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# Does body mass index predict changes in body composition and metabolic markers in response to testosterone therapy?

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ABSTRACT

*Background:* Despite reported improvements in body composition, metabolic profile, skeletal outcomes and symptoms from testosterone (T) therapy in men with hypogonadism, it remains unclear whether baseline body mass index (BMI) affects response. The objective of this study is to determine if baseline BMI influences response to T therapy.

*Methods:* This is a secondary analysis of data from a clinical trial on pharmacogenetics of response to T therapy in men with average morning $T < 300$ ng/dl, given intramuscular T cypionate 200 mg every 2 weeks for 18 months. Participants were divided into BMI categories: BMI 1 ($< 30$ kg/m$^2$), BMI 2 (30–34.99 kg/m$^2$), and BMI 3 ($\geq$35 kg/m$^2$). T, estradiol, adipokines, metabolic markers, body composition, bone mineral density, bone turnover markers and total symptom score were assessed at baseline, 6, 12 and 18 months.

*Results:* All 3 groups had improvement in body composition, though BMI 1 and 2 had greater increases in truncal fat-free and truncal lean mass than BMI 3. Appendicular fat mass declined the most in BMI 1 and the least in BMI 3.. Leptin declined in all 3 groups with the most in BMI 1, while adiponectin significantly decreased only BMI 2. All groups showed increase in lumbar spine BMD. BMI 1 and 2 had improvement in symptoms but not BMI 3.

*Conclusion:* Our study shows that men with a BMI <35 derived the greatest benefit from T therapy in terms of changes in body composition and symptoms, while those with higher BMI benefited the least.

## 1. Introduction

Obese men have low testosterone (T) levels compared to men with normal Body Mass Index (BMI). This is hypothesized as due to low sex hormone binding globulin (SHBG) levels in combination with a component of hypogonadotropic hypogonadism [1]. Low T levels are associated with a host of abnormalities, such as metabolic syndrome in men; increased fat mass, low lean mass, insulin resistance and elevated total and low-density lipoprotein (LDL) cholesterol [2]. In addition to the derangement in these metabolic markers are changes in adiponectin and leptin, two important adipokines that have been linked to Type 2 Diabetes Mellitus (T2DM) and obesity [3]. Leptin and adiponectin are secreted by the adipose tissues and function to regulate energy homeostasis [4]. Adiponectin levels are decreased, and leptin levels are elevated in obese individuals with insulin resistance [5,6]. While leptin has some pro-inflammatory activity, adiponectin is anti-inflammatory [7].

Findings have been conflicting on the beneficial effects of T therapy on metabolic markers. While some studies showed significant decreases in LDL and total cholesterol, decrease in fasting insulin levels [8,9], and improvement in body composition with reduction in fat mass and an increase in lean and fat-free mass [10], others failed to demonstrate any

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metabolic benefit from T therapy despite reduction in body weight and fat mass [11]. Furthermore, while studies showed reduction in both leptin and adiponectin following T treatment [10,12], which was found to correlate with the concomitant decrease in waist circumference, others found a reduction only in leptin with either no change or even an increase in adiponectin [13]. A meta-analysis suggests that aromatization of T to estradiol (E2) in addition to the type and dose of T used could be responsible for the heterogeneity in findings between studies [14]. That the degree of aromatization, which may be dependent on the amount of body fat, would result in differences in outcome from T therapy has not been investigated.

Prior studies suggest increased aromatization of T to E2 as a main contributor to hypogonadotropic hypogonadism in obese men [15,16]. Studies on the efficacy of T therapy in obese men with low T have yielded mixed findings. While some randomized studies showed that T treatment may have modest benefits on hypogonadal symptoms, body composition and metabolic parameters and may even have additive effect if done in conjunction with lifestyle modification, others reported no benefit [13,17]. Although most of these studies are short-term, with mostly small sample sizes [18] to explain these conflicting findings, it is also possible that this disparity was influenced by the body size or degree of adiposity of the study population which could vary from one study to another. In a retrospective analysis of longitudinal data from obese men enrolled in 2 registry studies with participants divided into 3 categories of obesity (class 1 to 111, with 111 being the most obese), the authors showed that all 3 groups experienced comparable reductions in body weight and waist circumference, improvement in symptoms and glucometabolic parameters after 1 to 8 years of observation while on T therapy [19]. However, the study was confined to obese men only, thus no participant with normal weight or overweight was included.

T-deficiency is also associated bone loss and osteoporosis [20]. Majority of the studies demonstrated improvement in bone mineral density (BMD) with T therapy which is in general more robust in the spine than in the hip [21,22]. An earlier study demonstrated that BMD response to T therapy was much greater for those with baseline T levels of <200 ng/dl [22]. However, to our knowledge no study has explored the effect of BMI on the BMD response to T. E2 remains the main hormone regulating BMD in men [23]. It is possible that BMD response to T varies according to BMI due to varying degrees of aromatization of T to E2.

Thus, taken altogether, it remains unclear if obese men will have the same response as nonobese men. In the current study, we aimed to evaluate if T treatment in obese people is as beneficial as those with lower BMI. We hypothesized that response to T will vary according to BMI due to differences in aromatase activity resulting from varying amounts of adipose tissue volume. Thus, the objective of this study was to determine if baseline BMI influences response to T therapy in terms of changes in body composition, metabolic and bone parameters, and symptoms.

## 2. Methods

### 2.1. Study design and study population

This study is a secondary analysis of the data from an open label clinical trial investigating the genetics of response to T therapy, conducted from October 2011 to November 2016 (ClinicalTrials.gov identifier: NCT01378299). The original study was conducted at the University of New Mexico VA Health Care System (NMVAHCS) and at the Michael E. DeBakey VA Medical Center (MEDVAMC) in accordance with guidelines of the Declaration of Helsinki for the ethical treatment of human subjects. The protocol was approved by the Institutional Review Boards of the University of New Mexico and Baylor College of Medicine. The participants were recruited from the patients attending the Endocrine, Urology, and Primary Care Clinics of the NMVAHCS (HRPO #11139) and MEDVAMC (H-34812). Written informed consent was obtained from each participant. Information regarding study design, inclusion, and exclusion criteria of the participants, as well as details of T therapy had been published elsewhere [24]. Hypogonadism was defined as an average total T of <300 ng/dl from two fasting samples taken in the morning. The inclusion criteria were male patients between 40 and 75 years of age with no medical problems that may prevent them from finishing the study. Exclusion criteria included treatment with bone-acting drugs (e.g., bisphosphonates, teriparatide, denosumab, glucocorticoids, sex steroid compounds, selective estrogen receptor modulators, androgen deprivation therapy, and anticonvulsants) and finasteride. Additional exclusion criteria included osteoporosis and history of fragility fractures or diseases known to affect bone metabolism, such as hyperparathyroidism, chronic liver disease, uncontrolled or untreated hyperthyroidism, and significant renal impairment (creatinine of >1.5 mg/dl). Those with a history of prostate cancer, breast cancer, and untreated sleep apnea also met the criteria for exclusion.

### 2.2. Testosterone therapy

Therapy consisted of intramuscular injection of 200 mg of T cypionate administered every 2 weeks. Initially, the dose was adjusted to reach the T serum target level of 17.3 to 27.7 nmol/L (500–800 ng/dl). However, after the third year of the study, upon the direction of the FDA, this target was changed to 10.4 to 20.8 nmol/L (300–600 ng/dl). This change affected the last 6 months of data for 16 participants at NMVAHCS and of all 15 participants at MEDVAMC. We did not detect a significant difference in T levels among those affected and those not affected by the change as published previously [24]. For all participants, safety monitoring and dose adjustments were performed based on T and hematocrit levels; dose adjustment modalities have been published elsewhere [24]. The participants were given T therapy for 18 months.

### 2.3. Biochemical measurements

Blood samples were collected in a fasting state at baseline; serum and plasma samples were extracted and stored at –80 °C until analysis except for the baseline screening of T levels. Baseline serum T represents an average from 2 determinations taken 30 min apart between 8:00 and 11:00 a.m., and measured using automated immunoassay, detection range 10 to 3200 ng/dl (Vitros®, Ortho Clinical Diagnostics, Rochester, NY). The coefficient of variation (CV) for T assay was ≤ 20 % for T < 50 ng/dl and ≤ 10 % for T 200 to 1000 ng/ dl. At the end of the study, T and E2 for each time point were measured by liquid chromatography/mass spectrometry (Mayo Clinic Laboratories, Mayo Clinic, Rochester, MN, USA). T intra- assay CVs were 7.4 %, 6.1 %, 9.0 %, 2.3 %, and 0.9 % at 0.65, 4.3, 48, 118, and 832 ng/dl, respectively. Inter-assay CVs were 8.9 %, 6.9 %, 4.0 %, 3.6 %, and 3.5 % at 0.69, 4.3, 45, 117, and 841 ng/dl, respectively. The detection range was 0.5 to 2000 ng/dl. For analysis in this study, T values by Liquid Chromatography/Mass Spectroscopy (LC/MS) from blood obtained at screening were used (see Statistical Methods section). E2 assay sensitivity was 0.23 pg/ml to 405 pg/ml, intra-assay CV was 1.4 % to 11.8 %, and inter-assay CV was 4.8 % to 10.8 % [25]. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were assessed by a third-generation chemiluminescent assay. The following metabolic markers were measured at the New Mexico VA Health Care System and the Michael E. DeBakey VA Medical Center: fasting glucose was measured using a Unicel DxC 800 auto-analyzer (Beckman Coulter, Fullerton, CA, USA), hemoglobin A1c was measured by high performance liquid chromatography (HPLC) with the Tosoh Automated Glycohemoglobin Analyzer HLC-723G8. (Tosoh Bioscience, Inc. South San Francisco, CA 94,080); CV <3.5 %, total cholesterol and triglycerides were measured by fluorometric assay and LDL and HDL were measured by colorimetric assay by UNICEL DxC (Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92,821 USA). Detection limits for these measurements are: 7–750 mg/dL (0.13–19.43 mmol/L) for total cholesterol, 11–500 mg/dl for LDL, 5–135 mg/dL

(0.13–3.5 nmol/L) for HDL, 10–1000 mg/dL (0.1–11.3 mmol/L) for triglycerides; CVs <10 % for all measurements. The following were measured using enzyme-linked immunosorbent assay kits: C-terminal telopeptide of type I collagen (CTX), marker of bone resorption (Crosslaps; Immunodiagnostic System Inc., Gaithersburg, MD, USA), osteocalcin, marker of bone formation (Metra OC; Quidel Corporation, San Diego, CA, USA), sclerostin (TECO medical Sclerostin HS Enzyme Immunoassay Kit, Quidel Corp, San Diego, CA, USA), and adiponectin (Quantikine; R&D Systems; CV 5.5 %). The CVs for the above assays in our laboratory are <10 %. RIA kits were used to measure leptin (Leptin HL-81 K; Linco Research Inc; CV 5.6 %).

### 2.4. Body mass index

Body weight was measured using a standard weighing scale and height was obtained using a stadiometer. BMI was calculated as body weight in kilograms (kg) divided by the square of the height in meters ($m^2$) and expressed as $kg/m^2$.

### 2.5. Body composition

Total body mass, lean body mass (mineral-free and fat free), fat mass, and truncal fat were measured by whole body DXA (Enhanced Whole Body Software version 11.2; Hologic, Inc.) as previously described [26]. The percentages of the whole and regional fat mass (% fat) were obtained from the estimated readings given by the machine for each region of interest. Fat-free mass was calculated by adding whole body bone mineral content to the lean mass [27]. The CV for lean mass and fat mass in our laboratory is 1.5 % [27]. BMI and body composition were assessed at baseline, 6, 12, and 18 months.

### 2.6. Areal bone mineral density by dual energy X-Ray absorptiometry (DXA)

Areal Bone Mineral Density (BMD) was measured by DXA of the lumbar spine and proximal femur using Hologic Discovery (Hologic Inc, Bedford, MA, USA). Regions of interest in the femur include the total hip and femoral neck. The CVs at our center are ~1.1 % for the lumbar spine and 1.2 % for the proximal femur [24].

### 2.7. Total symptom score

A total symptom score was calculated based on a questionnaire that we designed for the study (**Supplement 1**). It included vital signs, as well as signs and symptoms such as daytime sleepiness, tiredness, loss of energy, loss of muscle strength, depression, acne, loss of libido, erectile dysfunction, gynecomastia, leg edema, shortness of breath, history of bleeding, chest pain, snoring and headache. Each symptom was given 1 point, and the sum of all was the total symptom score, with a higher score indicating worse symptoms.

### 2.8. Statistical methods

For this secondary study, we used BMI cut offs to divide the sample size into three categories; category 1 defined as BMI < 30 $kg/m^2$ **(BMI 1)**, category 2 as BMI 30–34.99 $kg/m^2$ **(BMI 2)** and category 3 as BMI ≥35 $kg/m^2$ **(BMI 3).**

Baseline values, percent and absolute changes from baseline data are presented as mean ± SD in the tables and mean±SE in the figures. Individual changes from baseline within each BMI group at the different visits were compared using T-test. To test whether the changes in body composition, metabolic parameters, and BMD/bone turnover markers are influenced by baseline BMI (as categorized above), these 3 outcome domains are considered separately. The analysis for each variable between the three BMI groups at each visit was done by analysis of covariance (ANCOVA) with the BMI as a grouping factor, and with baseline measure of each outcome variable as a covariate.

The overall ANCOVA results for body composition and BMD were additionally adjusted for age at baseline to account for known age effects on these parameters. The results of metabolic, hormonal, and safety profile, and bone turnover markers were adjusted for baseline only. A p of 0.05 or less is considered statistically significant. Significant post-hoc difference between BMI categories was analyzed using Fisher's Least Significant Difference method and indicated by A, B, C designations.

Data were managed using Excel 2013 (Microsoft, Redmond, WA) and analyzed using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA).

## 3. Results

### 3.1. Baseline characteristics

A total of 105 men participated in the study; there were 39 in BMI 1, 38 in BMI 2, and 28 in BMI 3. However, only 100 out of the 105 participants had T level by mass spectrometry available. The baseline characteristics of all the participants are presented in Table 1. Men in BMI 1 are older than the rest of the groups. Among the 3 groups, men in BMI 3 had the lowest baseline T level and the highest E2 level . As expected, men in BMI 1 had the lowest weight and BMI 3 had the highest. Also as expected, body composition parameters (total, truncal and appendicular fat-free mass [FFM]; total, truncal and appendicular fat mass; and total, truncal and appendicular lean mass) were highest in BMI 3 and lowest in BMI 1. BMD of the spine, total hip and femoral neck were also highest in BMI 3 compared to BMI 1 and 2. The remaining characteristics did not differ significantly between the three groups.

### 3.2. Body composition outcomes

Table 2 shows changes in body composition over the course of 18 months of T therapy between the three groups. All categories of BMI had a significant increase in total FFM at 18 months compared to baseline (all $p < 0.05$); with the greatest increase in BMI 2; however, the difference between groups was not significant. Similar results were noted for appendicular FFM.. Men in BMI 1 and 2 had significant increases in truncal FFM at 18 months compared to baseline, whereas those in BMI 3 did not; with a significant difference between groups with the change in BMI 3 significantly less than BMI 1 and 2.

All three groups showed a significant decline in total fat mass at 18 months compared to baseline (Table 2). However, this decline was not significant in BMI 3, and greatest for BMI 1. Nonetheless, no significant between-group difference was noted. Decline from baseline in truncal fat mass was only significant for those in BMI 1 at 18 months, with no significant difference between the groups (Table 2). Appendicular fat mass also declined from baseline in all three groups at 18 months (Fig. 1A) which were significant for BMI 1 and 2 but not BMI 3; the greatest change noted in BMI 1 (−10.8 ± 8.9 %) followed by BMI 2 (−6.5 ± 8.7 %) and the lowest in BMI 3 (−3.4 ± 8.1 %). There was a significant difference between groups **($p = 0.03$)**, with the change being significant for BMI 1 compared to BMI 3.

BMI 1 and 2 showed significantly greater increases in total lean mass (Table 2) at the end of 18 months relative to baseline, while the change in BMI 3 was not significant. The significance between groups was only borderline ($p = 0.07$). Compared to baseline, appendicular lean mass significantly increased at 18 months in all the BMI categories; the highest for BMI 1 with no significant between-group difference. Truncal lean mass also significantly increased at all timepoints in BMI 1 and BMI 2 but not in BM 3 and was significantly different between the groups at the end of 18 months (BMI 1:3.2 ± 4.7 %, BMI 2: 4.3 ± 3.6 %, BMI 3:0.2 ± 4.5 %, **$p = 0.03$**) (Fig. 1B).

**Table 1**
Baseline clinical characteristics.

| | BMI <30 kg/m$^2$ (N = 39) | BMI 30–34.9 kg/m$^2$ (N = 38) | BMI≥35 kg/m$^2$ (N = 28) | P |
|---|---|---|---|---|
| Age (years) | 61.8 ± 8.2 | 57.2 ± 8.8 | 59.7 ± 7.2 | 0.046 |
| Body Weight (kg) | 87.3 ± 9.5 | 99.9 ± 8.6 | 121.0 ± 13.4 | 0.001 |
| Testosterone (ng/dl) | 300.9 ± 81.1 | 257.8 ± 78.4 | 245.5 ± 85.8 | 0.017 |
| Estradiol (pg/ml) | 16.0 ± 5.4 | 15.8 ± 6.4 | 19.7 ± 6.6 | 0.020 |
| Body composition (kg) | | | | |
| Total fat-free mass | 61.7 ± 6.9 | 68.2 ± 5.9 | 72.7 ± 7.2 | <0.001 |
| Appendicular fat-free mass | 27.7 ± 4.0 | 30.9 ± 3.5 | 32.5 ± 4.3 | <0.001 |
| Truncal fat-free mass | 29.9 ± 3.1 | 33.2 ± 3.1 | 36.0 ± 3.5 | <0.001 |
| Total fat mass | 24.7 ± 5.4 | 30.9 ± 5.8 | 46.6 ± 8.2 | <0.001 |
| Truncal fat mass | 12.5 ± 3.4 | 16.0 ± 3.3 | 26.2 ± 5.5 | <0.001 |
| Appendicular fat mass | 11.0 ± 2.5 | 13.5 ± 2.8 | 19.0 ± 3.5 | <0.001 |
| Total lean mass | 58.9 ± 6.4 | 65.6 ± 5.7 | 69.9 ± 7.0 | <0.001 |
| Appendicular lean mass | 25.9 ± 3.7 | 29.6 ± 3.1 | 30.9 ± 4.1 | <0.001 |
| Truncal lean mass | 29.3 ± 3.1 | 32.5 ± 3.1 | 35.3 ± 3.4 | <0.001 |
| Bone mineral density (g/cm$^2$) | | | | |
| Lumbar spine | 1.086±0.1 | 1.108±0.2 | 1.211±0.2 | 0.004 |
| Total Femur | 1.029±0.1 | 1.038±0.1 | 1.193±0.1 | <0.001 |
| Femoral neck | 0.801±0.1 | 0.796±0.1 | 0.879±0.1 | 0.014 |
| Bone turnover markers (ng/ml) | | | | |
| Osteocalcin | 7.0 ± 4.1 | 6.3 ± 5.4 | 5.7 ± 3.1 | 0.583 |
| CTX | 0.34 ± 0.2 | 0.34 ± 0.2 | 0.33 ± 0.2 | 0.963 |
| Sclerostin | 0.82 ± 0.3 | 0.79 ± 0.3 | 0.76 ± 0.2 | 0.738 |
| Metabolic parameters | | | | |
| Hemoglobin A1c (%) | 6.5 ± 1.9 | 6.6 ± 1.5 | 7.0 ± 1.6 | 0.449 |
| Fasting blood sugar (mg/dl) | 120.9 ± 45.7 | 114.2 ± 37.0 | 141.3 ± 59.5 | 0.058 |
| Total cholesterol (mg/dl) | 176.3 ± 51.0 | 178.7 ± 41.9 | 163.1 ± 36.4 | 0.404 |
| Triglycerides (mg/dl) | 173.6 ± 91.4 | 196.2 ± 57.6 | 173.2 ± 76.3 | 0.722 |
| Low density lipoprotein (mg/dl) | 99.7 ± 43.4 | 106.5 ± 36.0 | 88.1 ± 31.8 | 0.279 |
| High density lipoprotein (mg/dl) | 44.8 ± 15.5 | 37.5 ± 10.1 | 41.3 ± 12.5 | 0.069 |
| Leptin (ng/ml) | 2.2 ± 1.5 | 2.7 ± 2.1 | 3.7 ± 1.9 | 0.028 |
| Adiponectin (μg/ml) | 54.6 ± 26.6 | 48.3 ± 30.4 | 38.2 ± 14.7 | 0.208 |
| Prostate specific antigen (ng/ml) | 0.97±0.73 | 0.94±0.67 | 1.05±0.61 | 0.796 |
| Hematocrit (%) | 43.1±2.9 | 44.3±3.0 | 43.8±2.8 | 0.193 |
| Total symptom score | 6.5 ± 2.3 | 6.8 ± 2.4 | 7.3 ± 2.4 | 0.335 |

Values are expressed mean±SD; Bolded p-values are statistically significant. Group comparisons by analysis of variance (ANOVA).
CTX: C-terminal telopeptide of type I collagen, BMI: body mass index.

### 3.3. Metabolic markers and Adipokines

#### 3.3.1. HbA1c and lipid profile

HbA1c, fasting blood glucose and LDL cholesterol did not change significantly at any visit with T therapy, both when compared to baseline, and between the three groups (Table 2). Total cholesterol significantly declined in BMI 3 at 6 months, however no difference was noted between the three groups at any timepoint. Triglycerides significantly increased in patients in BMI 2 at 12 months, but no difference was noted between the three groups at any timepoint. HDL significantly declined in BMI 1 at 6 and 12 months; and at 12 and 18 months for BMI 3, with no significant between-group difference in all timepoints. .

#### 3.3.2. Adipokines

Leptin levels significantly decreased from baseline at 18 months in all three groups with the biggest decline seen in those in BMI 1 compared

**Table 2**
Changes (%) in body composition and metabolic profile with testosterone therapy.

| | BMI <30 (N = 39) | BMI 30–34.9 (N = 38) | BMI≥35 (N = 28) | P |
|---|---|---|---|---|
| A. Body composition | | | | |
| Total Fat-free Mass | | | | |
| 6 months | 4.4* ±4.4 | 4.6* ±4.4 | 3.4* ±4.0 | 0.65 |
| 12 months | 2.9* ±4.5 | 5.6* ±3.8 | 4.1* ±5.3 | 0.22 |
| 18 months | 3.6* ±9.7 | 5.1* ±3.7 | 1.9* ±3.7 | 0.61 |
| Appendicular Fat-free Mass | | | | |
| 6 months | 6.1*±4.6 | 7.1*±12.3 | 4.9*±5.3 | 0.60 |
| 12 months | 4.3*±3.2 | 9.1*±13.4 | 4.6*±4.2 | 0.12 |
| 18 months | 2.3*±4.6 | 7.3*±13.4 | 3.7*±4.7 | 0.19 |
| Truncal Fat-free mass | | | | |
| 6 months | 4.5* ±5.1 | 6.4* ±12.0 | 2.1 ± 4.7 | 0.37 |
| 12 months | 3.4* ±5.7 | 4.7* ±4.6 | 1.2 ± 3.4 | 0.16 |
| 18 months | 3.2*A ± 4.6 | 4.3*A ± 3.6 | 0.3B ±4.4 | 0.04 |
| Total Fat Mass | | | | |
| 6 months | −7.1*±6.4 | −6.0*±6.7 | −5.9*±7.3 | 0.87 |
| 12 months | −10.2*±8.4 | −5.6*±6.9 | −8.5*±12.1 | 0.36 |
| 18 months | −8.3*±10.5 | −4.5*±8.8 | −2.8 ± 9.2 | 0.20 |
| Truncal Fat Mass | | | | |
| 6 months | −6.5*±9.6 | −7.3*±8.4 | −6.6*±8.9 | 0.85 |
| 12 months | −9.9*±12.0 | −6.1*±9.0 | −7.0 ± 12.7 | 0.60 |
| 18 months | −6.2*±14.3 | −3.7 ± 10.6 | −2.5 ± 11.8 | 0.67 |
| Total Lean Mass | | | | |
| 6 months | 5.1*±4.2 | 4.8*±4.6 | 3.5*±4.3 | 0.48 |
| 12 months | 3.5*±4.2 | 5.8*±4.0 | 2.7*±3.0 | 0.10 |
| 18 months | 2.7*±3.7 | 5.2*±3.9 | 1.8 ± 3.9 | 0.07 |
| Appendicular Lean Mass | | | | |
| 6 months | 7.7*±9.9 | 5.4*±6.2 | 5.2*±5.6 | 0.57 |
| 12 months | 6.5*±10.8 | 7.1*±6.9 | 4.8*±4.4 | 0.65 |
| 18 months | 10.4 ± 33.5 | 6.3*±6.0 | 3.9*±5.1 | 0.71 |
| B. Metabolic Profile | | | | |
| Hemoglobin A1c | | | | |
| 6 months | 1.8 ± 7.0 | −0.4 ± 9.4 | 0.1 ± 11.7 | 0.61 |
| 12 months | −1.2 ± 6.7 | 2.4 ± 15.0 | −3.1 ± 12.4 | 0.30 |
| 18 months | 1.7 ± 6.6 | 1.2 ± 15.1 | −0.5 ± 12.3 | 0.87 |
| Fasting Blood Sugar | | | | |
| 6 months | 1.2 ± 31.5 | 5.7 ± 25.3 | −6.6 ± 43.8 | 0.40 |
| 12 months | −1.2 ± 32.8 | 11.3 ± 36.4 | −6.6 ± 29.7 | 0.18 |
| 18 months | −6.4 ± 24.3 | 18.1 ± 58.9 | −6.2 ± 46.1 | 0.09 |
| Total Cholesterol | | | | |
| 6 months | −2.0 ± 24.0 | −3.0 ± 20.2 | −6.9* ±15.1 | 0.68 |
| 12 months | 1.0 ± 27.5 | −1.4 ± 24.1 | −4.9 ± 18.2 | 0.73 |
| 18 months | 1.6 ± 35.3 | −5.3 ± 19.2 | 0.9 ± 10.3 | 0.61 |
| Triglycerides | | | | |
| 6 months | 0.3 ± 41.5 | 2.9 ± 44.4 | −8.3 ± 30.2 | 0.59 |
| 12 months | 2.4 ± 45.7 | 37.1* ±70.9 | 15.7 ± 64.3 | 0.11 |
| 18 months | −13.9 ± 44.7 | 24.9 ± 61.5 | 16.7 ± 52.8 | 0.06 |
| Low Density Lipoprotein | | | | |
| 6 months | 6.7 ± 39.4 | 1.9 ± 41.0 | −1.1 ± 24.4 | 0.72 |
| 12 months | 6.1 ± 34.3 | −1.9 ± 48.4 | −4.6 ± 25.5 | 0.62 |
| 18 months | 6.2 ± 42.1 | −4.3 ± 42.5 | 5.3 ± 19.5 | 0.62 |
| High Density lipoprotein | | | | |
| 6 months | −6.8* ±17.9 | −1.3 ± 35.9 | −6.5 ± 19.0 | 0.66 |
| 12 months | −9.0* ±19.9 | −0.5 ± 51.5 | −8.4* ±14.5 | 0.62 |
| 18 months | −6.6 ± 17.5 | −4.6 ± 48.5 | −15.2* ±11.2 | 0.65 |
| Leptin | | | | |
| 6 months | −24.7* ±32.2 | −12.1 ± 58.0 | −27.2* ±17.4 | 0.45 |
| 12 months | −20.6 ± 59.5 | −0.3 ± 75.7 | −3.0 ± 47.4 | 0.54 |
| 18 months | −40.6* ±31.8 | −24.9* ±40.1 | −27.8* ±28.5 | 0.31 |
| Adiponectin | | | | |
| 6 months | 5.4 ± 74.1 | −8.9 ± 24.8 | 18.6 ± 35.3 | 0.25 |
| 12 months | −1.9 ± 65.5 | 0.7 ± 32.1 | 11.9 ± 23.0 | 0.74 |
| 18 months | −6.3 ± 40.4 | −23.7* ±46.5 | 5.5 ± 44.5 | 0.21 |

BMI: body mass index. Values are mean±SD; change scores are reported as % change from baseline value. The individual comparison to baseline in each BMI category was done by paired t-test. Asterisk* means those changes from baseline were significant. The overall between-group P-values for body composition

parameters was adjusted for age of the subjects at baseline and are calculated by analysis of covariance (ANCOVA); while between-group p-values for the metabolic parameters are reported by analysis of variance (ANOVA). Bolded p-values are statistically significant. Significant post-hoc differences between BMI categories are labelled by A, B, C superscripts; those with the same letters are not significantly different and those with different letters are significantly different.

to BMI 2 and BMI 3 (Table 2). However, no significant between-group difference was noted. There was a significant decrease in adiponectin level in BMI 2 at 18 months, while levels for BMI 1 and BMI 3 did not significantly change. There was also no significant difference between the three groups.

### 3.4. Bone outcomes

#### 3.4.1. Areal bone mineral density (BMD)

All three groups showed a significant and progressive increase in BMD at the lumbar spine over 18 months (Table 3), with no significant between-group difference in BMD changes. Similarly, there was no significant between-group difference in BMD changes at the total hip and femoral neck. .

#### 3.4.2. Bone turnover markers

There were no significant differences in the changes in osteocalcin levels among the different BMI groups at 6 and 12 months (Table 3). However, there was an increase in osteocalcin level at 18 months in BMI 3 which was significant compared to BMI 1 and 2 ($p = 0.05$).

CTX levels declined at 6 months in all the groups which were significant for BMI 2 and 3, followed by increase in levels close to or above baseline at 18 months (Table 3). There were no significant differences in the changes in CTX levels between the groups at all timepoints. Changes in sclerostin levels were not significantly different compared to baseline for those in BMI 1 and 3 at all timepoints (Table 3). However, levels for those in BMI 2 decreased significantly from baseline at 18 months; with a significant between-group difference ($\mathbf{p = 0.03}$).

### 3.5. Symptoms

BMI 1 and BMI 2 had the significant decreases in TSS from baseline at all timepoints suggesting improvement, which was not observed in BMI 3 (Table 4), with significant between-group differences at 6 months ($\mathbf{p = 0.040}$). However, no significant difference between the groups was observed for 12 and 18 months.

### 3.6. Hormonal and safety profile

The greatest increase in E2 and T levels was seen at 6 months for all groups, with no significant difference noted between groups (Table 4). Greater increases in E2 by the end of the study were seen in BMI 2 and 3, and the greatest increase in T was seen in BMI 3 but there was no significant difference between the groups. There was also no significant between-group difference in the changes in PSA and hematocrit at any of the visits.

### 3.7. Adverse events

Details of adverse events have been published elsewhere [24]. Along with other events, there were 7 cardiac, 2 neurologic, 10 hematologic and 1 urologic event (increased prostate size, benign by biopsy). There were no significant differences in the occurrence of adverse events among the 3 BMI groups.

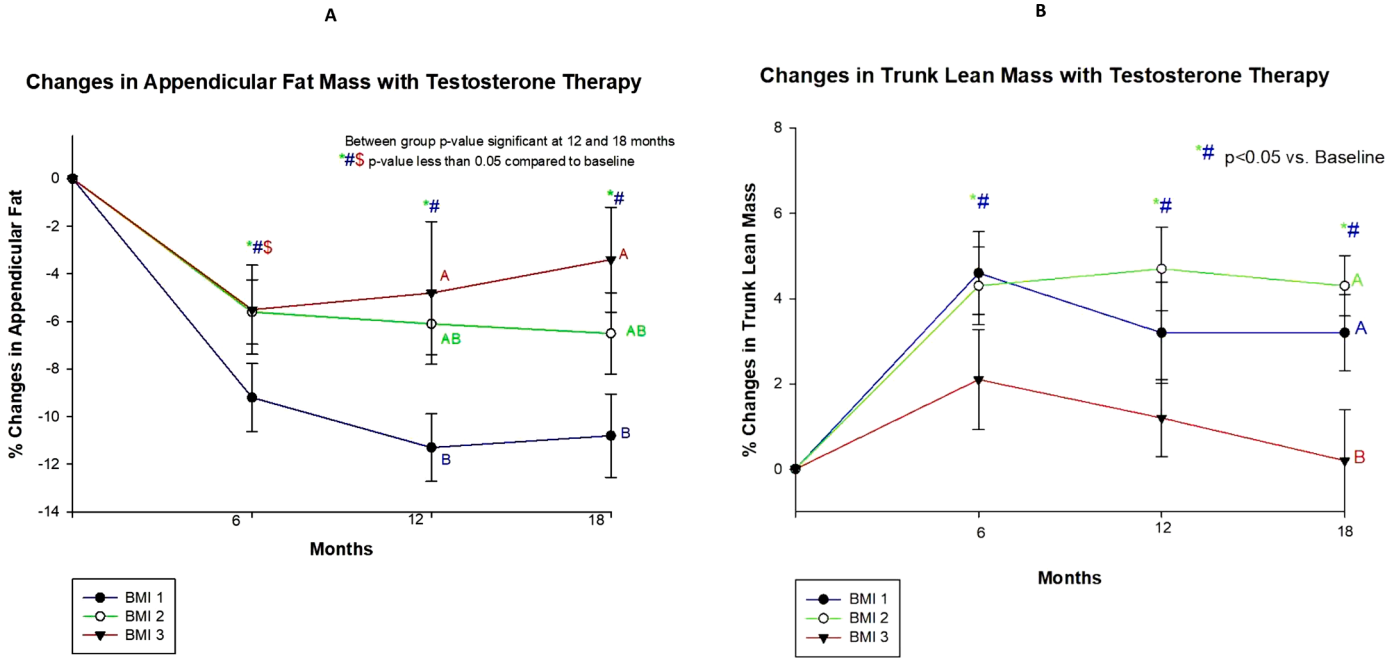


**Fig. 1.** A. Changes (%) in appendicular fat mass with testosterone (T) therapy according to baseline Body Mass Index (BMI). BMI 1: BMI < 30 (blue line), BMI 2:BMI 30–34.99 (green line) and BMI 3:BMI ≥35 (red line). The symbols *, # and $ are color coded by BMI category, and denote a p-value less than 0.05 for percentage change compared to baseline for that visit. Adjusted P-value is <0.05 for percentage change compared to baseline for BMI 1 and 2 at 6, 12 and 18 months, and for BMI 3 at only 6 months. Significant post-hoc difference between BMI categories is labelled by A, B and C; those with the same letters are not significantly different and those with different letters are significantly different. B. Changes (%) in truncal lean mass with testosterone (T) therapy according to baseline Body Mass Index (BMI). BMI 1: BMI < 30 (blue line), BMI 2: BMI 30–34.99 (green line) and BMI 3-:BMI ≥35 (red line). The symbols *, # and $ are color coded by BMI category, and denote a p < 0.05 for percentage change compared to baseline for that visit. Age-adjusted P is <0.05 for percentage change compared to baseline for BMI 1 and 2 at 6, 12 and 18 months; not significant for BMI 3 at any visit. Significant post-hoc difference between BMI categories is labelled by A and B; those with the same letters are not significantly different and those with different letters are significantly different.

**Table 3**
Changes (%) in bone mineral density and bone turnover markers with testosterone therapy.

| | BMI <30 (N = 39) | BMI 30–34.9 (N = 38) | BMI≥35 (N = 28) | p |
|---|---|---|---|---|
| A. Bone mineral density ($gm/cm^2$) | | | | |
| Lumbar Spine | | | | |
| 6 months | 2.3* ± 2.7 | 1.4* ± 2.7 | 2.0 ± 4.2 | 0.25 |
| 12 months | 2.6* ± 2.8 | 2.8* ± 2.5 | 3.7* ± 2.9 | 0.55 |
| 18 months | 3.2* ± 3.9 | 4.3* ± 2.9 | 4.1* ± 3.6 | 0.31 |
| Total Femur | | | | |
| 6 months | −0.1 ± 2.7 | 0.3 ± 3.6 | 1.2 ± 2.9 | 0.41 |
| 12 months | 0.3 ± 4.4 | 1.3* ±3.1 | −0.5 ± 2.1 | 0.30 |
| 18 months | 0.9 ± 3.5 | 1.6* ±2.5 | −0.3 ± 2.8 | 0.18 |
| Femoral Neck | | | | |
| 6 months | −1.0 ± 4.5 | −0.4 ± 4.3 | 2.0 ± 4.3 | 0.07 |
| 12 months | −0.8 ± 4.2 | 0.7 ± 3.9 | 0.8 ± 4.5 | 0.43 |
| 18 months | 0.5 ± 4.8 | 0.0 ± 3.8 | 0.2 ± 6.6 | 0.94 |
| B. Bone Turnover Markers | | | | |
| Osteocalcin | | | | |
| 6 months | −14.1 ± 51.2 | 3.0 ± 60.8 | 12.6 ± 58.9 | 0.25 |
| 12 months | −5.1 ± 86.2 | 23.0 ± 106.3 | 18.6 ± 90.8 | 0.55 |
| 18 months | −1.6 ± 62.3 | −4.7 ± 64.1 | 63.2 ± 140.1 | 0.05 |
| CTX (ng/ml) | | | | |
| 6 months | −23.4 ± 67.2 | −22.3* ±42.2 | −17.1* ±32.0 | 0.92 |
| 12 months | 2.4 ± 43.5 | −3.8 ± 45.2 | −0.3 ± 66.8 | 0.91 |
| 18 months | 16.0 ± 72.3 | 2.4 ± 55.9 | 5.9 ± 76.3 | 0.76 |
| Sclerostin | | | | |
| 6 months | −1.8 ± 33.8 | −2.6 ± 34.6 | 7.8 ± 35.4 | 0.63 |
| 12 months | −1.1 ± 36.8 | −10.7 ± 38.1 | 15.2 ± 44.6 | 0.26 |
| 18 months | 1.1A ±39.2 | −24.9*A ± 28.0 | 2.3A ±20.8 | 0.03 |

Values are mean±SD; change scores are reported as percent (%) change from baseline value. The individual comparison to baseline in each BMI category was done by paired t-test. Asterisk* means within-group changes from baseline were significant. The overall between-group p-values for bone mineral density comparisons were adjusted for age of the subjects at baseline and calculated by analysis of covariance (ANCOVA), while between-group p-values for bone turnover markers were obtained using analysis of variance (ANOVA). Bolded p-values are statistically significant. Significant post-hoc differences between BMI categories are labelled by A, B, C superscripts; those with the same letters are not significantly difference and those with different letters are significantly different.
BMI: body mass index, CTX: beta- C-terminal telopeptide.

**Table 4**
Changes (%) in hormone levels, safety profile and symptoms with testosterone therapy.

| | BMI <30 (N = 39) | BMI 30–34.9 (N = 38) | BMI≥35 (N = 28) | P |
|---|---|---|---|---|
| A. Hormones | | | | |
| Testosterone | | | | |
| 6 months | 455.1* ±364.0 | 340.5* ±292.9 | 367.3* ±319.0 | 0.33 |
| 12 months | 424.9* ±357.8 | 305.5* ±293.1 | 288.0* ±277.4 | 0.17 |
| 18 months | 293.3* ±311.7 | 296.2* ±250.2 | 325.9* ±236.8 | 0.95 |
| Estradiol | 24.5* ±24.6 | | | |
| 6 months | 12.5* ±18.4 | 22.7* ±19.2 | 29.0* ±25.2 | 0.82 |
| 12 months | 11.7* ±21.2 | 15.6* ±14.4 | 22.4* ±25.4 | 0.11 |
| 18 months | | 18.2* ±16.4 | 18.2* ±17.1 | 0.37 |
| B. Safety Profile | | | | |
| Hematocrit | | | | |
| 6 months | 4.6* ±3.6 | 4.1* ±3.3 | 5.2*±2.8 | 0.50 |
| 12 months | 3.1*±4.4 | 4.4 ± 3.7 | 4.1*±2.6 | 0.18 |
| 18 months | 3.7* ±4.7 | 4.7* ±3.6 | 4.3* ±2.8 | 0.49 |
| Prostate Specific Antigen | | | | |
| 6 months | 0.43* ±0.4 | 0.26* ±0.4 | 0.39* ±0.5 | 0.29 |
| 12 months | 0.38* ±0.5 | 0.40* ±0.5 | 0.43* ±0.4 | 0.95 |
| 18 months | 0.59* ±0.8 | 0.40* ±0.5 | 0.48* ±0.6 | 0.53 |
| C. Total Symptom Score | | | | |
| 6 months | −30.6* ±34.2 | −19.4* ±41.1 | −4.7 ± 50.2 | 0.04 |
| 12 months | −20.6* ±50.1 | −30.8* ±44.2 | 3.7 ± 75.0 | 0.10 |
| 18 months | −29.9*±53.7 | −31.0* ±49.0 | −8.6 ± 62.7 | 0.40 |

Values are mean±SD; change scores are reported as absolute change (Δ) from baseline value. The individual comparison to baseline in each BMI category was done by paired t-test. Asterisk* means those changes from baseline were significant. Between-group p-values are reported by analysis of variance (ANOVA).

## 4. Discussion

T replacement therapy has been shown to improve body composition and metabolic profile in several studies [9,10,18,28]. In one study, T treatment resulted in a reduction in visceral adiposity as assessed by waist circumference and waist/hip ratio [28]. T decreases visceral fat mass with consequent reduction in the waist circumference, and reduces lipoprotein lipase activity a key enzyme involved in the uptake of TGs into adipocytes [29]. Moreover, a meta-analysis showed that aside from reduction in body weight, BMI, waist conference, and fat mass, T therapy is also associated with an increase in lean mass [18].

Our study showed that T therapy results in improvement in the different parameters of body composition in men regardless of their baseline BMI. However, BMI 1 and 2 had the greater reductions in total and appendicular fat mass and increase in total and regional FFM and lean mass relative to men in BMI 3. Our results suggest that men in BMI 3, with the highest BMI, had the least benefit, both in terms of gaining lean muscle and losing fat compared to BMI 1 and 2. This finding supports our initial hypothesis that there may be variable response to T therapy depending on body adiposity. Because the aromatase enzyme primarily resides in the adipose tissues, very obese men have greatly enhanced aromatase activity relative to normal weight and less obese individuals resulting in varying degrees of conversion of T to E2. Hence, response to T may vary with severely obese men not responding as well compared to nonobese and less obese counterparts not only in the gain in lean or fat-free mass but may also be in the loss of total and regional fat mass.

Weight loss alone can improve the hormonal profile of obese participants [30]. Our group previously showed that a weight loss of ~10 % from diet and exercise is associated with increase in T levels in elderly obese men [31]. However, whether this improvement in hormonal profile also results in symptomatic improvement is addressed by another study where every participant was subjected to weight loss from a very low caloric diet for 10 weeks and then randomized to either T or placebo for 46 weeks [13]. This study found that despite significant increase in T levels from weight loss, symptoms only improved after T was added. Nevertheless, others advocate primarily diet and lifestyle intervention and refrain from prescribing T therapy to reverse hormonal and metabolic abnormalities and improve symptoms in obesity-associated hypogonadism [32]. Because of this lack of agreement, large randomized studies are needed to address the efficacy of T in obese men with low T levels. At this point, it may be sensible to encourage patients with higher BMI to lose weight first before starting them on T therapy.

In our study, HbA1c, and fasting glucose did not change significantly at any interval in time with T therapy in any BMI category. Prior studies showed that T therapy resulted in significant improvement in insulin sensitivity, and glycemic control [28,33]. The likely reason that no significant changes in HbA1c and fasting glucose were seen in our study

was the fact that the baseline values were not very elevated to begin with. Baseline HbA1C was 6.5, 6.6 and 7.0 in the three categories, respectively.

Regarding lipid profile, data in the literature on the effect of T therapy on lipid levels have been inconsistent [34]. Two meta-analyses suggested that T replacement therapy has no effect on levels of either LDL cholesterol or HDL cholesterol [14,35]. On the contrary, Whitsel et al. demonstrated that intramuscular T replacement therapy caused a reduction in the levels of both HDL and LDL cholesterol with no effect on triglyceride levels [36]. In our study, while total cholesterol decreased in men with BMI $\geq$ 35 at 6 months, there was no significant changes in LDL in all the groups while HDL decreased in all 3 groups (with BMI 3 having a significant decrease from baseline) at the end of the study. The reduction in HDL levels in our study appears concerning most especially among those in BMI 3. Nonetheless, the contribution of the reduction in HDL in the overall atherogenic environment in the different BMI groups when taken in context with changes in other lipid parameters remains undetermined.

As far as adipokines are concerned, obesity is associated with high leptin and low adiponectin levels [37]. Consistently, our study, showed that baseline leptin levels were the lowest in BMI 1, and highest in BMI 3 while baseline adiponectin levels were the highest in BMI 1 and lowest in BMI 3. A previous study showed that both leptin and adiponectin are reduced following biweekly T propionate treatment for 3 months [12]. One of the oldest studies showed normalization of leptin levels with T therapy, [38] yet, none of these studies compared improvement in metabolic markers and adipokines based on baseline BMI.

In our study, leptin levels decreased in all 3 groups. It was interesting to note that the biggest decline was seen in BMI 1. This group had the biggest decrease in fat mass, both total and regional and likely account for the magnitude of leptin decline. While loss of fat mass may explain for the decrease in leptin levels, this could not account for the changes in adiponectin. There was a significant decline in adiponectin in BMI 2, while no significant changes were seen in BMI 1 and 3. Our results suggest that the association between loss of fat mass and changes in levels of adiponectin, another factor produced by fat tissues, is not as strong as that of leptin. It is possible that leptin levels are more sensitive to T therapy and changes in the volume of fat mass than adiponectin.

All three groups showed a comparable and progressive increase in BMD at the lumbar spine at 18 months: with a small but significant increase at the total hip only among men in BMI 2. Regardless, there was no significant between-group differences in BMD changes in all sites at 18 months. It is well known that the magnitude improvement in BMD from T therapy at the hip is less compared to that of the spine [21,22], which likely explains the small or lack of improvement in BMD at the total hip and femoral neck in all the BMI categories in our study.

T therapy has been shown to decrease markers of bone turnover [39] (both resorption and formation) in general, although some investigators demonstrated increase in bone formation marker suggesting an anabolic effect of T [21,40]. In our study, although changes in osteocalcin levels were not significantly different from baseline, there was a trend for a reduction in BMI 1 at all timepoints while the changes were mixed for BMI 2. However, BMI 3 showed consistent increase in osteocalcin suggesting that perhaps T could be anabolic in a certain subset of men with low T. It has been suggested in other studies that T therapy may have differential effects on bone depending on the underlying pathophysiology, that is, mainly antiresorptive in those with high bone turnover and anabolic in those with low bone turnover [40]. Obesity is associated with low bone turnover [41]. In our study, baseline osteocalcin trended to go down with increasing baseline BMI with BMI 3 having the lowest osteocalcin level, and this likely explain the increase in osteocalcin levels in this group. As far as CTX changes are concerned, it seems that the antiresorptive effect of T therapy was only seen in the first 6 months of therapy in all the BMI categories and this effect disappeared with continued therapy. Although we have no explanation for our findings, to our knowledge there has been no study that examined the influence of BMI on the bone turnover response to T therapy.

Sclerostin levels significantly declined at 18 months in BMI 2. This decline in sclerostin could have a beneficial effect on bone remodeling as sclerostin inhibits osteoblast differentiation [42]. A prior study showed that reduction in sclerostin is influenced by estrogen rather than T [43]. In our study, the baseline E2 is lowest BMI 2 and could be the most sensitive group with the increase in E2 which may account for the greater reduction in sclerostin in this group.

Overall, despite small differences in bone turnover markers, our study shows that the BMD response to T is essentially the same for all the BMI groups. This is not surprising as baseline E2 levels, although significantly lower in BMI 1 and 2, did not reach the critical level for bone health in men i.e. <10 pg/ml [44]. It is possible that a difference in response could be appreciated if at least one of the groups had a baseline E2 level below this cut-off as those would be more sensitive to an increase in E2 level.

Per the Endocrine Society guidelines, to diagnose hypogonadism in men they must have both symptoms and signs of T deficiency along with low T levels [45]. T therapy has been shown to alleviate hypogonadal symptoms with improvement in sexual desire appearing after 3 weeks, erectile function and ejaculation may require up to 6 months and improved depressive mood within 3–6 weeks of T therapy [46].

In our study, men in BMI 1 and 2 had the greatest improvement in their symptoms at the end of 18 months. Men in BMI 3 did not have a significant change in their symptoms at the end of the study compared to baseline, despite increase in their total T levels. It is possible that their obesity may be contributing to most of their symptoms. Hence, they might benefit from weight loss as an adjunct to T therapy for improvement in symptoms.

Concerns about serious cardiovascular events had been raised previously by mostly retrospective and small randomized studies [47,48]. However, these concerns dissipated to some extent after the results from the Testosterone Replacement Therapy for Assessment of Long-term Vascular Events and Efficacy Response in Hypogonadal Men (TRAVERSE) trial did not show an increase in incidence of cardiovascular events in hypogonadal older men at high risk of cardiovascular disease [49]. In our study, there were only 7 cardiac events, thus, it would be hard to determine if this side effect favors one BMI over another. In our study, there were 10 participants who had an increase in hematocrit defined as a hematocrit of >52 %. Again, no particular BMI was affected more than the others. In the Traverse trial, there were only 6 participants who were dropped out from the study secondary to high hematocrit defined as hematocrit of 54 % [50]. Although we may have a relatively higher number of participants considered to have high hematocrit with T therapy, we used a lower cut-off than theirs. Finally, with regards to urologic side effects, only one of our participants had notable prostatic side effects.

Our study has several limitations. As this a secondary analysis of outcomes from a prior clinical trial, inclusion/exclusion criteria were based on the primary objective of the original study, i.e., pharmacogenetics of response to T therapy. Also, since the screening was done prior to harmonization of hormonal assays with LC/MS as the gold standard for T assays and using 264 ng/dl as the cut-off for normal level, some of the participants enrolled here are considered normal by the new Endocrine Society guidelines [45]. It is possible that results could be different if every participant is hypogonadal by the current standards. Additionally, we did not assess diet, physical activity, and sleep, and limited data on diabetes medications which are known to have significant effect on weight and metabolic profile. Furthermore, our sample size is small with a 28 % dropout. There was no a priori power calculation done for the sample size; a post-hoc power estimate calculated for a test that has already been performed cannot be interpreted as the probability of a desired future event. So, nonsignificant results cannot be asserted with adequate probability. Finally, there were not enough participants to validate the symptom questionnaire, although the symptoms are based on Endocrine Society recommendations for evaluation of symptoms of

hypogonadism as well as the androgen deficiency in aging males (ADAM) questionnaire, which has been validated for screening and evaluating T therapeutic response [51].

## 5. Conclusion

In conclusion, our study shows that men, regardless of BMI, derived benefit from T therapy, though men with a BMI <35 have greater improvements in body composition while BMI of >35 have the least benefit. All three groups showed similar benefits in terms of BMD, but bone turnover markers showed mixed results. Men with a BMI <35 experienced a significant improvement in their symptoms by the end of the study, while those with a BMI of >35 did not, despite having a significant increase in their T levels. From the public health standpoint, it is always recommended that obese individuals should lose weight. Given the positive effect of weight loss on cardiometabolic and hormonal profile, a sensible approach to severely obese men with low T is to promote weight loss before even considering T therapy. Nevertheless, given the above identified limitations, prospective studies with larger sample sizes, with longer periods of intervention, are needed to determine if baseline BMI will influence response to T therapy.

## Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

## Ethical approval

The protocol was approved by the Institutional Review Boards of the University of New Mexico and Baylor College of Medicine, and the study conducted in accordance with guidelines of the Declaration of Helsinki for the ethical treatment of human subjects.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ahr.2025.100265.

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