecture were minor. Testosterone reduced bone remodeling markers CTX, –48.1 ng/L [–81.1, –15.1], $P < 0.001$ and P1NP, –6.8 μg/L[–10.9, –2.7], $P < 0.001$. Testosterone significantly increased aBMD at the lumbar spine, 0.04 g/cm^2 (0.03, 0.05), $P < 0.001$ and the total hip, 0.01 g/cm^2 (0.01, 0.02), $P < 0.001$.

Conclusion: In men ≥ 50 years of age, testosterone treatment for 2 years increased volumetric bone density, predominantly via effects on cortical bone. Implications for fracture risk reduction require further study.

Key Words: testosterone, bone, microarchitecture, T4DM

Pathological hypogonadism due to pituitary or testicular disease is a risk factor for osteoporosis in men (1) and testosterone replacement increases bone mineral density (BMD) (2). Observational studies in community dwelling men suggest that age-related reductions in circulating sex steroids are associated with loss of BMD (3) and increased fracture risk (4), effects which are reversed by testosterone treatment in randomized controlled clinical trials (RCT) (5-7). Such RCTs have utilized dual-energy x-ray absorptiometry (DXA) (5, 6), measuring areal BMD (aBMD), or more recently quantitative computed tomography (QCT) (7) measuring volumetric BMD (vBMD). Previous findings suggest that testosterone treatment increases BMD predominantly at the lumbar spine, inferring a predominant effect on trabecular bone (5-7).

Although QCT measures cortical and trabecular bone at central sites, it lacks sufficient resolution to provide information about microstructure. By contrast, high resolution–peripheral QCT (HR-pQCT) can distinguish cortical from trabecular bone with sufficiently high precision to accurately elucidate microarchitecture of cortical and trabecular structures (8) and predict fracture risk independent of aBMD and Fracture Risk Assessment Tool (FRAX) score (9). RCTs assessing the effects of testosterone treatment on bone microarchitecture defined by HR-pQCT in men are lacking. Therefore we conducted Testosterone for Bone (T4Bone), a planned substudy of Testosterone for Diabetes Mellitus (T4DM), a 2-year placebo-controlled RCT testing whether testosterone treatment reduces the incidence of type 2 diabetes mellitus (10, 11). In T4Bone we investigated the effects of testosterone treatment on vBMD and bone microarchitecture as well as on aBMD at the lumbar spine and proximal femur. As longitudinal studies report an association between lowered sex steroids and bone loss at cortical sites (12-14) and changes in cortical bone may be more closely associated with fracture risk (15-18), cortical vBMD at the tibia, a weight-bearing site, was chosen as the primary prespecified endpoint. Areal bone mineral density at lumbar spine was the main outcome of the second aim, the DXA study.

Methods

Study Design

T4DM was a randomized, placebo-controlled, double-blind, 2-year trial conducted in 6 Australian academic centers testing whether testosterone treatment prevents or reverses type 2 diabetes in high-risk men beyond the effects of a lifestyle program (10, 11). The T4Bone substudy assessed the effects of testosterone treatment on skeletal outcomes. Bone

microarchitecture was assessed by HR-pQCT at 1 study center and aBMD at 5 centers. The T4Bone study protocol can be accessed at https://protocols.io/file/cszgbhqpf.pdf. We followed the CONSORT checklist of information to include when reporting a randomized trial. The checklist can be accessed at https://www.protocols.io/file/cszhbhqpf.docx.

The T4Bone primary endpoint was between-group change in distal tibial cortical vBMD by HR-pQCT measured over 2 years at baseline and 2 follow-up visits after 66 and 102 weeks. Secondary outcome measures were total and other cortical and trabecular parameters at the distal radius and tibia (HR-pQCT), and the bone markers beta carboxyl-terminal type I collagen telopeptide (CTX) and procollagen type 1 amino-terminal propeptide (P1NP), over 2 years. Areal bone mineral density at lumbar spine was the main outcome of the second aim, the DXA study.

Participants and Treatment

The T4DM trial design and entry criteria were previously reported (10, 11). Inclusion criteria included age 50 to 74 years, waist circumference ≥95 cm, impaired glucose tolerance or newly diagnosed type 2 diabetes, and a fasting serum testosterone drawn between 8 and 10 AM of ≤14 nmol/L (403 ng/dL) by immunoassay at an accredited pathology provider (Sonic Health Care, Australia). Serum testosterone was remeasured together with serum estradiol in baseline and on-study serum fasted samples drawn between 8 and 10 AM at the end of the study, using validated liquid chromatography–tandem mass spectrometry (LCMS/MS) methodology (19). Exclusions included hypothalamic-pituitary-testicular pathology, testosterone treatment in the past 12 months, or history of androgen abuse at any time. In T4Bone, men receiving osteoporotic drug therapy before or during the trial were excluded. Participants were enrolled in a lifestyle program (Weight Watchers) and randomized, in a concealed 1:1 allocation, to receive intramuscular testosterone undecanoate 1000 mg or matched placebo every 3 months (after a 6-week loading dose) for 2 years. The main T4DM study (including DXA) received ethics committee approval at each site (10, 11). Ethics approval for HR-pQCT and bone marker measurements at Austin Health was obtained (HREC/12/CRGH/79). An independent data and safety monitoring committee monitored the study (10, 11).

Assessments

Volumetric BMD and microarchitecture were measured at the distal tibia and radius at baseline and at weeks 66 and 102 using HR-pQCT (XtremeCT; Scanco Medical AG, Bruttisellen, Switzerland), acquiring a simultaneous stack of parallel computed tomography (CT) slices with a voxel size of 82 microns. At each site, 110 CT slices were obtained to deliver a 3-dimensional representation of 9.02 mm in the axial direction. The CT data were processed previously using Scanco analysis software as described (20). Outcome variables included total, trabecular, and cortical vBMD; cortical area, cortical thickness and trabecular thickness, number, separation, and trabecular bone volume/tissue volume (BV/TV) ratio. For follow-up studies, an algorithm automatically used the cross-sectional area within the periosteal boundary of the radius or tibia to match the volumes of interest on baseline and follow-up scans. Precision errors of volumetric densities in our hands were 0.6% to 1.4% (21). Quality control for the performance of the HR-pQCT was based on daily scanning of a phantom containing hydroxyapatite (HA) at densities of 0, 100, 200, 400, and 800 mg HA/cm^3) (Quality Assurance in Radiology and Medicine [QRM], Möhrendorf, Germany) (22).

CTX and P1NP were measured from fasting blood (8:00-10:00 AM) at baseline, week 66, and week 102 by electrochemiluminescence (Roche Cobas C8000, Roche Diagnostics). The coefficient of variation (CV) for CTX was 1.3% at level of 334 ng/L and 1.3% at 764 ng/L. The CV for P1NP was 2.8% at 34 µg/L and 2.6% at 205 µg/L (12).

Areal BMD was measured at the lumbar spine and the hip using DXA at baseline and at week 102 in 601 men participating in T4DM at 5 study sites. Prodigy (GE Lunar, Madison, WI) was used at Austin Health, Queen Elizabeth, and Princess Alexandra Hospitals. Hologic Discovery (Hologic Mississauga, ON) was used at Fremantle and Fiona Stanley Hospitals. For DXA, CVs at Austin were 1.1% to 2.6% (human data), Queen Elizabeth Hospital 2.4% to 3.5% (human data), Princess Alexandra Hospital 0.26% (phantom data), Fremantle Hospital 0.44% (phantom data), and Fiona Stanley Hospital 1% (human data).

To standardize the BMD values at the lumbar spine that were obtained with 2 different DXA methods across participating centers, the following harmonizing equations were used for the Lunar scanner 0.9683 * (BMD – 1.100) + 1.0436 and Hologic scanner 1.0550 * (BMD – 0.972) + 1.0436, respectively (23). Hip measurements were not standardized, as they are generally regarded as acceptably equivalent between the 2 machines (23, 24).

Statistical Analyses

The sample size estimate for the HR-pQCT study was based on a study (12) of 12-month effects of androgen

deprivation on tibial cortical vBMD (32.1 mg HA /cm^3, 3.9% from baseline) and a sample SD (55.9 mg HA/cm^3). This produced a sample size estimate of 49 patients per group. With a 1:1 allocation, a 2-sided type 1 error rate of 0.05 and power of 80% in a 2-sided *t* test, and attrition rate of 20%, the estimated total sample size was 118 participants, and a sample size of 156 men to achieve a power of 90%.

For the aBMD DXA-based study, the sample size was derived from a metanalysis (25) reporting testosterone treatment effects on lumbar spine BMD of 0.62, producing an estimated sample size of 42 patients per group. With a 1:1 allocation, a type 1 error of 0.05 and power 80%, the estimated total sample size was 101 participants, and 134 participants for a power of 90%.

Data were reported as mean (SD), except for skewed variables (BMI, bone markers) where median (interquartile range, [IQR]) was reported. Baseline characteristics between testosterone and placebo groups were compared using Welch *t* test except for nonnormal data tested with Wilcoxon rank sum test or chi-square test for proportions, substituted by Fisher exact test in case of low numbers. Statistical analyses of the treatment effect on the primary endpoint followed the intention to treat principle (ITT) including all randomized subjects and retaining them in their assigned group. The treatment effect on bone microarchitecture and bone markers was assessed using repeated measures linear mixed effects models based on restricted maximum likelihood estimates (REML) including fixed effects for the baseline level, treatment group, visit (time) and the interaction of time × treatment group, and random effects at the subject level. The treatment effect was represented by the interaction term and quantified as mean adjusted difference (MAD) with 95% CI between the groups. The significance level for the treatment effect was tested as a single *P* value over all visits with Kenward-Roger degrees of freedom. The mixed model was robust against missingness at random (MAR), which was an acceptable assumption for the primary outcome according to a nonsignificant Hawkins test ($P = 0.88$) (26). As a sensitivity analysis, we also conducted a per protocol analysis including only men who had outcome data available at all time points. Areal BMD outcomes lacking second follow-up visits were analyzed using generalized linear regression models with baseline adjusted group interaction as the treatment effect. For the multicentric part of the trial, trial site was included as a fixed covariate. The aBMD analysis relied on modified ITT including all data available for this variable in each randomized group. Specifically, 1 individual was excluded from the trial due to commencement of antiresorptive treatment. The decision of the study panel prior to, and independent of the analysis, was to exclude individuals taking or starting antiresorptive treatment as this would obscure the treatment effect of testosterone on bone microarchitecture. Also, some baseline values (n = 35 for the main outcome aBMD at the lumbar spine) were missing. A 2-sided *P* value of < 0.05 was considered indicative of statistical significance. No adjustments for multiple testing were made for the explanatory analysis of intercorrelated structural bone parameters. For comparison and ease of interpretation, effect sizes (Cohen's d) of some treatment effects were converted to percentage estimates. All statistical analyses were performed using R statistical package (version 4.0.2 for Mac), and the additional packages lme4 1.1-23 and effects 4.1-4 (27-29).

Results

Bone Microarchitecture and Remodeling Markers (Austin Health Cohort)

Participant flow and baseline data

Of 183 men assessed for eligibility at Austin Health, 177 men agreed to participate in T4Bone. 136 (77%) completed the baseline and 2-year assessment of bone microarchitecture by HR-pQCT (Fig. 1). Baseline characteristics are listed in Table 1.

Treatment outcomes

Microarchitectural parameters. Compared with placebo, testosterone treatment over 2 years significantly increased the primary outcome, tibial cortical vBMD, by a mean adjusted difference (MAD) of 9.33 mg HA/cm^3 [3.96, 14.71], $P < 0.001$, or 3.1% [1.2, 5.0]. Similarly, radial cortical vBMD increased by 8.96 mg HA/cm^3 [3.30, 14.62], $P = 0.005$ (2.9% [1.0, 4.9]). Testosterone significantly increased total vBMD at both tibia (MAD 4.16 mg HA/cm^3 [2.14, 6.19], $P < 0.001$, 1.3% [0.6, 1.9]), and radius (MAD 4.42 mg HA/cm^3 [1.67, 7.16], $P = 0.002$, 1.8% [0.4, 2.0]) (Fig. 2).

Testosterone treatment was also associated with significant increases in both cortical area (tibia MAD 4.11 mm^2 [2.78, 5.45], $P < 0.001$, 2.5% [1.6, 3.4]; radius MAD 1.60 mm^2 [0.62, 2.57], $P = 0.002$, 2.0% [0.7, 3.3]) and in cortical thickness (tibia MAD 0.03 mm [0.02, 0.05], $P < 0.001$, 1.7% [0.5, 2.9]; radius MAD 0.02 mm [0.01, 0.03], $P = 0.006$, 1.7% [0.5, 2.9]) (Table 2).

By contrast, the effects of testosterone treatment on trabecular parameters were less consistent. At the tibia, testosterone treatment was associated with an increase in trabecular vBMD (MAD 1.70 mg HA/cm^3 [0.51, 2.88], $P = 0.02$) and an increase in the BV/TV ratio (MAD

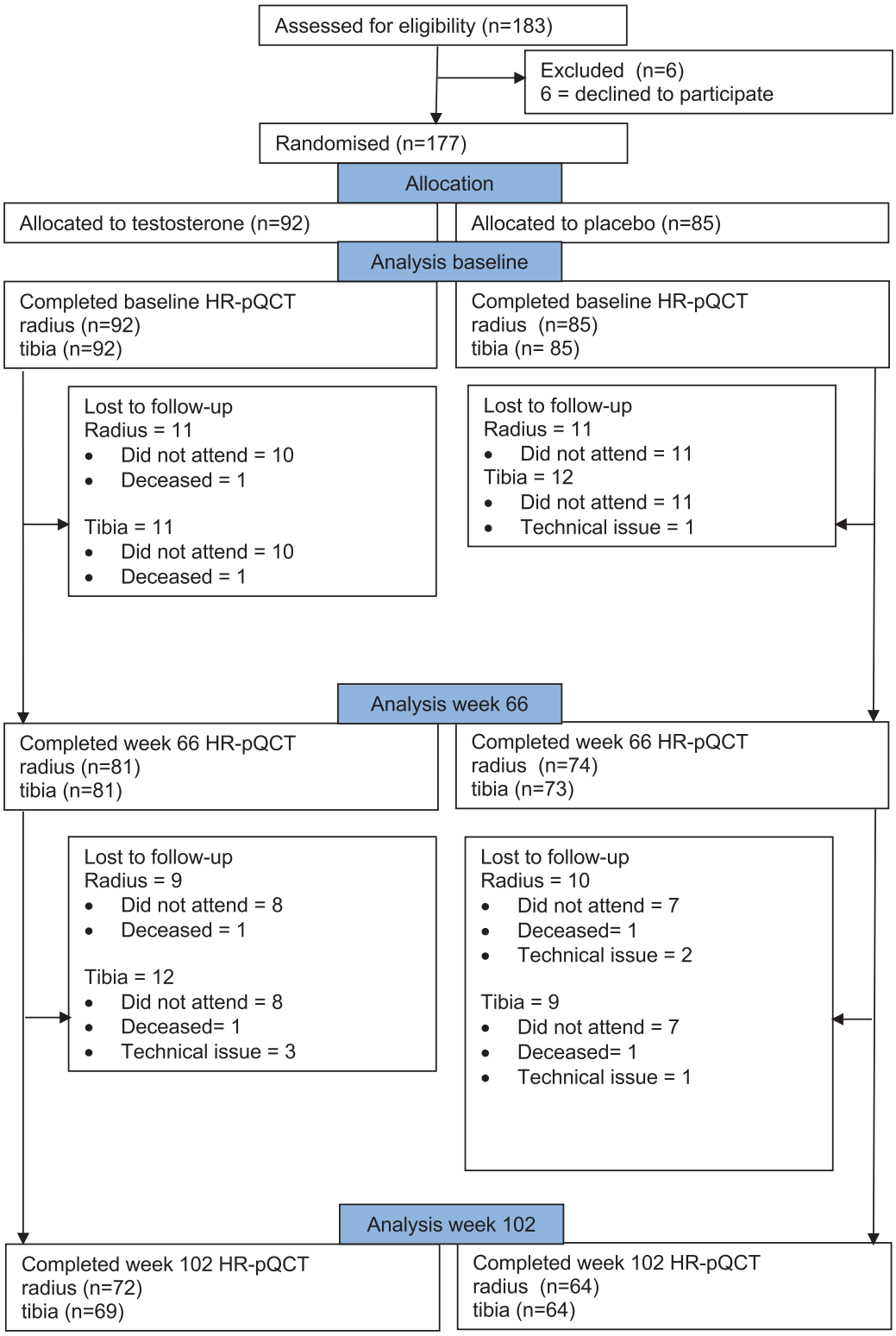

Figure 1. Consort flow diagram.
Shown is the flow of participants undergoing HR-pQCT analyses assigned to testosterone or placebo through each phase of the study, and the reasons for exclusion of participants following randomization.

0.13% [0.04, 0.24], $P = 0.02$), both at week 66, and a decrease in trabecular area (MAD –2.65mm^2 [–4.55, –0.75], $P = 0.02$) (Fig. 2, Table 2). The significant decrease in trabecular area with testosterone treatment is consistent with reduced medullary expansion due to increased cortical thickness (8, 13). Testosterone treatment had no

Table 1. Baseline participant characteristics

Characteristics	Placebo group (N = 85)	Testosterone group (N = 92)	Number with data at time point	*P* value
Age, years	60.3 (6.5)	60.0 (6.6)	177	0.74
Body mass index, kg/m^2	34.8 [31.1, 39.6]	34.1 [31.6, 38.8]	177	0.82
Smoking status			177	1.00
Current	2 (2.4%)	2 (2.2%)		
Non- or ex-smoker	83 (97.6%)	90 (97.8%)		
Alcohol, drinks/week	11.0 (20.9)	7.5 (10.3)	177	0.17
Calcium use			177	1.00
Yes	7 (8.2%)	7 (7.6%)		
No	78 (91.8%)	85 (92.4%)		
25-OH Vitamin D use			177	0.67
Yes	12 (14.1%)	16 (17.4%)		
No	73 (85.9%)	76 (82.6%)		
Glucocorticoid use			177	0.11
Yes	14 (16.5%)	14 (15.2%)		
No	71 (83.5%)	78 (84.8%)		
Serum testosterone,^ nmol/L	14.4 (4.4)	14.0 (4.4)	172	0.64
Serum testosterone,* nmol/L	10.1 (2.6)	10.0(2.0)	177	0.77
Serum estradiol, pmol/L^	202 (86)	202 (96)	172	0.99
SHBG, nmol/L	39.6 (14.7)	38.9 (13.8)	171	0.74
Serum 25-OH vitamin D, nmol/L	56.6 (20.0)	56.7 (16.9)	162	0.96
eGFR, mL/min/1.73m^2	83 (10)	81 (10)	177	0.30
TIBIA				
Total vBMD, mg HA/cm^3	316 (53.4)	321 (48.8)	177	0.49
Cortical vBMD, mg HA/cm^3	829 (48.3)	835 (46.8)	177	0.43
Cortical area, mm^2	145 (27.3)	145 (25.1)	177	0.86
Cortical thickness, mm	1.23 (0.29)	1.25 (0.25)	177	0.66
Trabecular vBMD, mg HA/cm^3	199 (35.6)	203 (35.4)	177	0.51
Trabecular area, mm^2	738 (160)	716 (138)	177	0.33
Trabecular BV/TV ratio (%)	16.6 (3.0)	16.9 (3.0)	177	0.51
Trabecular number, mm^{-1}	1.94 (0.32)	1.97 (0.34)	177	0.64
Trabecular thickness, mm	0.09 (0.01)	0.09 (0.01)	177	0.87
Trabecular separation, mm	0.44 (0.09)	0.44 (0.11)	177	0.87
RADIUS				
Total vBMD, mg HA/cm^3	348 (64.3)	347 (56.0)	177	0.88
Cortical vBMD, mg HA/cm^3	839 (50.3)	837 (47.7)	177	0.77
Cortical area, mm^2	72.6 (13.5)	72.7 (13.8)	177	0.96
Cortical thickness, mm	0.88 (0.19)	0.88 (0.19)	177	0.97
Trabecular vBMD, mg HA/cm^3	199 (38.2)	196 (31.3)	177	0.57
Trabecular area, mm^2	283 (61.4)	281 (60.1)	177	0.82
Trabecular BV/TV ratio (%)	16.6 (3.2)	16.3 (2.6)	177	0.57
Trabecular number, mm^{-1}	1.91 (0.24)	1.91 (0.25)	177	0.92
Trabecular thickness, mm	0.09 (0.01)	0.09 (0.01)	177	0.58
Trabecular separation, mm	0.45 (0.08)	0.45 (0.12)	177	0.76

Values are presented as mean (SD), median [interquartile range] or proportions N (%). Serum testosterone and estradiol were measured by LCMS/MS^ or by immunoassay*.

Glucocorticoid use denotes use at any time during the trial, with 19 men (8 placebo, 11 testosterone group) prescribed a course of oral, 7 men (6 placebo, 1 testosterone) inhaled, and 2 men (testosterone group) injectable glucocorticoids.

Abbreviations: BV/TV, bone volume/tissue volume; eGFR, estimated glomerular filtration rate; HA, hydroxyapatite; SHBG, sex hormone–binding globulin; vBMD, volumetric bone mineral density.

significant effect on tibial trabecular number, thickness, and separation and no significant effect on any trabecular parameter at the radius (all *P* values > 0.10) (Table 2).

Men with lower baseline serum testosterone or lower baseline serum estradiol were not more likely to have a larger treatment effect and gain more bone during

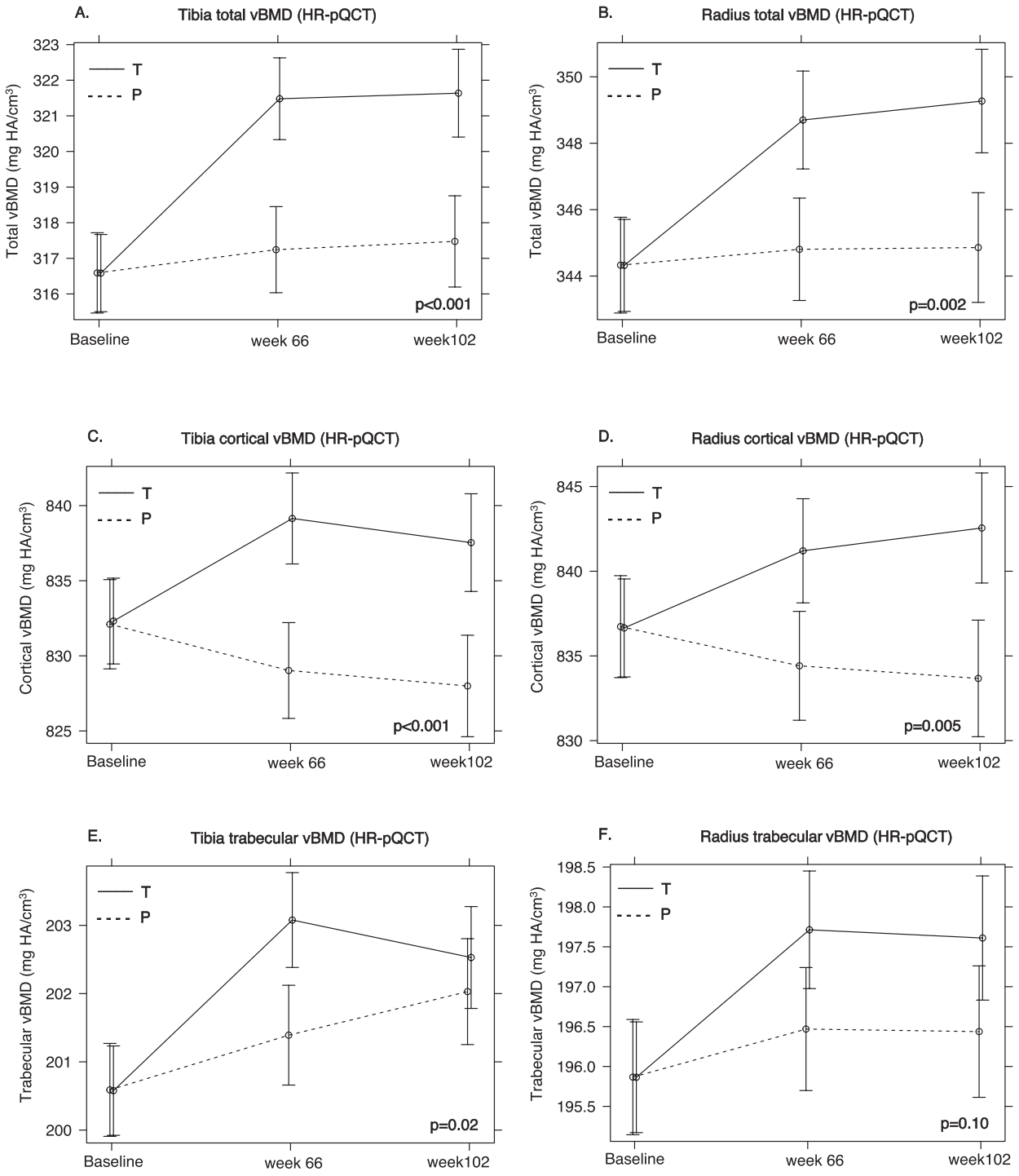

Figure 2. Volumetric bone mineral density in testosterone- and placebo-treated men.
Shown are adjusted mean (95% CI) total (A-B), cortical (C-D) and trabecular (E-F) vBMD, measured by HR-pQCT at the radius and at the tibia in testosterone- (T, solid lines) and placebo- (P, dashed lines) treated men at baseline (week 0), week 66, and week 102. HR-pQCT was performed at 1 site, Austin Health only.

testosterone treatment. Adding either baseline testosterone or estradiol concentrations or the respective changes in sex steroid concentrations as covariables did not result in any improvements to model performance in overall models that already explained 94% (conditional r squared) of the observed variation. Consequently, in tables and figures we

Table 2. Bone microarchitecture and bone remodeling markers

	Placebo group (N = 85)	Testosterone group (N = 92)	Number with data at time point	Mean adjusted difference [95% CI]	*P* value
TIBIA					
Total vBMD, mg HA/cm^3					
Baseline	316 (53.4)	321 (48.8)	177	4.24 [2.31, 6.17]	
Week 66	315 (53.2)	323 (50.8)	154	4.16 [2.14, 6.19]	
Week 102	316 (55.0)	319 (51.6)	133		<0.001
Cortical vBMD, mg HA/cm^3					
Baseline	829 (48.3)	835 (46.8)	177		
Week 66	826 (50.1)	841 (46.1)	154	9.91 [4.78, 15.03]	
Week 102	827 (51.6)	840 (47.3)	133	9.33 [3.96, 14.71]	<0.001
Cortical area, mm^2					
Baseline	145 (27.3)	145 (25.1)	177		
Week 66	144 (27.1)	147 (25.6)	154	2.90 [1.63, 4.17]	
Week 102	144 (27.5)	146 (26.0)	133	4.11 [2.78, 5.45]	<0.001
Cortical thickness, mm					
Baseline	1.23 (0.29)	1.25 (0.25)	177		
Week 66	1.23 (0.29)	1.25 (0.25)	154	0.02 [0.01, 0.03]	
Week 102	1.22 (0.29)	1.25 (0.26)	133	0.03 [0.02, 0.05]	<0.001
Trabecular vBMD, mg HA/cm^3					
Baseline	199 (35.6)	203 (35.4)	177		
Week 66	199 (35.5)	206 (36.8)	154	1.70 [0.51, 2.88]	
Week 102	200 (37.1)	201 (35.3)	133	0.51 [−0.73, 1.75]	0.02
Trabecular area, mm^2					
Baseline	738 (160)	716 (138)	177		
Week 66	743 (162)	727 (139)	154	−1.63 [−3.44, 0.18]	
Week 102	742 (162)	722 (140)	133	−2.65 [−4.55, −0.75]	0.02
Trabecular BV/TV ratio (%)					
Baseline	16.6 (3.0)	16.9 (3.0)	177		
Week 66	16.6 (3.0)	17.1 (3.1)	154	0.13 [0.04, 0.24]	
Week 102	16.7 (3.1)	16.6 (3.0)	133	0.39 [−0.06, 0.14]	0.02
Trabecular number, mm^{-1}					
Baseline	1.94 (0.32)	1.97 (0.34)	177		
Week 66	1.93 (0.28)	1.98 (0.30)	154	0.01 [−0.03, 0.06]	
Week 102	1.91 (0.32)	1.96 (0.31)	133	0.02 [−0.03, 0.07]	0.71
Trabecular thickness, mm					
Baseline	0.09 (0.01)	0.09 (0.01)	177		
Week 66	0.09 (0.01)	0.09 (0.01)	154	0.00 [0.00, 0.00]	
Week 102	0.09 (0.01)	0.09 (0.01)	133	0.00 [0.00, 0.00]	0.62
Trabecular separation, mm					
Baseline	0.44 (0.09)	0.44 (0.11)	177		
Week 66	0.44 (0.08)	0.43 (0.10)	154	−0.01 [−0.02, 0.01]	
Week 102	0.45 (0.09)	0.44 (0.10)	133	−0.01 [−0.02, 0.00]	0.32
RADIUS					
Total vBMD, mg HA/cm^3					
Baseline	348 (64.3)	347 (56.0)	177		
Week 66	348 (65.2)	344 (51.2)	155	3.90 [1.27, 6.54]	
Week 102	348 (70.0)	341 (51.4)	136	4.42 [1.67, 7.16]	0.002
Cortical vBMD, mg HA/cm^3					
Baseline	839 (50.3)	837 (47.7)	177		
Week 66	835 (51.6)	840 (47.7)	155	6.87 [1.43, 12.30]	
Week 102	833 (53.8)	841 (48.3)	136	8.96 [3.30, 14.62]	0.005
Cortical area, mm^2					
Baseline	72.6 (13.5)	72.7 (13.8)	177		
Week 66	73.1 (13.8)	72.5 (12.8)	155	1.32 [0.38, 2.25]	
Week 102	73.1 (14.4)	72.2 (12.9)	136	1.60 [0.62, 2.57]	0.002

Table 2. Continued

	Placebo group (N = 85)	Testosterone group (N = 92)	Number with data at time point	Mean adjusted difference [95% CI]	*P* value
Cortical thickness, mm					
Baseline	0.88 (0.19)	0.88 (0.19)	177		
Week 66	0.88 (0.20)	0.87 (0.17)	155	0.02 [0.02, 0.03]	
Week 102	0.88 (0.21)	0.87 (0.17)	136	0.02 [0.01, 0.03]	0.006
Trabecular vBMD, mg HA/cm^3					
Baseline	199 (38.2)	196 (31.3)	177		
Week 66	199 (39.6)	195 (31.0)	155	1.24 [–0.03, 2.51]	
Week 102	199 (41.7)	192 (27.9)	136	1.17 [–0.15, 2.50]	0.10
Trabecular area, mm^2					
Baseline	283 (61.4)	281 (60.1)	177		
Week 66	285 (62.3)	286 (57.6)	155	–0.81 [–2.08, 0.47]	
Week 102	287 (66.2)	287 (60.2)	136	–0.78 [–2.11, 0.55]	0.37
Trabecular BV/TV ratio (%)					
Baseline	16.6 (3.2)	16.3 (2.6)	177		
Week 66	16.6 (3.3)	16.2 (2.6)	155	0.96 [0.01, 0.20]	
Week 102	16.6 (3.5)	16.0 (2.3)	136	0.96 [0.02, 0.21]	0.13
Trabecular number, mm^{-1}					
Baseline	1.91 (0.24)	1.91 (0.25)	177		
Week 66	1.90 (0.25)	1.90 (0.26)	155	0.00 [–0.05, 0.04]	
Week 102	1.89 (0.25)	1.89 (0.23)	136	0.00 [–0.04, 0.05]	0.98
Trabecular thickness, mm					
Baseline	0.09 (0.01)	0.09 (0.01)	177		
Week 66	0.09 (0.01)	0.09 (0.01)	155	0.00 [0.00, 0.00]	
Week 102	0.09 (0.01)	0.09 (0.01)	136	0.00 [0.00, 0.00]	0.84
Trabecular separation, mm					
Baseline	0.45 (0.08)	0.45 (0.12)	177		
Week 66	0.45 (0.08)	0.46 (0.12)	155	0.00 [–0.01, 0.01]	
Week 102	0.45 (0.08)	0.46 (0.10)	136	0.00 [–0.02, 0.01]	0.75
C-telopeptide, ng/L					
Baseline	320 [223, 428]	308 [237, 376]	171		
Week 66	340 [255, 436]	236 [182, 289]	151	–86.0 [–118.2, –53.7]	
Week 102	332 [220, 440]	244 [183, 330]	142	–48.1 [–81.1, –15.1]	<0.001
P1NP, µg/L					
Baseline	40.0 [32.0, 49.0]	39.0 [32.0, 48.2]	175		
Week 66	40.0 [31.0, 57.0]	31.0 [25.0, 41.2]	153	–9.5 [–13.5, –5.5]	
Week 102	39.5 [28.5, 56.2]	30.0 [25.8, 38.2]	146	–6.8 [–10.9, –2.7]	<0.001

Mean (SD) or median [interquartile range] are reported. The treatment effect is reported as mean adjusted difference between the testosterone and placebo group and its 95% confidence interval and was assessed with a mixed model (see "Methods").
Abbreviations: BV/TV, bone volume/tissue volume; CTX, C-telopeptide; P1NP, procollagen type 1 N-terminal propeptide; vBMD, volumetric bone mineral density.

refrained from including these potential covariables to avoid reporting overfitted models.

Bone remodeling markers

Testosterone treatment, compared with placebo, reduced bone markers CTX (MAD –48.1ng/L [–81.1, –15.1], $P < 0.001$) and P1NP (MAD –6.8µg/L [–10.9, –2.7], $P < 0.001$) (Fig. 3, Table 2).

Sensitivity analysis

In a per protocol sensitivity analysis, HR-pQCT and bone marker outcomes were similar (data not shown).

aBMD (5-center cohort)

Baseline aBMD data at the lumbar spine were available for 566 men, and week 102 data for 443 men. There were no differences in baseline characteristics (Table 3) among T4Bone participants recruited at Austin Health compared with men undergoing DXA at other centers except for baseline testosterone, which was lower (by –1.8 nmol/L by LCMS/MS) at men at 1 center. Testosterone treatment, compared with placebo over 2 years, increased BMD at the standardized lumbar spine (MAD 0.04 g/cm^2 [0.03, 0.05], $P < 0.001$, 3.3% [2.7, 3.9]), at the total hip (MAD 0.01 g/cm^2 [0.01, 0.02], $P < 0.001$, 1.9% [1.2, 2.7]), and

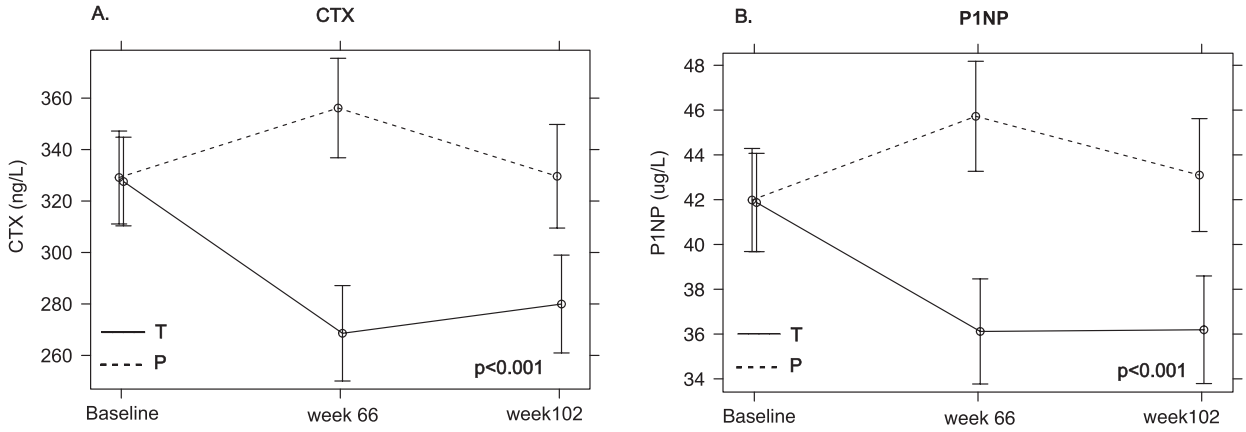

Figure 3. Bone remodeling markers in testosterone- and placebo-treated men.
Shown are adjusted mean (95% CI) circulating CTX (A) and P1NP (B) concentrations in testosterone- (T, solid lines) and placebo- (P, dashed lines) treated men at baseline (week 0), week 66, and week 102. Bone remodeling markers were measured at 1 site, Austin Health only.

the femoral neck (MAD 0.02 g/cm^2 [0.01, 0.02], $P < 0.001$, 1.7% [0.8%, 2.7%]) (Fig. 4, Table 4).

Sex steroid concentrations

As expected, trough serum testosterone and estradiol were significantly higher in men on testosterone treatment (Table 4).

Adverse effects

As in T4DM (11), testosterone treatment was associated with a greater risk of experiencing a hematocrit ≥0.54% occurring in 20% (61/308) of men assigned to testosterone versus 1% (3/293) in men assigned to placebo treatment. As in T4DM (11), testosterone treatment was not associated with a greater incidence of serious adverse events (Table 5).

Discussion

As measured by HR-pQCT, testosterone treatment (given as testosterone undecanoate over 2 years) significantly increased vBMD at both the tibia and the radius, compared with matching placebo. The increased vBMD was predominantly in cortical bone at both sites with highly significant treatment effect sizes ranging from 2.9% to 3.1%. By contrast, effects on trabecular bone parameters were not significant in the treatment group over placebo with the exception of an isolated increase of tibial trabecular vBMD and in BV/TV ratio observed at week 66, but not at study end.

Our findings that testosterone treatment predominantly increases cortical bone over placebo are consistent with some, but not all, previous data. No previous RCT assessing effects of testosterone on bone microarchitecture using HR-pQCT is available to compare our findings. However, longitudinal studies in men using HR-pQCT have suggested that lowered sex steroids (13, 14), especially if severe (12), are associated with accelerated bone loss, particularly at cortical sites (12-14). Small (n = 21-36) uncontrolled studies report that testosterone replacement in men with pathologic hypogonadism increased both cortical (using ^{125}I photon absorptiometry) (30) and trabecular bone (using quantitative CT at the spine) (2, 30). Other small (n = 10-15) uncontrolled studies using magnetic resonance microimaging (μMRI) at the tibia have reported that testosterone replacement in men with pathologic hypogonadism improves structural parameters of trabecular bone (31, 32), but effects on cortical bone parameters were mixed (32).

In a large placebo-controlled study of men aged 60 years or older (n = 211) with a baseline testosterone of <9.54 nmol/L, testosterone treatment over 1 year increased vBMD (by QCT) predominantly at the trabecular spine (+6.8% [4.8-8.7]), with lesser effects at the peripheral spine (+2.9% [2.1-3.7]) and total hip (+1.3% [0.8-1.7]), all $P < 0.001$ (7). This study used standard resolution QCT (resolution of ~500 μm), lower than that of the HR-pQCT (resolution ~82 μm) (8) used in the present study, which facilitates assessment of microarchitectural parameters of cortical and trabecular bone with high precision (8). In the present study treatment effects on cortical bone were consistent, with significant increases on total cortical vBMD, cortical thickness, and area at both bone sites, whereas effects on trabecular bone were minor. The predominant effects of testosterone treatment on cortical bone are consistent with observational HR-pQCT studies in older men with lower sex steroid concentrations displaying deficits

Table 3. Baseline characteristics for DXA trial participants

Characteristics	Placebo Group (N = 293)	Testosterone Group (N = 308)	Number with data at time point	*P* value
Age, years	59.7 (6.22)	59.6 (6.30)	601	0.78
Body mass index, kg/m^2	34.5 [31.0, 38.1]	34.6 [31.4, 38.1]	601	0.36
Smoking status				
Current	14 (4.7%)	15 (4.9%)	600	1.00
Non- or ex-smoker	282 (95.3%)	292 (95.1%)		
Alcohol				
Yes	244 (83.6%)	255 (82.8%)	600	0.89
No	48 (16.4%)	53 (17.2%)		
Calcium use				
Yes	26 (8.9%)	21 (6.8%)	601	0.43
No	267 (91.1%)	287 (93.2%)		
25-OH Vitamin D use				
Yes	37 (12.6%)	40 (13.0%)	601	0.99
No	256 (87.5%)	268 (93.2%)		
Glucocorticoid use				
Yes	28 (9.6%)	40 (13.0%)	601	0.23
No	265 (90.4%)	268 (87.0%)		
Serum testosterone, nmol/L^	14.0 (4.52)	13.2 (4.09)	555	0.02
Serum testosterone, nmol/L*	10.1 (2.5)	9.8 (2.4)	601	0.21
Serum estradiol, pmol/L^	206 (104)	194 (93.6)	555	0.17
SHBG, nmol/L	37.6 (13.8)	37.3 (14.2)	551	0.80
eGFR, mL/min/1.73m^2	82 (11)	82 (10)	601	0.44
Standardized BMD lumbar spine, g/cm^2	1.22 (0.18)	1.21 (0.18)	566	0.91
BMD total hip, g/cm^2	1.13 (0.14)	1.12 (0.13)	575	0.83
BMD femoral neck, g/cm^2	1.00 (0.15)	1.00 (0.14)	572	0.75
T score lumbar spine	0.47 (1.56)	0.45 (1.54)	563	0.88
T score total hip	0.35 (0.98)	0.31 (0.97)	573	0.68
T score femoral neck	−0.30 (0.98)	−0.32 (0.96)	574	0.77

Values are presented as mean (SD), median [interquartile range] or proportions N (%). Serum testosterone and estradiol were measured by LCMS/MS^ or by immunoassay*. Glucocorticoid use denotes use at any time during the trial, with 42 men (17 placebo, 25 testosterone group) prescribed a course of oral, 21 men (10 placebo, 11 testosterone) inhaled, 2 men (1 placebo, 1 testosterone) topical and 3 men (3 testosterone group) injectable glucocorticoids.
Abbreviations: BMD, bone mineral density; eGFR, estimated glomerular filtration rate; SHBG, sex hormone–binding globulin.

predominantly in cortical, rather than trabecular, bone (12-14).

While our RCT did not evaluate fractures, the testosterone-induced increases in cortical bone, which comprises 80% of the human skeleton, suggests that it might have an effect on fracture risk. Animal studies report that the loss of cortical bone has greater effect on lost bone strength compared with loss of trabecular bone (33). Of note in some (15-18), but not all (34) observational HR-pQCT studies in men, deficits in cortical parameters were more consistently associated with fractures than trabecular parameters.

The standardized effect sizes, ranging from 1.3% to 3.1% for total and cortical vBMD in this study, compare favorably with those reported for antiresorptive drug treatments. In postmenopausal women with low BMD, antiresorptive drug therapy over 12 to 24 months produced effect sizes on HR-pQCT indices ranging from 0.3% to 3.8% using alendronate (35, 36), denosumab (35), or zoledronic acid (37). In one RCT, teriparatide led to either no change (trabecular) or reduction (total and cortical) in vBMD (38).

In our larger cohort of 601 men, testosterone treatment significantly increased aBMD (by DXA) at the lumbar spine (effect size 3.3% [2.7, 3.9]), compared with a treatment effect of +1.2% (0.3, 2.1) reported in the 1-year T-trials bone study (7). Despite the higher mean baseline serum testosterone concentration of 13.6 nmol/L in our cohort compared with approximately 8.2 nmol/L in the T-trial bone study (7), we found significant treatment effect sizes at the

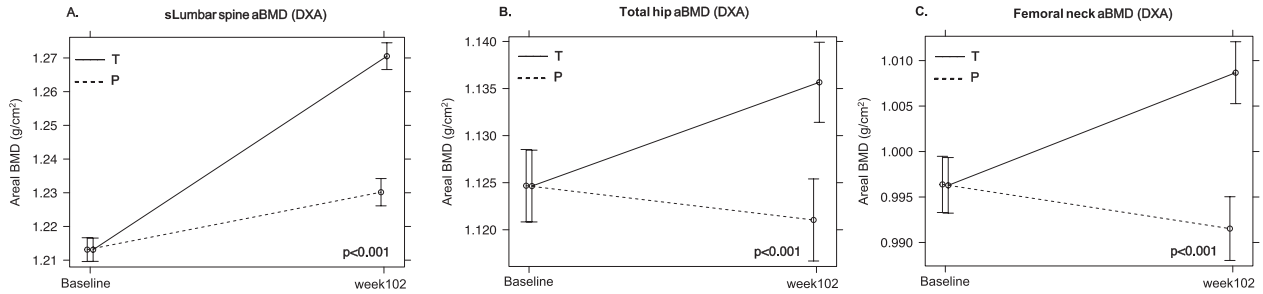

Figure 4. Areal bone mineral density in testosterone- and placebo-treated men.

Shown are adjusted mean (95% CI) aBMD at the standardized lumbar spine (sLumbar spine) (A), total hip (B), and femoral neck (C) measured by DXA in testosterone- (*T*, solid lines) and placebo- (*P*, dashed lines) treated men at baseline (week 0) and at week 102. DXA was performed at 5 academic sites.

Table 4. Areal bone mineral density (DXA) and sex steroids

	Placebo group (N = 293)	Testosterone group (N = 308)	Number with data at time point	Mean adjusted difference [95% CI]	*P* value
Standardized BMD lumbar spine (g/cm^2)					
2003 Baseline	1.22 (0.18)	1.21 (0.18)	566		
Week 102	1.23 (0.19)	1.26 (0.18)	443	0.04 [0.03, 0.05]	<0.001
BMD total hip, g/cm^2					
Baseline	1.13 (0.14)	1.12 (0.13)	575		
Week 102	1.12 (0.15)	1.13 (0.14)	472	0.01 [0.01, 0.02]	<0.001
BMD femoral neck, g/cm^2					
Baseline	1.00 (0.15)	1.00 (0.14)	572		
Week 102	0.99 (0.16)	1.01 (0.14)	473	0.02 [0.01, 0.02]	<0.001
T score lumbar spine					
Baseline	0.47 (1.56)	0.45 (1.54)	563		
Week 102	0.63 (1.66)	0.82 (1.58)	446	0.34 [0.28, 0.41]	<0.001
T score total hip					
Baseline	0.35 (0.98)	0.31 (0.97)	573		
Week 102	0.30 (1.03)	0.34 (0.97)	472	0.14 [0.10, 0.18]	<0.001
T score femoral neck					
Baseline	-0.30 (0.98)	–0.32 (0.96)	574		
Week 102	-0.34 (1.04)	–0.24 (0.98)	472	0.12 [0.07, 0.17]	<0.001
Serum testosterone, nmol/L					
Baseline	14.1 (4.6)	13.2 (4.1)	558		
Week 18	14.9 (5.1)	14.7 (5.5)	539	0.96 [0.11, 1.82]	
Week 66	14.7 (5.3)	16.1 (5.8)	476	1.99 [1.10, 2.88]	
Week 102	14.9 (5.6)	15.5 (6.2)	463	1.42 [0.53, 2.33]	<0.001
Serum estradiol, pmol/L					
Baseline	206 (104)	194 (94)	558		
Week 18	197 (94)	236 (145)	539	48.1 [28.5, 67.6]	
Week 66	196 (103)	238 (122)	476	50.2 [29.9, 70.5]	
Week 102	205 (114)	222 (126)	463	24.6 [4.1, 45.0]	<0.001

Mean (SD) are presented. Serum testosterone and estradiol were measured by LCMS/MS.

total hip (1.9% [1.2, 2.7]) and femoral neck 1.7% [0.8, 2.7]). This greater effect in the present study may be due to the use of injectable versus transdermal testosterone, the longer duration of treatment, and/or larger number of men in the current study.

The strengths of the present study include its first evaluation of testosterone treatment on bone microarchitecture using HR-pQCT, a low–radiation exposure technology (8) that predicts fracture risk independent of aBMD and FRAX (9), in a randomized, placebo-controlled study

Table 5. Adverse events

	Placebo group (N = 293)	Testosterone group (N = 308)	*P* value
Death	2 (0.7%)	2 (0.6%)	1.00
Total cardiac adverse events	13 (4.4%)	14 (4.6%)	1.00
- Cardiac death	0 (0%)	1 (0.3%)	
- AMI	3 (1.0%)	1 (0.3%)	
- Unstable angina	6 (2.0%)	3 (1.0%)	
- Heart failure	1 (0.3%)	1 (0.3%)	
- Arrhythmia	1 (0.3%)	7 (2.3%)	
- Stroke	2 (0.7%)	1 (0.3%)	
Total prostate adverse events	7 (2.4%)	8 (2.6%)	1.00
- BPH	4 (1.4%)	7 (2.3%)	
- Prostate cancer	3 (1.0%)	1 (0.3%)	
Raised hematocrit ≥54%	3 (1%)	61 (20%)	<0.001
DVT adverse events	0 (0.0%)	1 (0.3%)	1.00
Other adverse events	6 (2.1%)	14 (4.6%)	0.11
- Psychiatric illness	3 (1.0%)	1 (0.3%)	
- Cancer	2 (0.7%)	5 (1.6%)	
- Noncardiac chest pain	1 (0.3%)	4 (1.3%)	
- Noncardiac syncope	0 (0.0%)	2 (0.6%)	
- Neurological	0 (0.0%)	2 (0.6%)	

Values are presented as proportions N (%). Given low numbers of events, the 2 groups were compared using Fisher exact test. Deaths in placebo group were due to stroke and suicide, deaths in testosterone group due to cardiac death and suicide.

Abbreviations: AMI, acute myocardial infarction; BPH, benign prostatic hypertrophy, DVT, deep vein thrombosis.

design featuring a relatively large size and long duration of testosterone treatment, including the administration of study drug by study personnel eliminating compliance issues. In T4DM, adherence to all study visits was 74% for men receiving placebo and 77% for men receiving testosterone (11).

Study Limitations

Our study was not designed to assess the effects of testosterone on fracture outcomes. Similar to the T-trials bone study (7), baseline aBMD was relatively normal. Whether similar effects of testosterone on bone microarchitecture would be seen in men with osteoporosis remains unknown. Moreover, men participating in T4Bone were selected on the basis of high risk for diabetes and had central obesity (11). Given that previous studies have reported important relationships between obesity and body composition, and especially abdominal obesity, with bone microarchitecture (39) whether our findings can be generalized to other male populations remains unknown. Given potential concerns that the risk of adverse effects of testosterone treatment may be increased in older men (40), the upper bound of the age limit in this study was set at 74 years (11). Therefore, the effects of testosterone treatment in men older than 74 years, a population with substantially increased fracture risk, remain unknown. Men participating in T4Bone had a baseline serum testosterone of 13.6 nmol/L, compared with approximately 8.2 nmol/L in the T Bone trial (7). In using injectable testosterone, measured trough concentrations (blood drawn immediately prior to the next dose) represent a minimal estimate of time-averaged blood testosterone concentration since previous injections. The serum testosterone entry criterion for T4DM was defined to a testosterone level below which the risk of diabetes increases steeply (10, 11). Serum testosterone eligibility was based on immunoassay but analyses were based on remeasurements by validated LCMS/MS (19), which yielded higher concentrations than the immunoassay. Of note, given that the objective of T4DM was to assess the effects of testosterone treatment on diabetes incidence in high-risk men, participants were not required to have symptoms or signs of testosterone deficiency. Thus, testosterone treatment was pharmacological, rather than replacement, and findings may not apply to men with pathological or age-related hypogonadism. Some studies have suggested that skeletal health is compromised especially at testosterone concentrations <6.9 nmol/L (41, 42), lower than the baseline concentration in our study. However, a recent experimental study in older men has reported that bone resorption increases below serum testosterone concentrations <17.3 nmol/L (43). Moreover, endogenous testosterone thresholds for androgen deficit effects are not necessarily the same as those for effects of testosterone treatment. For example, dose-dependent testosterone effects on muscle mass and strength extend well into the supraphysiologic range (44, 45). The threshold-based method using standard Scanco analysis software may have limitations in accurately segmenting cortical from trabecular bone (21). Ongoing cortical fragmentation of the inner cortex adjacent to the medullary canal may result in cortical fragments in the placebo group erroneously measured as "trabecular," underestimating the decrease in the loss of trabecular bone in the placebo and thereby failing to detect a protective effect of testosterone against trabecular bone loss (21). Physical activity, a potential confounder, while not different between

men assigned to testosterone or placebo, was measured by self-report (11). Moreover, we did not measure bone strength, such as finite element analysis. Finally, the number of men recruited for the HR-pQCT study slightly exceeded the number estimated by our power calculations. Of note, the total cumulative radiation exposure of a participant completing all 3 scans at both sites (radius and tibia) is around ~18 μSv (46), 5-fold less than that of a single chest x-ray. Moreover, the power analysis was based on the microstructural decay occurring in men subjected to androgen deprivation therapy causing profound sex steroid deficiency (12). While the best data available at the time T4Bone was designed, reducing sex steroids to near zero may have effects on bone decay that may well be more substantial than the effects of testosterone treatment on men eligible for this study. Moreover, a larger study size had the potential to identify changes on HR-pQCT parameters other than the primary endpoint. Likewise, we exceeded the recruitment target for the DXA study, which allowed achieving significance for femoral aBMD sites. Of note, DXA scanning for monitoring of changes in lean and fat mass was routinely performed for participants in the main T4DM study (11), and the BMD data were available at minimal additional risk to participants.

In summary, testosterone treatment given over 2 years to men older than 50 years with prediabetes or early diabetes improves bone microarchitecture as measured by HR-pQCT at both the tibia and the radius. This effect is predominantly due to increases in cortical, rather than trabecular, bone. Consistent with the findings in bone microarchitecture, testosterone treatment reduces bone remodeling, and increases aBMD at lumbar spine and femur. This is the first RCT to address the effect of testosterone treatment on bone microarchitecture; however, the implications for fracture outcomes require further studies. Of note, from a clinical perspective, our findings are clearly not meant to endorse testosterone treatment to improve skeletal health in men without pathological hypogonadism and with a normal serum testosterone.

Acknowledgments

The authors thank the men who generously took part in this study for the benefit of future medical knowledge. We gratefully acknowledge the professionalism of the clinical research nurses, Jenny Healy (Austin Hospital), Glenda Fraser (Concord Hospital), Helen Daniels and Chyn Soh (Fremantle Hospital and Fiona Stanley Hospital), Jody Sawyer (Princess Alexandra Hospital), Rosemary Cox and Fiona Cossey (The Queen Elizabeth Hospital), and Lee Mahoney (The Keogh Institute for Medical Research); the NHMRC Clinical Trials Centre (Simone Marschner, Andrzej Januszewski, Caitlin Van Holst Pellekaan, Sandra Healey) and the University of Adelaide (Margaret McGee, Dr Susan Shanley), and The Queen Elisabeth Hospital (Mr Christopher Seaborn).

Financial Support: There was no funding for this T4Bone substudy. The T4DM study was supported by grants from the National Health and Medical Research Council (NHMRC) Project Grant #1030123, Bayer, Lilly, and the University of Adelaide with in-kind support from Weight Watchers and Sonic Healthcare.

Clinical Trial Information: Australian New Zealand Clinical Trials Registry No. ACTRN12612000287831. https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=362125

Author Contributions: M.N.T.F., R.H., K.B., D.J.H., W.J.I., B.G.A.S., B.B.Y., A.G.Z., K.P.R., D.J., J.D.Z, G.A.W., and M.G. were involved in the design, applications for funding and/or conduct of the study. M.N.T.F., R.H., K.B., D.J.H., W.J.I., B.G.A.S., B.B.Y., A.G.Z., K.P.R., D.J., J.D.Z., G.A.W., and M.G. had input into and approved the statistical plan. R.H. performed the statistical analyses. M.N.T.F., R.H., K.B., D.J.H., W.J.I., B.G.A.S., B.B.Y., A.G.Z., K.P.R., D.J., J.D.Z, G.A.W., and M.G. had input into the interpretation and write-up of the data. M.N.G.T.F. and M.G. wrote the first draft of the article. M.N.T.F., R.H., K.B., D.J.H., W.J.I., B.G.A.S., B.B.Y., A.G.Z., K.P.R., D.J., J.D.Z., G.A.W., and M.G. revised and approved the article.

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Disclosures: M.G. has received research funding from Bayer, Otzuka, and speaker's honoraria from Besins Health Care and Novartis. D.J.H. has received institutional grants without personal funding for investigator-initiated testosterone pharmacology studies (Besins, Lawley) and served as an expert witness in antidoping and professional standards tribunals and for testosterone litigation. B.G.A.S. has received speaker fees (Besins). B.B.Y. has received speaker honoraria and conference support from Bayer, Lilly, and Besins Healthcare, and research support from Bayer, Lilly, and Lawley Pharmaceuticals, and has held advisory roles with Lilly, Besins Healthcare, Ferring, and Lawley Pharmaceuticals. A.G.Z. is a shareholder in StraxCorp. G.A.W. has received research funding for testosterone pharmacology studies (Lawley, Bayer, Lilly), speakers (Besins, Bayer) and consultancy (Elsevier) fees. J.D.Z. has received speakers fees from Besins. M.N.T.F., R.H., K.B., W.J.I., K.P.R., and D.J. have nothing to disclose.

Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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