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Testosterone implants in women: Pharmacological dosing for a physiologic effect

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ABSTRACT

Objectives: The objectives of this study were to determine therapeutic serum testosterone (T) levels/ranges and inter-individual variance in women treated with subcutaneous T implants.
Study design: In study group 1, T levels were measured at two separate time intervals in pre- and post-menopausal women treated with subcutaneous T for symptoms of androgen deficiency: (i) four weeks after pellet insertion, and (ii) when symptoms of androgen deficiency returned.

In a separate pharmacokinetic study (study group 2), 12 previously untreated postmenopausal women each received a 100 mg T implant. Serum T levels were measured at baseline, 4 weeks and 16 weeks following T pellet implantation.

In study 'group' 3, serial T levels were measured throughout a 26 h period in a treated patient.
Results: In study group 1, serum T levels measured at 'week 4' (299.36 ± 107.34 ng/dl, $n = 154$), and when symptoms returned (171.43 ± 73.01 ng/dl, $n = 261$), were several-fold higher compared to levels of endogenous T. There was significant inter-individual variance in T levels at 'week 4' (CV 35.9%) and when symptoms returned (CV 42.6%). Even with identical dosing (study group 2), there was significant inter-individual variance in T levels at 'week 4' (CV 41.9%) and 'week 16' (CV 41.6%). In addition, there was significant intra-individual circadian variation (CV 25%).
Conclusions: Pharmacologic dosing of subcutaneous T, as evidenced by serum levels on therapy, is needed to produce a physiologic effect in female patients. Safety, tolerability and clinical response should guide therapy rather than a single T measurement, which is extremely variable and inherently unreliable.

1. Introduction

Testosterone (T) is becoming increasingly recognized as a vital hormone in women. T elicits a physiologic effect via functional androgen receptors (ARs), which are located in almost all tissues including the breast, heart, blood vessels, gastrointestinal tract, lung, brain, spinal cord, peripheral nerves, bladder, uterus, ovaries, endocrine glands, vaginal tissue, skin, bone, bone marrow, synovium, muscle and adipose tissue [1,2]. T is also the major substrate for estrogen in both men and women and thus has an indirect effect via the estrogen receptor. Until recently, outside of its role in sex drive and libido, T has been virtually ignored as an essential hormone in female physiology and erroneously labeled as a 'male hormone'. Healthy pre-menopausal women have 15–20-fold higher levels of T than estradiol. In addition, there are exponentially higher levels of androgen precursors, including dihydroepiandrosterone sulfate (DHEAS) and androstenedione, producing an immeasurable amount of T locally, at the cellular level, which is able to bind to the AR. Unlike the acute decline of estrogen at menopause, T and its prohormones decline gradually with age [3,4].

Pre- and post-menopausal patients may experience symptoms of androgen deficiency including sexual dysfunction, dysphoric mood (anxiety, irritability and depression), lack of well-being, physical fatigue, changes in cognition, memory loss, insomnia, hot flashes, rheumatoid complaints, pain, vaginal dryness, urinary complaints and incontinence, which are becoming increasingly recognized and treated [5,6]. There is a paucity of data guiding T replacement therapy in women. Although some authors recommend following T levels and adjusting doses based on these levels, there is no evidence supporting that a single testosterone

Abbreviations: T, testosterone; CV, coefficient of variation; IRB, Institutional Review Board; BMI, body mass index; PK, pharmacokinetic; DHEAS, dihydroepiandrosterone sulfate; AR, androgen receptor.

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measurement is accurate, nor that it correlates with physiologic effect. More importantly, there is no evidence to support that testosterone levels on therapy should remain within ranges for endogenous production. This paper investigates the inherent variability of single measurement of testosterone and supports that pharmacologic dosing of subcutaneous T implants is both safe, and necessary, to produce a physiologic effect.

2. Methods

2.1. Study group 1: serum T levels (ranges) on therapy, 'week 4' and prior to re-implantation, 'end'

All patients in this group are part of an ongoing, 10 year, prospective IRB approved trial on the effect of subcutaneous T implants on the incidence of breast cancer [5]. Pre- and post-menopausal patients participating in the trial were either self-referred or referred by their physician to this private clinical practice (RG) for symptoms of relative androgen deficiency including; hot flashes, sweating, sleep disturbance, heart discomfort, depressive mood, irritability, anxiety, pre-menstrual syndrome, fatigue, memory loss, menstrual or migraine headaches, vaginal dryness, sexual problems, urinary symptoms, pain and bone loss. As reported elsewhere, no patient was excluded from therapy based on baseline serum hormone levels (we previously reported that there was no correlation between baseline hormone levels (estradiol, free T, total T) and incidence/severity of presenting symptoms as reported on the validated Menopause Rating Scale; and that all symptoms improved on subcutaneous T therapy alone, independent of baseline hormone levels [5]). Written informed consent was obtained on all patients.

285 patients treated with testosterone implant therapy for at least one year (mean 28.1 ± 10.4 months), seen at the clinic between February and April 2010, were included in a follow-up clinical, questionnaire study. 3.1 mm (diameter) T implants were compounded by a pharmacy in Cincinnati, OH. The mean testosterone implant dose in this cohort of patients was 133.3 ± 26.8 mg, range 55–240 mg. Dosing was based on weight and adjusted based on clinical response to therapy. Testosterone implants had been inserted, on average, every 13.8 ± 3.8 weeks. All patients were offered, but not required to have, blood testing. 154 of these patients had serum testosterone levels drawn 4 weeks after their testosterone pellets were inserted.

In addition, 'end' serum testosterone levels were collected on a separate cohort of 261 patients treated at the clinic between November 2011 and March 2012. Depending on the laboratory used and insurance coverage, free T levels were also performed on 153 of these patients. Patients were instructed to have serum T levels drawn when their symptoms of androgen deficiency returned, prior to their subsequent T pellet implant. Only serum T levels obtained within 2 weeks of the patient becoming symptomatic (i.e., 'end' levels) were included in this analysis.

2.2. Study group 2: pharmacokinetic study, inter-individual variation in T levels

In a separate IRB approved trial (Miami Valley Hospital, Premier Health Partners, MVH Study # 06-0090;6859), pharmacokinetic (PK) studies were performed in 12 previously untreated, postmenopausal women receiving identical doses (100 mg) of T as a subcutaneous implant. Serum T levels were measured at baseline (prior to therapy), 4 weeks and 16 weeks after T pellet insertion. BMI was calculated and correlated with serum T levels at baseline and on therapy. Written informed consent was obtained on all patients.

2.3. Study 'group' 3: circadian (intra-individual) variation in T levels

A 26 h PK pilot study was performed on a female patient treated with a 112.5 mg T implant. Venous bloodspot specimens were collected every 2 h during waking hours, throughout a 26 h period, 6 weeks after T pellet implantation.

3. Methodologies

3.1. Serum testosterone testing

In group 1, total testosterone levels were measured using liquid chromatography tandem mass spectrometry, LCMS (intra-assay CV 9%) or by immune-assay using Bayer Advia Centaur immunoassay (intra-assay CV 11.8%). The methodology used (IA vs. LCMS) depended on the lab, which was determined by insurance coverage.

Free testosterone was performed by tracer equilibrium dialysis calculation (intra-assay CV 11.3%).

In the 12 patients from group 2, total testosterone was measured by immune-assay using Bayer Advia Centaur immunoassay. A duplicate specimen was sent to a second lab (LC) for comparison. T was measured using ammonium sulfate precipitation radioassay (intra-assay CV 12%).

3.2. Venous bloodspot

Drops of venous blood from a forearm venipuncture were dropped onto specialized filter paper (Schleicher and Schuell 903; Bioscience, Keene, NH) and allowed to dry. Samples were stored at room temperature. Standard and control, 6.4 mm discs were punched from dried blood spot samples using the Wallac Multipuncher Dried Bloodspot Puncher (Perkin Elmer-Wallac). The samples, along with standards, were added to a 96 deep-well (2 ml per well) plates and re-hydrated in 200 ml per disk of assay buffer containing phosphate-buffered saline (Diamedix, Miami, FL), 0.025% Tween 20, and 0.01% ProClin 950 antimicrobial (Sigma–Aldrich, St. Louis, MO). From this point the standard procedure for serum testing using enzyme immunoassay for testosterone (DRG) was followed and results given in ng/dl (ZRT lab, Beaverton, OR).

3.3. Statistical analysis

The statistical program R (R Development Core Team, 2012) was used for all data analysis [7]. The Spearman's rank correlation coefficient, Spearman's rho (ρ), analysis was used to screen relationships between individual variables (T dose, BMI, T level). Coefficient of variation (CV) was calculated and expressed as a percentage.

4. Results

4.1. Testosterone dose and week 4 T levels (study group 1)

The mean serum testosterone level, 4 weeks after T implantation, was 299.36 ± 107.34 ng/dl (range 101–633, $n = 154$, CV 35.9%). This mean value is 4–6 times the upper limit of normal for endogenous production (i.e., 42–72 ng/dl).

As expected with weight based dosing, there was a positive correlation between the patients BMI and their testosterone implant dose (0.566, $P < 0.0001$). Conversely, there was no correlation between serum T levels at week 4 and BMI ($\rho = -0.043$, $P = 0.59$) (Fig. 1).

In this group of patients, treated with testosterone therapy for over one year, there were no reported adverse drug events. As