Original Article
Hormonal regulation of male reproduction and hypogonadism

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Comparison of Intratesticular Testosterone between Men Receiving Nasal, Intramuscular, and Subcutaneous Pellet Testosterone Therapy: Evaluation of Data from Two Single-Center Randomized Clinical Trials

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Purpose: Testosterone replacement therapy (TRT) can potentially cause decreased spermatogenesis and subsequent infertility. Recent studies have suggested that 17-hydroxyprogesterone (17-OHP) is a reliable surrogate for intratesticular testosterone (ITT) that is essential for spermatogenesis. We evaluated data from two ongoing open-label, randomized, two-arm clinical trials amongst different treatment preparations (Trial I) subcutaneous testosterone pellets (TP) and (Trial II) intranasal testosterone (NT) or intramuscular testosterone cypionate (TC).

Materials and Methods: Seventy-five symptomatic hypogonadal men (2 serum testosterone <300 ng/dL) were randomized into open label randomized clinical trials. Eligible subjects received 800 mg TP, 11 mg TID NT or 200 mg ×2 weeks TC. 17-OHP and Serum testosterone were evaluated at baseline and follow-up. The primary outcome was changes in 17-OHP. Secondary outcome was changes in serum testosterone. Data was analyzed by two-sample and single-sample t-tests, and determination of equal or unequal variances was computed using F-tests.

Results: Median participant age was 45 years old, with overall baseline 17-OHP of 46 and serum testosterone of 223.5 ng/dL. 17-OHP significantly decreased in subjects prescribed long-acting TP or TC. The 4-month change in 17-OHP in the NT group (-33.3% from baseline) was less than the change seen in TC (-65.3% from baseline) or TP (-44% from baseline) (p=0.005). All testosterone formulations increased serum testosterone levels at follow-up, with the largest increase seen in TC (+157.6%), followed by NT (+114.3%) and TP (+79.6%) (p=0.005).

Conclusions: Short-acting nasal testosterone appear to have no impact on serum 17-OHP especially in comparison to long-acting testosterone formulations. All modalities saw significant increases in serum testosterone levels at follow-up. NT and other short acting testosterone formulations may better preserve ITT and be beneficial for hypogonadal men seeking to maintain fertility potential while on TRT.

Keywords: 17-alpha-Hydroxyprogesterone; Androgens; Fertility; Hypogonadism; Testosterone

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INTRODUCTION

Hypogonadism in men presents as a clinical syndrome of low serum testosterone and decreased spermatogenesis combined with symptoms of erectile dysfunction, suppressed mood, and decreased libido [1,2]. Current mainstay treatment exists as testosterone replacement therapy (TRT) in the forms of testosterone pellets, intramuscular injections, and nasal testosterone gel. Classic adverse effects of TRT are secondary erythrocytosis, increases in estrogen, and suppression of the hypothalamic-pituitary-gonadal axis impacting fertility [3].

As of 2006, it was reported over 4 million men are impacted by hypogonadism in the United States [4]. Travison et al [5] first described hypogonadism can occur in younger men [6]. In fact, the average age of fathers has been consistently increasing since 1972. Specifically, fathers' average ages have increased 4.4 years over the past 45 years [7,8]. The combination of younger men being prescribed TRT and the average age of fathers increasing raises the importance of fertility preservation in men of reproductive age.

Intratesticular testosterone (ITT) is regarded as a critical component for spermatogenesis [9]. Unfortunately, ITT is decreased in men who receive TRT leading to impaired fertility potential. In recent years, 17-hydroxyprogesterone (17-OHP), an intermediate product of steroid metabolism, has been proposed as a surrogate biomarker for ITT and in turn, male fertility (Fig. 1) [7,10,11]. Existing evidence shows strong evidence of an inverse relationship between exogeneous TRT and serum 17-OHP along with an association between favorable semen parameters and increased 17-OHP serum levels [12]. As different TRT formulations have emerged in the market with each its own unique regimen schedule, it is unclear how each therapy impacts ITT. This study presents the first report of two open-label randomized control trials consisting of patients receiving either subcutaneous testosterone pellets (TP), intramuscular (IM), or intranasal testosterone (NT). We were able to quantify the differences each TRT formulation has on ITT using 17-OHP as a biomarker. We hypothesized that long-acting T formulations would decrease ITT compared to short-acting formulations due to short-acting T more closely mimicking the pulsatile secretion of gonadotropin-releasing hormone (GnRH).

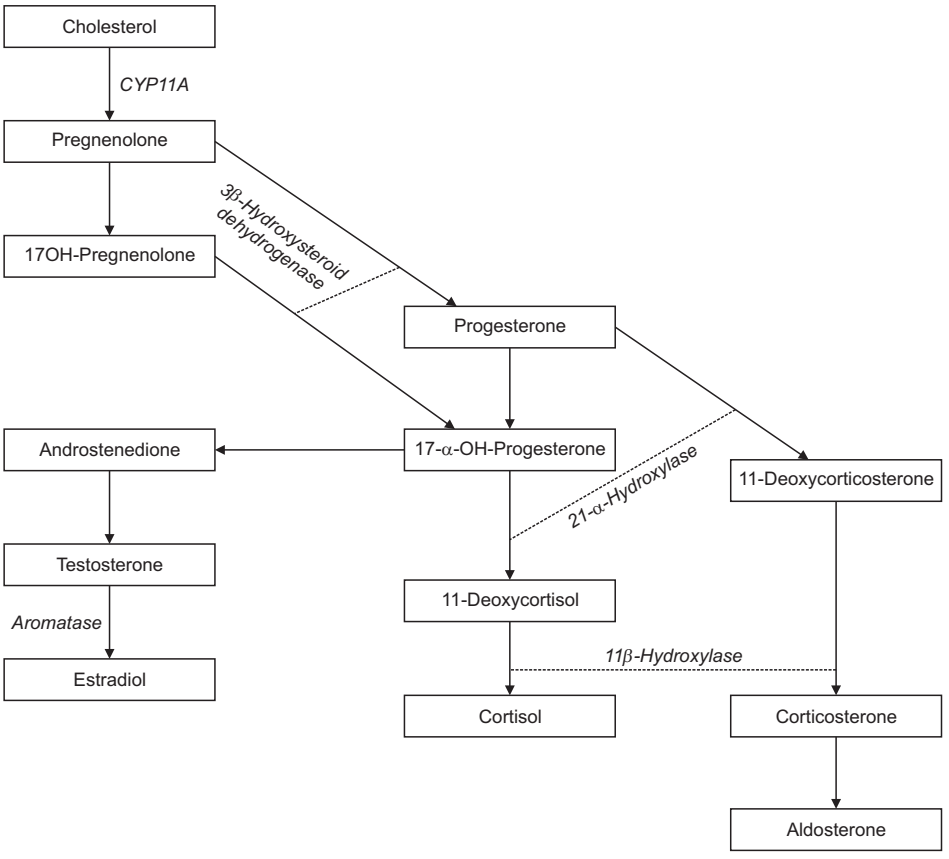

Fig. 1. Pathway of testosterone production and the 17-hydroxyprogesterone intermediary. Data from Patel et al. (Transl Androl Urol 2019;8(Suppl 1):S58-63) [11].

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MATERIALS AND METHODS

A retrospective study between January 1, 2011 and December 31, 2020, was conducted to compare the influence of TRT delivery methods on ITT by using the surrogate biomarker 17-OHP. Protocols for two single-center open label randomized clinical trials were approved by the Institutional Review Board of University of Miami Miller School of Medicine and performed in accordance with the World Medical Association's Declaration of Helsinki (IRB #20192539 - NCT04523480, #20200971 - NCT04983940). Written informed consent was obtained from the subjects.

1. Participants selection and variable evaluation

Diagnoses of hypogonadism were confirmed with two separate AM serum testosterone measurements less than 300 ng/dL by liquid chromatography–mass spectrometry combined with the presence of symptoms. Inclusion criteria consisted of age between 18 and 80 years, documented onset of hypogonadism prior to 65 years old, and TRT naïve or off TRT for at least four months. Exclusion criteria consisted of abnormal prostate digital rectal examination, prostate-specific antigen >4 ng/mL, hematocrit <35% or >50%, abnormal electrocardiogram, history of stroke or myocardial infarction in past five years, history or suspected prostate or breast cancer, and history of diagnosed severe or untreated obstructive sleep apnea. Patients with genetic conditions such as congenital adrenal hyperplasia or Klinefelter's disease were also excluded.

A total of 75 men (25 in each treatment cohort) were identified. Eligible subjects received either 800 mg for six months TP, 11 mg three times a day of NT, or 200 mg every two weeks testosterone cypionate (TC) for 16 weeks. Age and testosterone formulation were recorded for all patients. Additionally, pre and post treatment serum testosterone along, pre and post 17-OHP and pre and post hematocrit were recorded for all patients.

2. Outcomes

17-OHP and serum testosterone of all patients were evaluated at baseline and follow-up. The primary outcome was change in serum 17-OHP following 16 weeks of TRT. The secondary outcome was changes in serum testosterone after TRT. During clinical trial enrollment, subjects were instructed on the timing of laboratory bloodwork following medication administration. Subjects receiving TP underwent laboratory testing +/- 10 days of timepoint, 5 days after TC injections, and 30 minutes–1 hour after morning NT administration.

3. Statistical analysis

Data analysis was performed with SPSS ver. 28 (IBM Corp., Armonk, NY, USA), continuous variable was presented as mean±standard deviation or median [interquartile range 25th–75th] in accordance with data distribution on normality test, and comparation between groups were performed with the ANOVA or Kruskal–Wallis test as required. Changes of testosterone and 17-OHP were analyzed with the Wilcoxon test. A $p<0.05$ was considered statistically significant.

Table 1. Serum testosterone and ITT Before and after replacement therapy

Variable	Overall	TP	NT	TC	p-value
Total	75 (100.0)	25 (33.3)	25 (33.3)	25 (33.3)	
Age (y)	44.6±10.8	43.7±7.6	43.5 ±10.3	46.5±13.7	0.554
BMI (kg/m^2)	30.4±5.7	29.5±7.4	32.5 ±4.6	29.3±4.4	0.086
Before treatment					
Testosterone (ng/dL)	223.5 [190.0–261.0]	210 [144.5–239.8]	237 [199.8–262.3]	242 [192.3–289.8]	0.101
17-OHP (ng/dL)	46 [24.0–60.0]	29 [15.0–54.0]	48 [30.5–62.5]	49 [24.5–75.0]	0.102
After treatment					
Testosterone (ng/dL)	476 [321.0–670.0]	377 [249.5–496.0]	508 [383.0–700.5]	624 [319.0–848.5]	0.017
17-OHP (ng/dL)	20 [12.0–44.0]	16 [0.1–34.0]	32 [18.5–60.0]	17 [11.5–31.5]	0.005

Values are presented as number (%), mean±standard deviation, or median [interquartile range].
ITT: interatesticular testosterone, TP: subcutaneous testosterone pellets, NT: intranasal testosterone, TC: testosterone cypionate, BMI: body mass index, 17-OHP: 17-hydroxyprogesterone.

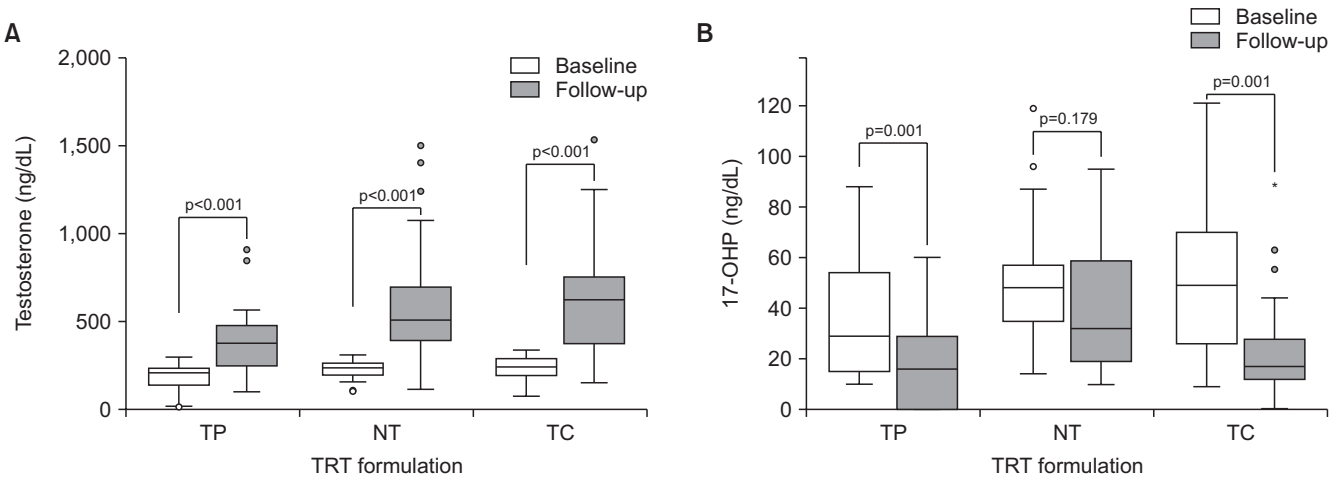

Fig. 2. Box plot figure showing the changes within group of serum (A) testosterone levels and (B) 17-OHP. TP: subcutaneous testosterone pellets, NT: intranasal testosterone, TC: testosterone cypionate, TRT: testosterone replacement therapy, 17-OHP: 17-hydroxyprogesterone.

RESULTS

A total of 75 men with hypogonadism receiving either NT, TC, or TP within the University of Miami Health System. Overall mean age was 44.6±10.8 years and body mass index (BMI) 30.4±5.7 kg/m^2, baseline serum testosterone of 223.5 ng/dL [190–261 ng/dL] and 17-OHP of 46 ng/dL [24–60 ng/dL]. Age, BMI, testosterone, and 17-OHP at baseline were similar among groups (p>0.05), at baseline hematocrit was higher in men in NT (p=0.012). Among groups, all three TRT regimen cohorts had significantly different testosterone and 17-OHP at follow-up (p<0.05) (Table 1).

Within men in each group, all T formulations increased testosterone levels at follow-up (p<0.001), with TP from 210 ng/dL [144.5–239.8 ng/dL] to 377 ng/dL [249.5–496.0 ng/dL]; NT from 237 ng/dL [199.8–262.3 ng/dL] to 508 ng/dL [383.0–700.5 ng/dL]; and TC from 242 ng/dL [192.3–289.8 ng/dL] to 624 ng/dL [319.0–848.5 ng/dL]. On the other hand, 17-OHP was not significantly affected overtime in the men receiving NT with a baseline 17-OHP of 48 ng/dL [30.5–62.5 ng/dL] and follow-up 32 ng/dL [18.5–60.0 ng/dL] (p=0.179). Furthermore, in patients with TP and TC 17-OHP levels significantly decrease after treatment from 29 ng/dL [15.0–54.0] ng/dL to 16 ng/dL [0.1–34.0 ng/dL] and 49 ng/dL [24.5–75.0 ng/dL] to 17 ng/dL [11.5–31.5 ng/dL], respectively (p=0.001) (Fig. 2).

DISCUSSION

Usage of long-acting TRT in hypogonadal men was associated with significantly decreased 17-OHP at follow-up. However, in line with the hypothesis, short-acting NT had the smallest change in 17-OHP following treatment compared to long-acting injectables or pellets. Our study is the first to simultaneously compare the impact of intranasal, intramuscular, and subcutaneous testosterone treatment on endogenous testosterone in hypogonadal males. The results of this study might suggest that short-acting formulations may have less of an impact on local testicular environment.

A plausible explanation for this finding is dosing schedule paired with the short half-life of NT more closely mimics normal physiology allowing for maintenance of GnRH pulsatility (LH) and its downstream products lutenizing hormone and follicle stimulating hormone (FSH) [13]. LH then stimulates Leydig cells within the testis to create endogenous testosterone that contribute to subsequent spermatogenesis. Given the increase of serum testosterone and maintenance of ITT, the result of this study should be considered when discussing the optimal TRT regimen for hypogonadal men who are concerned about testis atrophy and future fertility potential.

Long-acting testosteroned has been shown to diminish endogenous testosterone production *via* suppression of the hypothalamic–pituitary–adrenal axis [14]. Men with low endogenous testosterone often suffer from decreased testes volume, decreased sperm concentration with evidence of increased risk of all-cause and CVD death [15,16]. With an incidence of hypogonadism in 3% to 8% of 20 to 45-year-old men [9], and an average

age of first-time fathers 31 years old [17] many hypogonadal males may be unwilling to begin treatment due to contraceptive effects from TRT. To date, physicians have countered this decrease in ITT by prescribing the LH-agonist human chorionic gonadotropin (HCG) to stimulate the testes to produce testosterone, and existing evidence shows sperm count recovery after TRT to 90% within 12 months [18]. However, the cost, lack of insurance coverage, and heavy restrictions imposed by the U.S. Food and Drug Administration (FDA) make HCG inaccessible for many. This study contributes to a clearer understanding of short-acting testosterone formulations ability to maintain endogenous testosterone and support previous research suggesting that intranasal testosterone gel can treat hypogonadism while maintaining semen parameters. There is a lack of information on whether hypogonadal men can recover endogenous testosterone production following TRT, and further studies should be done to evaluate this among the various delivery methods.

This study is not without limitations. While 17-OHP is helpful as a surrogate for ITT, it is an imperfect marker as approximately 30% is made by the adrenal glands. Future projects should be sure to include the collection of serum LH and FSH in subjects to better elucidate the pulsatility of GnRH between the various testosterone delivery methods. Given the retrospective study design, we are unable to quantify missed doses, medication non-adherence, nor the proficiency of the clinicians in counseling patients on TRT. In addition, while subjects were instructed regarding the timing of laboratory bloodwork their personal schedules or testing center availability may have precluded optimal profiling. Finally, our data only includes hypogonadal men recruited and treated at a single hospital system and may not be applicable to patients in different settings with replacement regimens beyond FDA recommendations [19-21]. Nevertheless, our study is the first to compare the impact of nasal, intramuscular, and subcutaneous testosterone treatment on 17-OHP in humans using randomized clinical trial data.

CONCLUSIONS

Short-acting nasal testosterone appear to have no impact on serum 17-OHP especially in comparison to long-acting testosterone formulations. However, all TRT modalities demonstrated significant increases in serum testosterone levels of hypogonadal men at follow-up. Maintenance of 17-OHP gives insight into the testicular micro-environment which is especially important in spermatogenesis and overall male fertility. Intranasal T and other short acting testosterone formulations may better to preserve ITT and be beneficial for hypogonadal men seeking to maintain fertility potential while on TRT.

Conflict of Interest

The authors have nothing to disclose.

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Authors Contributions

Conceptualization: RoRe, PD, RBB, RR. Data curation: RoRe, PD, RR, IZ, AD, DCG, EK. Formal analysis: RoRe, PD, RBB, IZ, EK, RR. Investigation: PD, RoRe, RBB, DCG, EK, RR. Methodology: RoRe, PD, DCG, EK, RR. Project administration: PD, RR. Resources: RR, RBB. Software: RoRe, RBB, IZ, AD, DCG, EK, RR. Supervision: RoRe, RR, RBB, IZ, AD, DCG, EK, RR. Validation: RoRe, RBB, IZ, AD, DCG, EK, RR. Visualization: RoRe, RR. Writing – original draft: PD, RR, RBB, IZ, AD, DCG, EK, RR. Writing – review & editing: RR, PD.

Data Sharing Statement

The data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

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