ORIGINAL ARTICLE

Open Access

Effects of Testosterone Therapy on Erythrocytosis and Prostate Adverse Events in Obese Males with Functional Hypogonadism and Type 2 Diabetes in a 2-Year Clinical Trial

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Abstract

Aims: Testosterone therapy (TTh) has been postulated to increase the risk of prostate adverse events (PAEs) and erythrocytosis, risk further exacerbated in high-risk obese patients with type 2 diabetes (T2D) and functional hypogonadism (FH). We investigated safety aspects of TTh in obese males with FH and T2D by observing the incidence of PAEs and erythrocytosis and determining when statistically significant difference from the baseline manifests in hematocrit (Hct) and prostate-specific antigen (PSA) levels.

Materials and Methods: Fifty-five obese Caucasian men with FH and T2D participated in a two-part prospective observational clinical study (first year: double-blind randomized placebo-controlled trial employing testosterone undecanoate; second year: open-label follow-up with all participants receiving TTh). Outcomes were assessments of Hct and PSA levels at the baseline, and 3, 6, and 12 months into each of 2 years of the study.

Results: No adverse cardiovascular events or PAEs were observed. Hct first increased at statistically significant level from the baseline after 3 months of TTh in group T and after 6 months of TTh in group P. Individual Hct values for all participants remained <0.52 throughout 2-year course of the study. PSA increased from the baseline in both groups within 3–6 months of trial start regardless of intervention applied (placebo or TTh). Fifty-two patients never exceeded PSA level of $4.0 \mu\text{g/L}$ nor experienced year-on-year PSA increase $>1.4 \mu\text{g/L}$. No subject ever reached supraphysiological concentration of total testosterone.

Conclusions: Our results show that TTh may be safe in obese males with FH and T2D. ClinicalTrials.gov ID: NCT0379232.

Keywords: testosterone; prostate-specific antigen; erythrocytosis; hematocrit; functional hypogonadism; prostate adverse events

Introduction

Male hypogonadism is a clinical syndrome resulting from failure of testes to produce physiological concentrations of testosterone and/or normal amounts of spermatozoa due to pathology of hypothalamic-pituitary-testicular axis.¹ Functional hypogonadism (FH) or late-onset hypogonadism is associated with

aging and its related comorbidities, including obesity, metabolic syndrome, and type 2 diabetes (T2D).^{2–4} Approximately 50% of males with T2D, aged >40 years, exhibit decreased total testosterone (TT) levels.^{3,5} Obesity is considered to be the most frequent cause of FH.^{6–9} Obesity and T2D are high risk factors for cardiovascular disease (CVD),^{2,10–12} and are also

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risk factors for benign prostatic hyperplasia (BPH); men with T2D are twice as likely to have an enlarged prostate as men without T2D.¹³ Pathophysiological mechanisms explaining the correlation between obesity, T2D, risk of BPH, and prostate carcinoma (PCa) are hyperinsulinemic state, increased estrogen-to-androgen ratio, increased sympathetic nervous activity, promotion of inflammation processes, which in turn contribute to ischemia, oxidative stress, and an intra-prostatic environment favorable to BPH and PCa.¹⁴

FH has recently come under greater scrutiny with the widespread use of testosterone therapy (TTh), and concerns regarding the efficacy and safety of TTh have been raised.¹⁵ TTh may exert several benefits with regard to metabolic profile, body composition, psychological, and sexual parameters. Multiple studies have shown that TTh applied to obese men with FH and T2D improves glycemic control; reduces insulin resistance (IR), inflammation, and symptoms of hypogonadism; increases bone mineral density; and improves vascular function and morphology along with improvements in cognitive function.^{10,16–21}

TTh has been postulated to potentially increase the risk of prostate adverse events (PAEs) and erythrocytosis.¹¹ This increased risk for both types of adverse events stems from physiological effects of testosterone. The normal function of the prostate gland is dependent on testosterone, and it has been well documented that administration of testosterone to men with hypogonadism results in a small increase in serum prostate-specific antigen (PSA) level.²² Meta-analyses of testosterone trials did not show that testosterone increases the risk of PCa.^{9,11,23} “Saturation model” hypothesis postulates that once testosterone concentrations have stabilized as a result of TTh, further effects of testosterone on prostate will diminish.²⁴

Evidence supports obesity as a risk factor for both BPH and PCa.¹⁴ Increased waist circumference is positively associated with prostate volume, PSA level, and worsened lower urinary tract symptoms (LUTS).²⁵ Adiposity is associated with increased erythrocyte aggregation; abdominal fat increases blood viscosity due to a rise in hematocrit (Hct), and increased body mass index (BMI) is associated with increased plasma viscosity.²⁶

Several challenges exist whenever TTh is being considered. Contraindications must be excluded according to the clinical guidelines.^{4,27,28} TTh is not recommended to patients with a history of, or at a high level of risk of PCa, severe BPH, or with high CVD risk.^{4,27,28} It is recommended that TTh be applied with caution

in men diagnosed with BPH and mild or moderate LUTS, whereas men with severe LUTS should undergo urological evaluation before commencing treatment. Maintaining physiological serum levels of testosterone and monitoring PSA and Hct in patients on TTh is mandatory so that appropriate measures (such as dosage reduction, the withholding of testosterone, and therapeutic phlebotomy) can be performed if erythrocytosis develops. A PSA value $>4.0 \mu\text{g/L}$ or year-on-year increase of $1.4 \mu\text{g/L}$ or more are the standard indications for prostate biopsy.^{4,27,28}

It is the combination of effects of testosterone and the way both obesity and T2D affect prostate and erythropoiesis that makes administering TTh to obese patients with FH and T2D more challenging than to nondiabetic nonobese men. This article attempts to clarify whether TTh can be safely applied to such a high-risk population and to analyze the effect of TTh on Hct and PSA levels.

Objectives

The goal of this report is to evaluate safety aspects of TTh in high-risk population of obese males with T2D and FH by (1) observing the incidence of PAEs and erythrocytosis and by (2) determining the time point where statistically significant difference (increase) from the baseline manifests in Hct and PSA. Three individual cases of significantly elevated PSA in study participants are also detailed.

Materials and Methods

Study design

SETH2 study (Study on Effects of Testosterone Replacement Therapy in Hypogonadal Type 2 Diabetic Patients) was a two-part single-center prospective observational clinical study (first year double-blind randomized placebo-controlled trial; second year open-label follow-up), conducted from January 2014 to March 2018 at General Hospital Celje (Slovenia). Study was approved by the National Medical Ethic Committee (54/04/12) and was conducted in accordance with the Declaration of Helsinki and with all applicable local laws and regulations. Written informed consent was obtained from all study subjects before their participation in the study. The study has been registered at ClinicalTrials.gov (identifier: NCT03792321).

Study population

Fifty-five obese Caucasian male patients with confirmed symptomatic FH, aged 40–70 years, with T2D,

participated in SETH2 trial. FH was diagnosed as a biochemical deficiency of circulating testosterone levels (TT <11 nmol/L and free testosterone <220 pmol/L) on at least two separate morning measurements after an overnight fast in addition to exhibiting at least two symptoms of sexual dysfunction (less frequent morning erections, erectile dysfunction, and decreased libido).^{4,5,27}

Inclusion criteria were male, confirmed FH, age >35 years, BMI ≥ 30 kg/m², and T2D treated exclusively with noninsulin antidiabetic medications (metformin and sulfonylureas). Participants did not use concomitant medications that can reduce weight, BMI, and waist circumference.

Exclusion criteria were previously treated FH, history of current prostate or breast cancer, severe BPH or PSA >4.0 μ g/L, severe heart failure, acute coronary event or procedure during the 6 months before the study, chronic obstructive lung disease, severe obstructive sleep apnea, and active infection.

Study protocol

Participants were randomized into groups T and P before the first part of the study, and received either intramuscular testosterone undecanoate (TU; Nebido 1000 mg; Bayer AG) or matching placebo for 1 year. Participants and trial investigators were blinded to treatment allocation. Use of placebo was limited to 1 year due to ethical concerns over withholding TTh from hypogonadal patients, so group P subjects were switched to TU for the second part. Group T subjects continued receiving TU for a total of 2 years. First injection of TU/placebo was administered at the first visit (baseline), second injection 6 weeks later (second visit), and each subsequent injection 10 weeks after the previous injection. All participants received TU starting with week 56.

Methods

All patients underwent clinical, biochemical and hormonal assessment at the beginning, after 12 months and after 24 months of the study. Fasting blood samples were taken between 07:00 a.m. and 11:00 a.m. to measure serum TT, estradiol, sex hormone binding globulin (SHBG), albumin, luteinizing hormone, follicle-stimulating hormone, fasting plasma glucose, glycated hemoglobin (HbA_{1c}), lipids (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides), PSA, routine blood tests (complete blood count, electrolytes, urea, creatinine, and liver tests). TT was

assessed using IMMULITE 2000 chemiluminescent enzyme immunoassay (Siemens Healthcare GmbH). Intra-assay coefficients of variation (CVs) in the relevant result range are 11.7% (at 2.99 nmol/L), 10.0% (5.27 nmol/L), 8.3% (9.70 nmol/L), 7.2% (14.35 nmol/L), and 5.1% (34.36 nmol/L). Interassay CVs at those same respective means are 13.0%, 10.3%, 9.1%, 8.2%, and 7.2%. Safety parameters (complete blood count, PSA, and markers of hepatic and renal functions) were performed at 0, 3, 6, 12, 15, 18, and 24 months.

We calculated free testosterone (cFT) and bioavailable testosterone (BT) from TT, SHBG, and albumin values using online calculator, which employs formulae of Vermuelen.²⁹

Outcomes

Assessments of Hct and PSA levels were performed in accordance with the clinical guidelines.^{4,27,28} We performed one additional check each year of the study during the first 6 months to get more detailed insight into how Hct and PSA levels respond to introduction of TTh, resulting in three safety tests per each year of the study (at baseline, approximately at 3, 6, and 12 months into each year).

Statistical methods

Results were compared within groups to determine the time point when Hct and PSA changed from their respective baseline values at statistically significant level ($\alpha = 0.05$). Shapiro–Wilk test was used to assess normality of data. Repeated measures analysis of variance (RM-ANOVA) was used to examine normally distributed results and Friedman's nonparametric test was used to examine non-normally distributed results. Adjusted *p*-values are reported for *post hoc* tests with Bonferroni correction. SPSS Statistics 22.0 (IBM Corporation, New York) was used for statistical analysis.

Results

Study subjects

Baseline demographic, anthropometrical, and laboratory parameters of the study population are provided in Table 1.

Changes in outcome measures

Results for groups P and T are presented separately due to slight differences in TTh administration protocol at the point of introduction of TTh in each study group (lack of initial 6-week loading period for group P upon switch from placebo to TTh).

Table 1. Baseline Study Population Characteristics: Key Anthropometrical, Biochemical, and Clinical Parameters

Parameter	Group T (n=28)	Group P (n=27)	Total (n=55)
Age (years)	58.18 ± 7.93	62.19 ± 5.90	60.15 ± 7.23
BMI (kg/m ²)	34.03 ± 4.37	32.63 ± 3.67	33.34 ± 4.07
HbA _{1c} (%)	8.12 ± 1.04	7.89 ± 0.77	8.00 ± 0.91
HOMA-IR index	11.45 ± 7.34	10.70 ± 6.52	11.08 ± 6.90
Total serum testosterone (nmol/L)	7.24 ± 1.97	7.96 ± 1.34	7.59 ± 1.71
Bioavailable testosterone (nmol/L)	3.74 ± 0.97	4.61 ± 0.95	4.17 ± 1.05
Calculated free testosterone (pmol/L)	155.54 ± 41.11	192.07 ± 44.13	173.47 ± 46.07
Hct level	0.428 ± 0.020	0.436 ± 0.024	0.432 ± 0.022
PSA level (μg/L)	0.645 (0.503 to 1.235)	0.670 (0.390 to 1.190)	0.650 (0.440 to 1.220)
Arterial hypertension, n (%)	25 (89.3)	26 (96.3)	51 (92.7)
Dyslipidemia, n (%)	28 (100.0)	27 (100.0)	55 (100.0)
Current smoker, n (%)	8 (28.6)	13 (48.1)	21 (38.2)

Values are reported as mean ± standard deviation when normally distributed, as median (interquartile range) in the case of PSA and as count (percentage of n) for clinical parameters.

BMI, body mass index; HbA_{1c}, glycated hemoglobin; Hct, hematocrit; HOMA-IR, homeostatic model of insulin resistance index; PSA, prostate-specific antigen.

Hct and testosterone levels are reported as mean ± standard deviation. PSA is reported as median (interquartile range).

Changes in Hct are shown in Figure 1 and Table 2. With assumption of sphericity not met for either group P ($p=0.001$) or T ($p=0.027$), Greenhouse–Geisser correction was applied to RM-ANOVA, showing that mean

Hct level differed significantly between time points in both group P [$F(4.106, 106.757)=10.708, p<0.001$] and T [$F(4.303, 116.188)=12.903, p<0.001$]. *Post hoc* pairwise test with Bonferroni correction revealed that mean Hct level changed from 0.444 ± 0.024 at the introduction of TTh in group P to 0.465 ± 0.027 after 12 months of TTh, and from 0.427 ± 0.020 to 0.449 ± 0.028 after 6 months of TTh in group T. Hct increased by 0.021 ± 0.025 in group P and by 0.025 ± 0.026 in group T after first year of TTh in each respective group, whereas no additional increase was observed after extended TTh in group T during second year of the trial ($p=1.000$ for all pairwise observations). Individual Hct values never exceeded the upper safety limit of 0.52 in either study group at any point of the study.

Changes in PSA level are outlined in Figure 2 and Table 3. Friedman's test confirmed that PSA levels differed at statistically significant level between the time points in both groups P [$\chi^2(6)=70.529, p<0.001$] and T [$\chi^2(6)=87.274, p<0.001$]. Dunn–Bonferroni pairwise *post hoc* test showed that PSA increased at statistically significant level from the baseline after 3 months of TTh in group P and after 6 months of TTh in group T. This was reaffirmed by performing RM-ANOVA with Greenhouse–Geisser correction on log-transformed PSA data [$F(2.559, 66.524)=20.086, p<0.001$ for group P and $F(3.315, 89.496)=20.791, p<0.001$ for group T], with pairwise Bonferroni-corrected *post hoc* test detecting mean change in log(PSA) after 3 months of TTh in both groups. Median PSA increased from the baseline of $0.645 (0.503–1.235) \mu\text{g/L}$ to $0.825 (0.610–1.368) \mu\text{g/L}$ after 12 months of TTh to $1.175 (0.803–1.580) \mu\text{g/L}$ after 24 months of TTh in group T. Similarly, PSA in group P went from the

FIG. 1. Hct levels in obese males with FH and T2D on TTh over the 2-year course of the study. Group P received placebo during first year of the study and was switched to testosterone undecanoate afterward. Group T received testosterone undecanoate throughout entire course of the study. FH, functional hypogonadism; Hct, hematocrit; T2D, type 2 diabetes; TTh, testosterone therapy.

Table 2. Changes in Hematocrit in Obese Males with Functional Hypogonadism and Type 2 Diabetes on Testosterone Therapy over the 2-Year Course of the Study

Parameter	Time point	Group T (n=28)	Change from baseline	Group P (n=27)	Change from baseline	Change from introduction of TTh
Hct	Baseline	0.427 ± 0.020		0.436 ± 0.024		
	3 Months	0.438 ± 0.026	0.010 ± 0.018 (p = 0.097)	0.434 ± 0.026	−0.001 ± 0.013 (p = 1.000)	
	6 Months	0.449 ± 0.028	0.021 ± 0.020 (p < 0.001)	0.442 ± 0.024	0.007 ± 0.018 (p = 1.000)	
	12 Months	0.453 ± 0.031	0.025 ± 0.026 (p < 0.001)	0.444 ± 0.024	0.009 ± 0.027 (p = 1.000)	
	15 Months	0.456 ± 0.027	0.028 ± 0.027 (p < 0.001)	0.443 ± 0.024	0.007 ± 0.021 (p = 1.000)	−0.002 ± 0.021 (p = 1.000)
	18 Months	0.458 ± 0.033	0.030 ± 0.028 (p < 0.001)	0.450 ± 0.029	0.015 ± 0.023 (p = 0.051)	0.006 ± 0.027 (p = 1.000)
	24 Months	0.460 ± 0.027	0.033 ± 0.027 (p < 0.001)	0.465 ± 0.027	0.030 ± 0.026 (p < 0.001)	0.021 ± 0.025 (p = 0.005)

Group P participants were receiving placebo during first year of the study and was switched to testosterone undecanoate afterward. Group T participants were receiving testosterone undecanoate throughout the entire course of the study. Values are reported as mean ± standard deviation. Statistically significant changes from the point where TTh was introduced in each respective group are marked in bold.

TTh, testosterone therapy.

baseline of 0.670 (0.390–1.190) $\mu\text{g/L}$ to 0.890 (0.500–1.220) $\mu\text{g/L}$ after receiving placebo for 12 months, and then to 0.950 (0.700–1.560) $\mu\text{g/L}$ after 12 months of TTh.

Of 55 patients, 52 never exhibited PSA >4 $\mu\text{g/L}$ or a year-on-year increase >1.4 $\mu\text{g/L}$; PSA levels for the three exceptions are outlined in Table 4. Study participant number 30 (group T) has been enrolled with pre-existing (diagnosed) BPH, confirmed by prostate biopsy, and was under regular supervision of a urologist; BPH was not severe enough to warrant exclusion

from the study. His baseline serum PSA was 4.0 $\mu\text{g/L}$, and did not change during the first year of TTh. PSA increased to 5.00 $\mu\text{g/L}$ after the second year of TTh, resulting in TTh termination.

Participants numbers 15 and 36 (both group P) were diagnosed with BPH during the second year of the trial, after a marked increase in their PSA after commencing TTh. Patient number 15 PSA increased from 1.17 $\mu\text{g/L}$ at 12 months (the point of introduction of TTh) to 2.67 $\mu\text{g/L}$ 3 months later, and again from 2.70 $\mu\text{g/L}$ at the 18-month mark to 5.20 $\mu\text{g/L}$ after 24 months of the study. Baseline PSA for patient number 36 was 3.03 $\mu\text{g/L}$ and remained steady until the 18-month mark when it first rose >4.00 $\mu\text{g/L}$ (to 4.04 $\mu\text{g/L}$), and then to 5.40 $\mu\text{g/L}$ at 24 months. Both patients stopped TTh and underwent prostate biopsy; PCa was excluded and BPH was diagnosed in both.

Changes in testosterone levels are detailed in Table 5. Group T mean TT increased from 7.24 ± 1.97 to 17.04 ± 3.07 nmol/L after 12 months of TTh and to 23.50 ± 4.91 nmol/L after 24 months. All group T participants but one reached serum TT concentration above the 11 nmol/L reference value after 12 months of TTh, whereas the sole exception went from baseline TT of 7.5 to 10.6 nmol/L. Statistically significant increase of mean TT in group P after 12 months (from 7.96 ± 1.34 to 9.83 ± 2.21 nmol/L) is of little clinical significance. Mean TT increased considerably more (to 17.92 ± 2.21 nmol/L) after 12 months of TTh (24 months into the study). All group P participants reached TT >11 nmol/L at this point. The calculated values of BT and cFT correlated highly with values of the TT, with clinically relevant increase manifesting in both groups after the first year of TTh (12 months into the study for group T and at 24 months for group P).

FIG. 2. PSA levels in obese males with FH and T2D on TTh over the 2-year course of the study, displayed on a logarithmic scale due to log-normal distribution of values. Group P received placebo during first year of the study and was switched to testosterone undecanoate afterward. Group T received testosterone undecanoate throughout entire course of the study. PSA, prostate-specific antigen.

Table 3. Changes in Prostate-Specific Antigen in Obese Males with Functional Hypogonadism and Type 2 Diabetes on Testosterone Therapy Over the 2-Year Course of the Study

Parameter	Time point	Group T (n = 28)	Change from baseline	Group P (n = 27)	Change from baseline	Change from introduction of TTh
PSA ($\mu\text{g/L}$)	Baseline	0.645 (0.503 to 1.235)		0.670 (0.390 to 1.190)		
	3 Months	0.700 (0.525 to 1.280)	0.065 (0.020 to 0.120; $p = 1.000$)	0.650 (0.440 to 1.190)	0.020 (−0.010 to 0.070; $p = 1.000$)	
	6 Months	0.735 (0.520 to 1.180)	0.085 (0.025 to 0.203; $p = 0.030$)	0.750 (0.460 to 1.280)	0.070 (0.010 to 0.130; $p = 1.000$)	
	12 Months	0.825 (0.610 to 1.368)	0.180 (0.093 to 0.325; $p < 0.001$)	0.890 (0.500 to 1.220)	0.090 (−0.030 to 0.220; $p = 0.087$)	
	15 Months	0.970 (0.713 to 1.595)	0.245 (0.105 to 0.515; $p < 0.001$)	0.800 (0.520 to 1.250)	0.120 (0.010 to 0.250; $p = 0.031$)	0.030 (−0.070 to 0.100; $p = 1.000$)
	18 Months	1.050 (0.720 to 1.488)	0.255 (0.158 to 0.643; $p < 0.001$)	0.860 (0.660 to 1.490)	0.230 (0.090 to 0.420; $p < 0.001$)	0.100 (0.000 to 0.290; $p = 0.071$)
	24 Months	1.175 (0.803 to 1.580)	0.370 (0.205 to 0.920; $p < 0.001$)	0.950 (0.700 to 1.560)	0.290 (0.160 to 0.520; $p < 0.001$)	0.180 (0.010 to 0.350; $p = 0.004$)

Group P participants were receiving placebo during first year of the study and was switched to testosterone undecanoate afterward. Group T participants were receiving testosterone undecanoate throughout the entire course of the study. Values are reported as median (interquartile range). Statistically significant changes from the point where TTh was introduced in each respective group are marked in bold.

Table 4. Prostate-Specific Antigen Levels for Three Individuals Who Required Urological Examination at Some Point During the Study due to Elevated Prostate-Specific Antigen

Parameter	Time point	Patient no. 30 (group T)	Patient no. 15 (group P)	Patient no. 36 (group P)
PSA ($\mu\text{g/L}$)	Baseline	4.00^a	1.22	3.03
	3 Months	4.00	1.16	3.00
	6 Months	4.00	1.30	3.33
	12 Months	4.61	1.17	2.99
	15 Months	5.00	2.67^b	3.35
	18 Months	5.00	2.70	4.04^c
	24 Months	5.00	5.20	5.40

Points where each individual was referred to a urologist are marked in bold.

^aPatient no. 30 was enrolled into study with known (previously diagnosed) BPH and was under regular supervision of a urologist throughout entire course of the study. Patient was receiving testosterone undecanoate during both years of the study.

^bPatient no. 15 was referred to a urologist after an increase in PSA (by 1.50 $\mu\text{g/L}$ between two successive tests) 3 months after having been switched from placebo to testosterone undecanoate. Patient was diagnosed with BPH; PCa was excluded.

^cPatient no. 36 was referred to a urologist after an increase in PSA >4.00 $\mu\text{g/L}$ 6 months after having been switched from placebo to testosterone undecanoate. Patient was diagnosed with BPH; PCa was excluded.

BPH, benign prostatic hyperplasia; PCa, prostate carcinoma.

Harms

No adverse events (PCa, erythrocytosis, and CVD events) or other side effects of TTh have been observed.

Discussion

This study was conducted to assess the safety of TTh in obese men with T2D and FH. Published evidence of TTh safety in this population, which is at higher risk for CVD and PAEs, is scarce.

We showed that Hct increased gradually from the baseline in both groups within 3–6 months after the start of TTh. Our findings are in accordance with several studies, which showed that the effects of TTh on Hct become apparent after 3 months and the plateau is reached within 9–12 months.^{30,31} No trial participant ever exceeded the upper Hct limit of 0.52 at any point over the 2-year course of this study.

Increased red blood cell mass (erythrocytosis) is the most common adverse event associated with TTh in clinical practice and in testosterone trials.^{4,32} TTh-induced erythrocytosis is associated with stimulation of erythropoietin and reduced ferritin and hepcidin concentrations.^{33,34} Large epidemiological studies show that increased Hct levels are associated with increased risk of adverse CVD, because of increase in blood viscosity.^{35,36} Guidelines on TTh recommend measuring Hct at baseline, at 3–6 months and annually

Table 5. Changes in Serum Testosterone Levels in Obese Males with Functional Hypogonadism and Type 2 Diabetes on Testosterone Therapy over the 2-Year Course of the Study for Both Study Groups

Parameter	Time point	Group T (n=28)	Change from baseline	Group P (n=27)	Change from baseline	Change from introduction of TTh
Total testosterone (nmol/L)	Baseline	7.24 ± 1.97		7.96 ± 1.34		
	12 Months	17.04 ± 3.07	9.80 ± 3.59 (<i>p</i> < 0.001)	9.83 ± 1.51	1.87 ± 1.54 (<i>p</i> < 0.001)	
	24 Months	23.50 ± 4.91	16.26 ± 4.89 (<i>p</i> < 0.001)	17.92 ± 2.21	9.96 ± 2.92 (<i>p</i> < 0.001)	8.09 ± 2.08 (<i>p</i> < 0.001)
Calculated bioavailable testosterone (nmol/L)	Baseline	3.74 ± 0.97		4.61 ± 0.95		
	12 Months	9.50 ± 1.89	5.76 ± 2.16 (<i>p</i> < 0.001)	5.73 ± 0.89	1.12 ± 0.93 (<i>p</i> < 0.001)	
	24 Months	13.93 ± 3.39	10.19 ± 3.42 (<i>p</i> < 0.001)	10.72 ± 1.84	6.11 ± 1.93 (<i>p</i> < 0.001)	4.99 ± 1.91 (<i>p</i> < 0.001)
Calculated free testosterone (pmol/L)	Baseline	155.54 ± 41.11		192.07 ± 44.13		
	12 Months	403.84 ± 90.34	248.29 ± 99.45 (<i>p</i> < 0.001)	242.00 ± 42.23	49.93 ± 40.67 (<i>p</i> < 0.001)	
	24 Months	594.04 ± 147.09	438.50 ± 146.87 (<i>p</i> < 0.001)	454.56 ± 87.78	262.49 ± 90.11 (<i>p</i> < 0.001)	212.56 ± 84.06 (<i>p</i> < 0.001)

Group P participants were receiving placebo during first year of the study and was switched to testosterone undecanoate afterward. Group T participants were receiving testosterone undecanoate throughout the entire course of the study. Values are reported as mean ± standard deviation.

after initiating TTh.^{4,27} It is recommended that individuals with baseline (before initiation of TTh) Hct >0.50 undergo a workup before TTh because they have an increased chance of developing Hct level >0.54.

Prostate safety remains one of the most important controversial issues of TTh. We observed a sporadic increase of PSA in both study groups within 3–6 months after the start of the study regardless of intervention (testosterone or placebo), although the rate of increase was higher in group T. The small increase PSA in P group without prostatic symptoms was statistically significant but of questionable clinical relevance. We also observed small but statistically significant increase of testosterone levels in group P, which was attributed to loss of weight and improved glucose control, which we also in this group.³⁷ In addition to increased testosterone concentrations, decreased BMI, IR, and HbA_{1c},^{38,39} some other factors can also influence PSA changes: older age, recent ejaculation, certain medications (statin and betamethasone).^{40–42} We observed no cases of PCa.

PSA increase after TTh is a response of the physiological stimulation of the prostate by testosterone and its metabolites.⁴³ Rhoden and Morgentaler⁴⁴ showed that even in men with a predisposition to PCa (high-grade intraepithelial neoplasia), 1 year of TTh did not increase PCa incidence. TTh causes a mild increase in serum PSA in most patients without resulting in prostatic changes.⁴⁵ According to “saturation hypothesis,” although testosterone acts as a critical factor to prostatic tissue growth, there is a saturation point for androgen receptors (AR) at which the human prostate AR are “saturated” by the circulating androgens and, therefore, rather insensitive to further testosterone in-

crease, such as that derived from TTh in cases of mild hypogonadism, so any further increase in testosterone will have no detrimental effects.²⁴ Studies on the administration of supraphysiological doses of testosterone in healthy volunteers have not demonstrated an increase in PSA or prostate volume for up to 9 months, supporting the central hypothesis of the prostate saturation model that testosterone stimulates prostate tissue, but only up to the point of AR saturation.⁴⁶

In our study TT, cFT, and calculated BT concentrations have increased significantly after initiating TTh in both groups. Crucially, not a single subject in either P or T group ever reached supraphysiological concentrations of testosterone.

Strengths and limitations

Strengths of this study include its prospective randomized placebo-controlled trial design. TTh safety data from placebo-controlled RCTs, especially pertaining to high-risk populations such as obese males with T2D, are invaluable in assessing potential risks of TTh. The fact that no adverse CVD or PAEs have been observed over the 2-year course of our study is potentially attributable to limited study population size and does not constitute conclusive evidence that TTh exerts no negative effects on CVD and prostate health. Our findings have clinical implication for obese male patients with T2D considering TTh and for those receiving treatment.

Conclusions

The results of safety investigations performed throughout the course of our study suggest that 2 years of TTh can be safe even in high-risk population of obese men

with FH and T2D when appropriate safety practices outlined by the clinical guidelines are diligently followed; men on long-term TTh should be monitored with PSA, Hct, and digital rectal examination.^{4,27,28,47}

TTh has been associated with modest increases in serum PSA, within safe clinical parameters, but without substantial evidence to support an increased risk of PCa. TTh has been associated with increase in Hct; however, data on the significance of this trend related to patient outcomes is lacking.

Future research should require a dedicated focus on the evaluation of large multicentric cohorts of obese males with T2D to better elucidate risks of TTh related to PCa and erythrocytosis.

Authors' Contributions

K.G.A. and M.P. designed the study. K.G.A. supervised the study, collected study data, and prepared initial article draft. B.A. performed data analysis and prepared figures and tables. K.G.A. and B.A. wrote the article with input from M.P.

Author Disclosure Statement

Authors declare that there are no conflicts of interest to report.

Funding Information

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. Bayer Pharma AG (Berlin, Germany) provided testosterone and placebo for this study, but had no role in design of the study protocol, data collection, data analysis, or writing of this article.

References

- Matsumoto A, Bremner WJ. Testicular disorders. In: Melmed S, Polonsky KS, Larsen PR, and Kronenberg HM (eds.). *Williams Textbook of Endocrinology*, 13th ed. New York, NY: Elsevier, 2016, pp. 695–784.
- Corona G, Rastrelli G, Vignozzi L, Mannucci E, Maggi M. Testosterone, cardiovascular disease and the metabolic syndrome. *Best Pract Res Clin Endocrinol Metab*. 2011;25(2):337–353.
- Grossmann M. Low testosterone in men with type 2 diabetes: Significance and treatment. *J Clin Endocrinol Metab*. 2011;96(8):2341–2353.
- Bhasin S, Brito JP, Cunningham GR, et al. Testosterone therapy in men with hypogonadism: An Endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2018;103(5):1715–1744.
- Wu FCW, Tajar A, Beynon JM, et al. Identification of late-onset hypogonadism in middle-aged and elderly men. *N Engl J Med*. 2010;363(2):123–135.
- Corona G, Rastrelli G, Monami M, et al. Body weight loss reverts obesity-associated hypogonadotropic hypogonadism: A systematic review and meta-analysis. *Eur J Endocrinol*. 2013;168(6):829–843.
- Grossmann M, Ng Tang Fui M, Cheung AS. Late-onset hypogonadism: metabolic impact. *Andrology*. [Epub ahead of print]; DOI: 10.1111/andr.12705.
- Carrageta DF, Oliveira PF, Alves MG, Monteiro MP. Obesity and male hypogonadism: Tales of a vicious cycle. *Obes Rev Off J Int Assoc Study Obes*. 2019;20(8):1148–1158.
- Fernandez CJ, Chacko EC, Pappachan JM. Male obesity-related secondary hypogonadism—pathophysiology, clinical implications and management. *Eur Endocrinol*. 2019;15(2):83–90.
- Kapoor D, Goodwin E, Channer KS, Jones TH. Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. *Eur J Endocrinol*. 2006;154(6):899–906.
- Snyder PJ, Peachey H, Berlin JA, et al. Effects of testosterone replacement in hypogonadal men. *J Clin Endocrinol Metab*. 2000;85(8):2670–2677.
- Wang C, Jackson G, Jones TH, et al. Low testosterone associated with obesity and the metabolic syndrome contributes to sexual dysfunction and cardiovascular disease risk in men with type 2 diabetes. *Diabetes Care*. 2011;34(7):1669–1675.
- Parsons JK, Carter HB, Partin AW, et al. Metabolic factors associated with benign prostatic hyperplasia. *J Clin Endocrinol Metab*. 2006;91(7):2562–2568.
- Parikesit D, Mochtar CA, Umbas R, Hamid ARAH. The impact of obesity towards prostate diseases. *Prostate Int*. 2016;4(1):1–6.
- Tharakan T, Miah S, Jayasena C, Minhas S. Investigating the basis of sexual dysfunction during late-onset hypogonadism. *F1000Research*. 2019;8: F1000 Faculty Rev-331.
- Haider A, Saad F, Doros G, Gooren L. Hypogonadal obese men with and without diabetes mellitus type 2 lose weight and show improvement in cardiovascular risk factors when treated with testosterone: An observational study. *Obes Res Clin Pract*. 2014;8(4):e339–e349.
- Yassin D-J, Yassin A, Hammerer PG, Traish A, Doros G. 705 Long-term testosterone treatment over 5 years leads to progressive weight loss and waist size reduction as well as improvement of metabolic syndrome parameters in elderly men with hypogonadism. *Eur Urol Suppl*. 2014; 13(1):e705.
- Yassin A, Haider A, Haider KS, et al. Testosterone therapy in men with hypogonadism prevents progression from prediabetes to type 2 diabetes: Eight-year data from a registry study. *Diabetes Care*. 2019;42(6): 1104–1111.
- Zitzmann M, Vorona E, Wenk M, Saad F, Nieschlag E. Testosterone administration decreases carotid artery intima media thickness as a marker of impaired vascular integrity in middle-aged overweight men. *J Mens Health*. 2009;6(3):243.
- Traish AM, Haider A, Doros G, Saad F. Long-term testosterone therapy in hypogonadal men ameliorates elements of the metabolic syndrome: An observational, long-term registry study. *Int J Clin Pract*. 2014;68(3): 314–329.
- Saad F, Caliber M, Doros G, Haider KS, Haider A. Long-term treatment with testosterone undecanoate injections in men with hypogonadism alleviates erectile dysfunction and reduces risk of major adverse cardiovascular events, prostate cancer, and mortality. *Aging Male*. 2020;23(1):81–92.
- Gerstenbluth RE, Maniam PN, Corty EW, Seftel AD. Prostate-specific antigen changes in hypogonadal men treated with testosterone replacement. *J Androl*. 2002;23(6):922–926.
- Cui Y, Zong H, Yan H, Zhang Y. The effect of testosterone replacement therapy on prostate cancer: A systematic review and meta-analysis. *Prostate Cancer Prostatic Dis*. 2014;17(2):132–143.
- Morgentaler A, Traish AM. Shifting the paradigm of testosterone and prostate cancer: The saturation model and the limits of androgen-dependent growth. *Eur Urol*. 2009;55(2):310–320.
- Lee RK, Chung D, Chughtai B, Te AE, Kaplan SA. Central obesity as measured by waist circumference is predictive of severity of lower urinary tract symptoms. *BJU Int*. 2012;110(4):540–545.
- Guiraudou M, Varlet-Marie E, Raynaud de Mauverger E, Brun J-F. Obesity-related increase in whole blood viscosity includes different profiles according to fat localization. *Clin Hemorheol Microcirc*. 2013; 55(1):63–73.
- Corona G, Goulis DG, Huhtaniemi I, et al. European Academy of Andrology (EAA) guidelines on investigation, treatment and monitoring of functional hypogonadism in males. *Andrology*. 2020;8(5):970–987.
- Barbonetti A, D'Andrea S, Francavilla S. Testosterone replacement therapy. *Andrology*. [Epub ahead of print]; DOI: 10.1111/andr.12774.
- Free and Bioavailable Testosterone calculator. www.issam.ch/freetestosterone.htm Accessed July 1, 2020.

30. Saad F, Gooren L, Haider A, Yassin A. An exploratory study of the effects of 12 month administration of the novel long-acting testosterone undecanoate on measures of sexual function and the metabolic syndrome. *Arch Androl.* 2007;53(6):353–357.
31. Saad F, Aversa A, Isidori AM, Zafalon L, Zitzmann M, Gooren L. Onset of effects of testosterone treatment and time span until maximum effects are achieved. *Eur J Endocrinol.* 2011;165(5):675–685.
32. Calof OM, Singh AB, Lee ML, et al. Adverse events associated with testosterone replacement in middle-aged and older men: A meta-analysis of randomized, placebo-controlled trials. *J Gerontol Ser A.* 2005;60(11):1451–1457.
33. Bachman E, Travison TG, Basaria S, et al. Testosterone induces erythrocytosis via increased erythropoietin and suppressed hepcidin: Evidence for a new erythropoietin/hemoglobin set point. *J Gerontol A Biol Sci Med Sci.* 2014;69(6):725–735.
34. Dhindsa S, Ghanim H, Batra M, et al. Effect of testosterone on hepcidin, ferroportin, ferritin and iron binding capacity in patients with hypogonadotropic hypogonadism and type 2 diabetes. *Clin Endocrinol (Oxf).* 2016;85(5):772–780.
35. Spitzer M, Huang G, Basaria S, Travison TG, Bhasin S. Risks and benefits of testosterone therapy in older men. *Nat Rev Endocrinol.* 2013;9(7):414–424.
36. Danesh J, Collins R, Peto R, Lowe GD. Haematocrit, viscosity, erythrocyte sedimentation rate: Meta-analyses of prospective studies of coronary heart disease. *Eur Heart J.* 2000;21(7):515–520.
37. Groti K, Žuran I, Antičić B, Foršnarič L, Pfeifer M. The impact of testosterone replacement therapy on glycemic control, vascular function, and components of the metabolic syndrome in obese hypogonadal men with type 2 diabetes. *Aging Male.* 2018;21(3):1–12.
38. Elrifai A, Kotb AF, Sharaki O, Abdelhady M. Correlation of body mass index with serum total PSA, total testosterone and prostatic volume in a sample of men. *Pol Ann Med.* 2016;23(1):1–5.
39. Parekh N, Lin Y, Marcella S, Kant AK, Lu-Yao G. Associations of lifestyle and physiologic factors with prostate-specific antigen concentrations: Evidence from the National Health and Nutrition Examination Survey (2001–2004). *Cancer Epidemiol Biomark Amp Prev.* 2008;17(9):2467.
40. Boudreau DM, Yu O, Buist DSM, Miglioretti DL. Statin use and prostate cancer risk in a large population-based setting. *Cancer Causes Control.* 2008;19(7):767–774.
41. Iguchi K, Hashimoto M, Kubota M, et al. Effects of 14 frequently used drugs on prostate-specific antigen expression in prostate cancer LNCaP cells. *Oncol Lett.* 2014;7(5):1665–1668.
42. Morgentaler A, Benesh JA, Denes BS, Kan-Dobrosky N, Harb D, Miller MG. Factors influencing prostate-specific antigen response among men treated with testosterone therapy for 6 months. *J Sex Med.* 2014;11(11):2818–2825.
43. Meikle AW, Arver S, Dobs AS, et al. Prostate size in hypogonadal men treated with a nonscrotal permeation-enhanced testosterone transdermal system. *Urology.* 1997;49(2):191–196.
44. Rhoden EL, Morgentaler A. Testosterone replacement therapy in hypogonadal men at high risk for prostate cancer: Results of 1 year of treatment in men with prostatic intraepithelial neoplasia. *J Urol.* 2003;170(6):2348–2351.
45. Rhoden EL, Morgentaler A. Risks of testosterone-replacement therapy and recommendations for monitoring. *N Engl J Med.* 2004;350(5):482–492.
46. Cooper CS, Perry PJ, Sparks AE, MacIndoe JH, Yates WR, Williams RD. Effect of exogenous testosterone on prostate volume, serum and semen prostate specific antigen levels in healthy young men. *J Urol.* 1998;159(2):441–443.
47. Wang C, Nieschlag E, Swerdloff R, et al. Investigation, treatment and monitoring of late-onset hypogonadism in males: ISA, ISSAM, EAU, EAA and ASA recommendations. *Eur J Endocrinol.* 2008;159(5):507–514.

Cite this article as: Groti Antičić K, Antičić B, Pfeifer M (2020) Effects of testosterone therapy on erythrocytosis and prostate adverse events in obese males with functional hypogonadism and type 2 diabetes in a 2-year clinical trial, *Androgens: Clinical Research and Therapeutics* 1.1, 85–93, DOI: 10.1089/andro.2020.0008.

Abbreviations Used

AR = androgen receptors
 BMI = body mass index
 BPH = benign prostatic hyperplasia
 BT = bioavailable testosterone
 cFT = calculated free testosterone
 CVD = cardiovascular disease
 CVs = coefficients of variation
 FH = functional hypogonadism
 HbA_{1c} = glycated hemoglobin
 Hct = hematocrit
 IR = insulin resistance
 LUTS = lower urinary tract symptoms
 PAEs = prostate adverse events
 PCa = prostate carcinoma
 PSA = prostate-specific antigen
 RM-ANOVA = repeated measures analysis of variance
 SHBG = sex hormone binding globulin
 T2D = type 2 diabetes
 TT = total testosterone
 TTh = testosterone therapy

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