



# Testosterone implants in women: Pharmacological dosing for a physiologic effect

Rebecca Glaser<sup>a,b,\*</sup>, Sophia Kalantaridou<sup>c</sup>, Constantine Dimitrakakis<sup>d,e</sup>

<sup>a</sup> Millennium Wellness Center, 228 E. Spring Valley Road, Dayton, OH 45458, USA

<sup>b</sup> Wright State University Boonshoft School of Medicine, Department of Surgery, 3460 Colonel Glenn Highway, Dayton, OH 45435, USA

<sup>c</sup> Department of Obstetrics and Gynaecology, Medical School, University of Ioannina, 45110 Ioannina, Greece

<sup>d</sup> 1st Department of Ob/Gyn, Athens University Medical School, 80 Vas. Sophias Street, 11528 Athens, Greece

<sup>e</sup> National Institutes of Health, NICHD, Building 10, 10 Center Drive, Bethesda, MD 20892-1103, USA

## ARTICLE INFO

### Article history:

Received 8 October 2012

Received in revised form 4 November 2012

Accepted 10 November 2012

### Keywords:

Testosterone

Implants

Women

Dosing

Efficacy

Serum levels

## 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' ( $299.36 \pm 107.34$  ng/dl,  $n=154$ ), and when symptoms returned ( $171.43 \pm 73.01$  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.

© 2012 Elsevier Ireland Ltd. All rights reserved.

## 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.

\* Corresponding author at: 228 E. Spring Valley Road, Dayton, OH 45458, USA. Tel.: +1 937 436 9821; fax: +1 937 436 9827.

E-mail addresses: [rglaser@woh.rr.com](mailto:rglaser@woh.rr.com), [rglaserm@gmail.com](mailto:rglaserm@gmail.com) (R. Glaser), [sophiakalantaridou@gmail.com](mailto:sophiakalantaridou@gmail.com) (S. Kalantaridou), [dimitrac@ymail.com](mailto:dimitrac@ymail.com) (C. Dimitrakakis).

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  $28.1 \pm 10.4$  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  $133.3 \pm 26.8$  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  $13.8 \pm 3.8$  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, post-menopausal 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 ( $\rho$ ), 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.

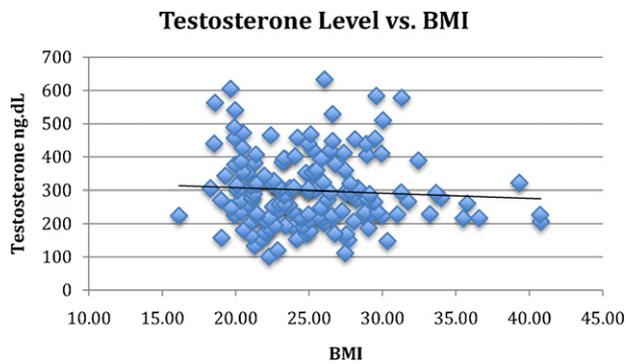
## 4. Results

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

The mean serum testosterone level, 4 weeks after T implantation, was  $299.36 \pm 107.34$  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



**Fig. 1.** There was no correlation between serum testosterone levels 4 weeks after T pellet implant and patients BMI ( $\rho = -0.043$ ,  $P = 0.59$ ,  $n = 154$ ).

previously published, 85.7% of patients reported a mild to moderate increase in facial hair while 6.4% of patients reported a severe increase [8]. 32 of 285 (11.2%) patients reported a moderate increase in acne, half of who had a prior history of adult acne. One patient, with a history of adult cystic acne, reported severe acne on therapy. 50% of patients reported skin improvement on therapy (e.g., moister skin, softer skin and fewer wrinkles). Although occasionally reported in clinical practice, no one in this cohort reported clitoromegaly. Three patients (1%) reported perceived voice changes: (i) voice cracking, (ii) raspy voice and (iii) deeper voice.

#### 4.2. 'End' serum testosterone levels: levels drawn when patient's symptoms returned prior to re-implantation (study group 1)

261 women in study group 1 had testosterone levels measured when their symptoms of androgen deficiency returned, prior to T pellet re-implantation. The mean testosterone level for this cohort was  $171.43 \pm 73.01$  ng/dl (range 22–461, CV 42.6%).

153 of these 261 patients had both free and total testosterone performed by a single lab (Quest) at the 'end' of their pellet implantation. The mean total T in this group was  $184.72 \pm 74.6$  ng/dl (range 47–461, CV 40.4%), over 4 times the upper limit of normal for endogenous production (reference range total T: 2–45 ng/dl). The mean free T level was  $18.82 \pm 11.51$  pg/ml (range 1.1–74.8, CV 61.2%). The average free T prior to re-implantation was over 3 times the upper limits of normal for endogenous production (reference range free T: 0.1–6.4 pg/ml). There was a positive correlation between total T and free T ( $\rho = 0.66$ ,  $P < 0.001$ ).

#### 4.3. Inter-individual variation (group 2)

Baseline serum testosterone level in the 12 post-menopausal patients, prior to T implant therapy, varied significantly,  $23.9 \pm 20.1$  ng/dl (range 1–52, CV 84%).

The mean serum T level measured 4 weeks after insertion of a 100 mg T implant, was  $190.8 \pm 80$  ng/dl (range 83–368, CV 41.9%) (Fig. 2). There was over a 4-fold difference between the lowest and highest testosterone level despite identical dosing. None of the patients had symptoms of androgen excess.

This significant variation in serum T levels persisted through week 16, past the time when symptoms normally return. Mean testosterone level 16 weeks after T pellet implantation was  $74.9 \pm 31.2$  ng/dl (range 44–136, CV 41.6%).

For quality control, duplicate serum specimens in these 12 patients had been sent to a second lab at each time interval (baseline, week 4, week 16). There was no correlation in serum T levels, between the two labs (both using immune-assay) at low baseline T levels ( $\rho = 0.4542$ ,  $P = 0.138$ ). However, there was a strong

correlation in serum T levels between the two labs at week 4 ( $\rho = 0.9021$ ,  $P < 0.01$ ) and week 16 ( $\rho = 0.8231$ ,  $P < 0.01$ ) when measuring higher serum T levels on therapy.

Interestingly, although all patients received a 100 mg T implant (non weight-based dosing), there was no correlation between BMI ( $24.5 \pm 3.9$ , range 20.1–32.4) and serum T levels measured at either week 4 ( $\rho = -0.1821$ ,  $P = 0.571$ ) or week 16 ( $\rho = 0.0841$ ,  $P = 0.0795$ ).

There was no correlation between baseline T levels, and T levels at week 4 ( $\rho = 0.3410$ ,  $P = 0.278$ ) or week 16 ( $\rho = 0.3269$ ,  $P = 0.300$ ). However, there was a positive correlation between T levels measured at week 4 and week 16 ( $\rho = 0.62949$ ,  $P = 0.0324$ ).

#### 4.4. Intra-individual (circadian) variation, 26 h study

Venous blood spot testosterone levels were measured every 2 h (while awake) over a 26 h period in a female patient 6 weeks after receiving a 112.5 mg testosterone implant. The mean testosterone level was  $268.4 \pm 67.1$  ng/dl (range 176–383, CV 25%) (Fig. 3). Notably, levels fluctuated significantly throughout the day, similar to the circadian release of endogenous hormones [9].

## 5. Discussion

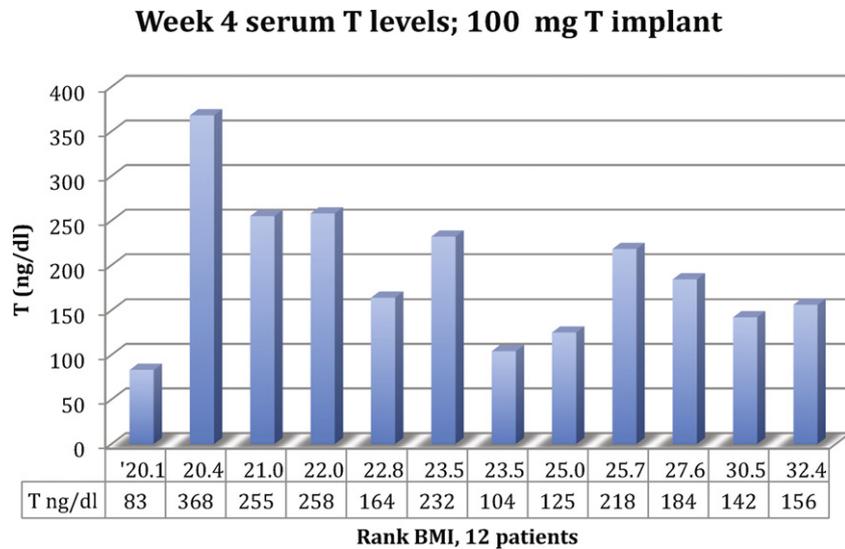
Testosterone therapy is becoming increasingly used in pre- and post-menopausal women to treat symptoms associated with hormone/androgen deficiency. We have previously reported that subcutaneous T alone (no estrogen) effectively treats many symptoms previously considered due to estrogen deficiency [5]. T exerts a direct effect by binding to ARs, which are located in almost all organs and tissues in both men and women. In addition, T is aromatized to estradiol in the ovary, adrenal gland and peripheral tissues; and has a secondary effect via the estrogen receptor. T has also been shown to effectively treat 'hormone deficiency symptoms' when used in combination with an aromatase inhibitor, supporting that testosterone's biologic effect is primarily via its cognate AR [10,11].

Subcutaneous testosterone implants have been used in women since 1938 in doses of 50–225 mg. Long-term data exists on the safety, tolerability and efficacy of these doses in up to 40 years of therapy [12–18]. In addition, significantly higher doses of T, used to treat breast cancer patients and 'female to male' transgender patients have been studied and found to be safe [16,19–21].

Long acting, sustained release T implant dosing is weight based. As previously published, the T doses used in this current study (55–240 mg), are both clinically effective and well tolerated [5,8,10,11,25,27]. Higher T doses have been shown to correlate with greater improvement in quality of life as evidenced by the 'Menopause Rating Scale' total score and somatic, psychological and urogenital sub-scores [5]. This is consistent with other studies reporting that T effect is dose dependent [18,22–24].

In addition, these T implant doses have been shown to increase scalp hair growth and were not associated with androgenic alopecia [8]. As expected, there was a concomitant increased facial hair growth in the majority of patients. However, no patient discontinued therapy because of increased hair growth. Similar dosing (100–180 mg) effectively treats migraine headaches in both pre and postmenopausal women [25] and has safely been used (with an aromatase inhibitor) in breast cancer survivors to treat symptoms of androgen deficiency [10]. There have been no adverse drug events related to subcutaneous T therapy; and other than increased facial hair and mild to moderate acne, side effects have been minimal at these doses.

It has been documented in the past that serum T levels on subcutaneous implant therapy are higher than endogenous ranges [8,10,12,13,25] and that 'more consistent benefit is seen with testosterone levels that exceed the normal range' [26]. Higher



**Fig. 2.** Serum testosterone levels in 12 female patients, 4 weeks after therapy with a 100 mg T implant. Mean T level was  $190.8 \pm 80$  ng/dl (range 83–368, CV 41.9%). There was no correlation between serum T levels and BMI ( $\rho = -0.1821$ ,  $P = 0.571$ ).

serum levels of T have been shown to correlate with greater clinical effect including a beneficial effect on lipids; higher HDL, lower VLDL and lower TG [18,27].

Contrarily, there is no clinical evidence supporting the recommendation that 'serum levels of T on therapy should remain within the upper limits of endogenous production for a young healthy female'. This theoretical 'physiologic dosing' of T in women has been shown to be clinically ineffective [18,24,28]. The simplistic concept of using a single serum T level to guide therapy ignores the complexity of physiologic events from production/release to biological effect; and totally disregards the significant contribution of local production, as well as, age related changes.

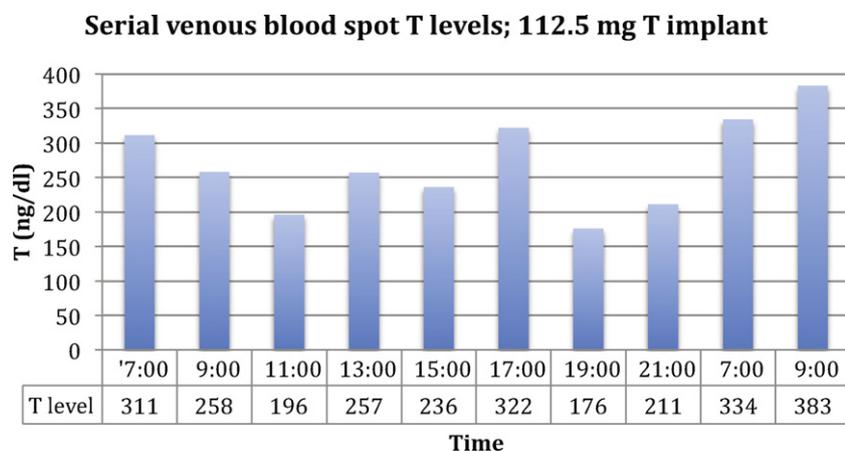
We have demonstrated that serum T levels, on the 'biologically effective' doses used in this study, were 4–6-fold higher on than endogenous T ranges at both 'week 4' ( $299.36 \pm 107.34$  ng/dl) and prior to re-implantation ( $171.43 \pm 73.01$  ng/dl). However, the majority of the circulating level of T measured in serum is tightly bound to SHBG, unable to bind to the AR and therefore, unable to elicit a biologic/clinical response. The relatively small proportion of T available to bind to the AR is a complex combination of unbound T, a portion of albumin bound T (i.e., low affinity binding protein compared to SHBG), and most importantly, the unquantifiable

amount of testosterone produced locally at the cellular level from the androgen precursors, DHEAS and androstenedione.

Similar to T's decline with age, DHEAS and androstenediones production also decreases with age [3]. This decline in proandrogens markedly reduces the amount of T available at the cellular level. While androstenedione is found in 5–10-fold higher concentrations than T in serum, DHEAS levels may be thousands of times higher than T levels [4]. Thus, in comparison to T, the contribution of these prohormones to bioavailable T at the AR exponentially declines with age. With this marked decline in local production, increasing amounts of T (i.e., from replacement therapy) would be needed to supply a greater portion of bioavailable T to the AR.

There is also concern of AR 'resistance' [29]. Theoretically, with aging the AR, similar to the insulin receptor, may become resistant to T and require higher levels to elicit the same response.

This study has shown that a single serum T level on therapy is extremely variable and inherently unreliable. There was significant variation between individuals (CV > 40%) in both groups of patients tested, independent of dosing and BMI. In addition, the broad range in T levels reflects significant inter-subject variability in pharmacodynamic response to these serum T concentrations.



**Fig. 3.** Serial venous blood spot testosterone levels were measured every 2 h (while awake) over a 26 h period in a female patient treated with a 112.5 mg testosterone implant. Mean T level,  $268.4 \pm 67.1$  ng/dl (range 176–383, CV 25%).

Our case presentation demonstrated significant circadian variation in a female patient over a 26 h period (CV 25%). A similar circadian variation (CV 21%) was also seen in a male patient treated with 1200 mg of subcutaneous T (data not shown). In addition, we have seen a variation in T levels in a female patient who inadvertently had consecutive serum samples analyzed (231 ng/dl vs. 310 ng/dl, CV 21%). Although these case findings are of limited value, in light of the significant inter-individual variation, we suggest that routinely monitoring T levels in clinical practice, and adjusting therapy based on a single value, should be viewed with skepticism; as well as clinical guidelines founded solely on serum levels on therapy.

We propose that T dosing should be based on adequate clinical efficacy, similar to insulin dosing, where individual biologic effect and tolerability determines dosing rather than serum levels based on endogenous production. We no longer routinely monitor serum T levels in all patients. However, because of aromatization and the adverse effects of excess estrogen in men and some women, we do measure estradiol and testosterone levels in subgroups of patients. Patients are treated with aromatase inhibitors, combined in the pellet implant based on history (e.g., breast cancer, endometriosis, fibroids etc.), symptoms (e.g., fluid retention, weight gain, anxiety etc.) and serum levels [11].

In this clinical practice (RG), in the past 7 years, over 16,000 T pellet insertions have been performed in over 1300 female patients on protocol. We have previously reported on the benefits and safety of T delivered by sustained release implants with an average starting dose of 2 mg/kg [5,8,25,27]. We have observed, that some symptoms (e.g., bone pain, memory loss, neurological complaints and tremor) and some diseases (e.g., multiple sclerosis, Parkinson's disease and Alzheimer's disease) require higher dosing (4 mg/kg) for optimal clinical effect. There have been no reported adverse drug events attributed to T therapy other than expected androgenic side effects, which are reversible with lowering T dose. Many patients prefer the clinical benefits of higher doses/levels of T and choose to take care of the side effects of therapy. We have also combined finasteride, a 5 alpha reductase inhibitor, with testosterone in a pellet implant (60 mg T + 6 mg of finasteride), which has markedly reduced the incidence of acne. Considering long term data on the use of testosterone in female to male transgender patients, excluding aromatization as mentioned above, there does not appear to be a 'maximum' dose based on safety [16,19,20].

A weakness of this study was that serum T levels were performed at different laboratories, by different methodologies (group 1). This was unavoidable as this is a private clinical practice and the study was not funded. We did demonstrate a strong correlation between methodologies ( $P < 0.01$ ) at the 'higher' T levels on therapy. In addition, findings were similar when evaluating results from a large subgroup of patients who had T levels drawn at a single laboratory and measured by the same methodology. Perhaps future studies, without insurance limitations, could include more consistent measurement profiles including SHBG.

## 6. Conclusion

T therapy delivered by subcutaneous implant, has been shown to be both safe and effective in pharmacologic doses. Although various authors have suggested 'physiologic' dosing of T, we have found that 'pharmacologic' dosing (based on serum levels on therapy) is necessary to provide adequate amounts of bioavailable T to the AR. We have shown that a single T measurement is extremely variable. In addition, there is inter-subject variability in pharmacodynamic response. As with any medication, clinical response to therapy (i.e., physiologic effect), safety and tolerability should determine dosing.

## Contributors

R.G. and C.D. contributed equally to the research, design of the study, analyzing the data, writing and editing the ms. R.G. recruited participants. S.K. contributed to writing and editing the ms. All authors approved the final manuscript.

## Competing interest

None of the authors (R.G., S.K. and C.D.) have any competing interests.

## Funding

None.

## References

- [1] Wilson CM, McPhaul MJ. A and B forms of the androgen receptor are expressed in a variety of human tissues. *Molecular and Cellular Endocrinology* 1996;120:51–7.
- [2] Takeda H, Chodak G, Mutchnik S, Nakamoto T, Chang C. Immunohistochemical localization of androgen receptors with mono- and polyclonal antibodies to androgen receptor. *The Journal of Endocrinology* 1990;126:17–25.
- [3] Burger HG. Androgen production in women. *Fertility and Sterility* 2002;77:3–5.
- [4] Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. *Journal of Clinical Endocrinology & Metabolism* 2005;90:3847–53.
- [5] Glaser R, York AE, Dimitrakakis C. Beneficial effects of testosterone therapy in women measured by the validated Menopause Rating Scale (MRS). *Maturitas* 2011;68:355–61.
- [6] Kalantaridou SN, Callis KA. Testosterone therapy in premenopausal women. *Seminars in Reproductive Medicine* 2006;24(2):106–14.
- [7] R Development Cor Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2012 <http://www.R-project.org/>
- [8] Glaser RL, Dimitrakakis C, Messenger AG. Improvement in scalp hair growth in androgen-deficient women treated with testosterone: a questionnaire study. *British Journal of Dermatology* 2012;166:274–8.
- [9] Spratt DL, O'dea LS, Schoenfeld D, Butler J, Rao PN, Crowley Jr WF. Neuroendocrine-gonadal axis in men: frequent sampling of LH, FSH, and testosterone. *American Journal of Physiology: Endocrinology and Metabolism* 1988;254:E658–66.
- [10] Glaser R. Subcutaneous testosterone–anastrozole implant therapy in breast cancer survivors. In: *American Society of Clinical Oncology Breast Cancer Symposium*. 2010. D:221.
- [11] Glaser R, Dimitrakakis C. Subgroups of patients treated with an aromatase inhibitor (anastrozole) delivered subcutaneously in combination with testosterone. In: *9th European Congress on Menopause and Andropause*. 2012. A424:0001:00048.
- [12] Burger HG, Hailes J, Menelaus M, Nelson J, Hudson B, Balazs N. The management of persistent menopausal symptoms with oestradiol–testosterone implants: clinical, lipid and hormonal results. *Maturitas* 1984;6:351–8.
- [13] Gambrell Jr RD, Natrajan PK. Moderate dosage estrogen–androgen therapy improves continuation rates in postmenopausal women: impact of the WHI reports. *Climacteric* 2006;9:224–33.
- [14] Dimitrakakis C, Jones RA, Liu A, Bondy CA. Breast cancer incidence in postmenopausal women using testosterone in addition to usual hormone therapy. *Menopause* 2004;11:531.
- [15] Thom MH, Collins WP, Studd JWW. Hormonal profiles in postmenopausal women after therapy with subcutaneous implants. *BJOG: An International Journal of Obstetrics & Gynaecology* 1981;88:426–33.
- [16] Traish AM, Gooren LJ. Safety of physiological testosterone therapy in women: lessons from female-to-male transsexuals (FMT) treated with pharmacological testosterone therapy. *The Journal of Sexual Medicine* 2010;7:3758–64.
- [17] Van Staa TP, Sprafka JM. Study of adverse outcomes in women using testosterone therapy. *Maturitas* 2009;62:76–80.
- [18] Braunstein GD. Safety of testosterone treatment in postmenopausal women. *Fertility and Sterility* 2007;88:1–17.
- [19] Gooren LJ, Giltay EJ, Bunck MC. Long-term treatment of transsexuals with cross-sex hormones: extensive personal experience. *Journal of Clinical Endocrinology & Metabolism* 2008;93:19–25.
- [20] Gooren LJ, Giltay EJ. Review of studies of androgen treatment of female-to-male transsexuals: effects and risks of administration of androgens to females. *The Journal of Sexual Medicine* 2008;5:765–76.
- [21] Segaloff A. Hormone therapy of breast cancer. *Cancer Treatment Reviews* 1975;2:129–35.

- [22] Davis SR, Van Der Mooren MJ, van Lunsen RHW, et al. Efficacy and safety of a testosterone patch for the treatment of hypoactive sexual desire disorder in surgically menopausal women: a randomized, placebo-controlled trial. *Menopause* 2006;13:387.
- [23] Davis SR, Moreau M, Kroll R, et al. Testosterone for low libido in postmenopausal women not taking estrogen. *New England Journal of Medicine* 2008;359:2005–17.
- [24] Kingsberg SA, Simon JA, Goldstein I. The current outlook for testosterone in the management of hypoactive sexual desire disorder in postmenopausal women. *The Journal of Sexual Medicine* 2008;5:182–93.
- [25] Glaser R, Dimitrakakis C, Trimble N, Martin V. Testosterone pellet implants and migraine headaches: a pilot study. *Maturitas* 2012;71:385–8.
- [26] Lobo RA. Androgens in postmenopausal women: production, possible role, and replacement options. *Obstetrical & Gynecological Survey* 2001;56:361.
- [27] Glaser R, Dimitrakakis C. Beneficial effects of subcutaneous testosterone on lipid profiles in women. In: 9th European Congress on Menopause and Andropause. 2012. A424:0009:00025.
- [28] Choi HH, Gray PB, Storer TW, et al. Effects of testosterone replacement in human immunodeficiency virus-infected women with weight loss. *Journal of Clinical Endocrinology & Metabolism* 2005;90:1531–41.
- [29] Carruthers M. The paradox dividing testosterone deficiency symptoms and androgen assays: a closer look at the cellular and molecular mechanisms of androgen action. *The Journal of Sexual Medicine* 2008;5:998.