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ORIGINAL ARTICLE

Monitoring testosterone replacement therapy with transdermal gel: when and how?

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Abstract

Purpose Testosterone replacement therapy (TRT) is recommended for the treatment of most cases of male hypogonadism. Transdermal testosterone (T) gels are commonly used in clinical practice; however, there is little evidence concerning how to monitor dosage to bring and maintain serum T levels in the normal physiologic range.

Methods We examined 30 hypogonadal patients undergoing treatment with 40 mg/day transdermal 2% testosterone gel. After a week from treatment onset, all patients underwent a total of four measurements to assess serum total T, bioavailable T and free T at +2 h (samples A and A′) and +23 h (samples B and B′).

Results No significant difference was found concerning total, free and bioavailable T between the two samples taken at the same time points (A vs A′ and B vs B′). A repeated-measures mixed effects regression model showed significantly lower serum levels of total, free and bioavailable T at +23 h compared to +2 h (total T, $\beta = -3.050 \pm 0.704$, $p < 0.001$; free T, $\beta = -85.187 \pm 22.746$, $p < 0.001$; bioavailable T, $\beta = -1.519 \pm 0.497$, $p = 0.003$) without a significant between-sample variability. Serum T > 3.5 ng/ml at +2 h was reached in 21/30 patients (70%), but only 11 (36.7%) still had adequate serum T at +23 h.

Conclusion Assessment of TRT with transdermal gels at its peak and at its minimum could be useful in providing a finely tailored treatment for hypogonadal men, both preventing supra-physiological levels and maintaining adequate concentrations through the day.

Keywords Testosterone replacement therapy · Monitoring · Male hypogonadism · Testosterone gel

Introduction

Male hypogonadism is classically defined as a clinical syndrome resulting from an impairment of the hypothalamic–pituitary–testicular axis, ultimately manifesting with the inability of the testis to produce physiological concentrations of testosterone and/or maintain normal spermatogenesis [1–4]. Given the significant daily fluctuations in serum testosterone levels, guidelines actually suggest that in no circumstance a single testosterone measurement should be considered adequate for diagnosis: at least two measurements on separate mornings in fasting state, using a reliable method, are therefore needed for diagnosis [1–3].

Testosterone replacement therapy (TRT) is recommended in most cases of testosterone deficiency (TD), as a means to improve the signs and symptoms resulting from impaired serum androgen levels [1–4]. Several formulations are available for TRT, including injectable esters, transdermal gels and patches, subcutaneous pellets and buccal bioadhesive tablets; each formulation has its own list of advantages and disadvantages, such as the fluctuation of testosterone levels for injectable preparations and the risk of interpersonal transfer for gels. Testosterone gels have also been preferred to i.m. injections in selected conditions, such as puberty induction, in which the shorter half-life of transdermal preparations can be an additional benefit for treatment [5].

In recent years, no topic in the field of andrology has been as debated as the side effects of TRT: several papers [6–9] have questioned whether undergoing TRT could increase

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the risk of severe cardiovascular events (for review, see [10]), and while more recent meta-analysis studies have proven otherwise [11–14], the warnings issued by the FDA still stand. However, the effects of TRT on sexual function, mood, body composition, metabolic profile and bone density have often been reported [4, 15, 16] and confirmed by the Testosterone Trials [17]. The aim of TRT is increasing serum testosterone levels, although there seems to be no definite consensus concerning the actual target for treatment. The EAU Guidelines on Male Hypogonadism [2] suggest that "there are as yet insufficient data to define optimal serum levels of testosterone during testosterone treatment" and that "testosterone treatment should restore the serum testosterone level to the mid-normal range of specific age groups of men", whereas the AUA Guidelines [3] suggest that clinicians should use the "minimal dosing necessary to drive testosterone levels to the normal physiologic range of 450–600 ng/dL", similarly to the Endocrine Society Guideline recommending "serum testosterone concentrations in the mid-normal range for healthy young men". These recommendations agree with the general consensus that supraphysiological serum testosterone levels should be avoided to minimize the risk of developing side effects [2, 15, 18].

An appropriate interval of treatment is needed before measuring serum testosterone to ensure the suggested target levels have been reached; however, measurement should be scheduled according to the formulation in use. Injectable testosterone esters have a longer half-life than transdermal preparations, which require daily application; guidelines suggest dosing serum testosterone midway through injections, and 2–8 h or 3–12 h following application of gels and patches, respectively [1].

In this study, we aimed to address two potential issues associated with the use of transdermal testosterone gel. First, given the fluctuations in testosterone levels, we investigated whether serum testosterone might differ between the two samples taken at the same time point among transdermal testosterone gel users. Second, we assessed serum testosterone concentrations at two time points, namely at + 2 h and + 23 h, to assess whether the correct dosage for + 2 h would result in inadequate testosterone concentration at + 23 h.

Materials and methods

Patients

30 patients with a diagnosis of hypogonadism were recruited for this study. Diagnosis was performed based on two measurements of serum testosterone: patients were considered hypogonadal with a serum testosterone level < 8 nmol/L or with a serum testosterone level < 12 nmol/L in the presence of specific signs and symptoms. Patients with any other endocrine or severe disease (including malnutrition as well as liver, kidney, neurological, pulmonary and cardiovascular affections), or with any contraindications to TRT (prostate/breast cancer, severe obstructive sleep apneas, or hematocrit > 54%) were excluded from the study [19]; likewise, patients undergoing treatment with drugs possibly interacting with TRT were excluded. Patients performing strenuous physical activity, such as endurance sports, were also excluded from the study: in these cases, hypogonadism is often the consequence of overtraining [20, 21], and TRT is not recommended. Given the availability of several TRT formulations, we also excluded all patients who were not willing to undergo treatment with transdermal gels. All study procedures and potential risks were explained to the participants and they provided written informed consent to participate in the study.

Testosterone replacement therapy

All patients recruited in the study received treatment with transdermal 2% testosterone gel. The Endocrine Society guidelines suggest starting treatment with 40–70 mg daily [1]: in this study, to minimize the risk of developing side effects associated with excessive dosage, all patients were treated with the minimum recommended dosage, i.e., 40 mg/day. For the duration of treatment, patients were asked to apply the gel to clean, dry skin every day at the same hour between 7:30 and 9:00 am, on the shoulders or upper arms. No titration was performed until unblinding of the serum testosterone levels at the end of the study.

Serum testosterone measurements

According to guidelines, a blood sample was taken shortly after gel application (sample A, + 2 h); additionally, to assess whether adequate concentrations of serum T would be maintained, we took another blood sample just before the application of a new dose of testosterone gel (sample B, + 23 h). To assess the possible intra-individual variation in serum T, both samples were taken twice, in two separate mornings: therefore, all patients underwent a total of four measurements (A and A′ at + 2 h, B and B′ at + 23 h). All samples were drawn in the morning, after a night's fasting; the first sample was taken after a week from treatment onset to ensure reaching of the steady state, and each following sample was taken after 7 days from the previous one. According to Guidelines, we also assessed SHBG and serum albumin to estimate free and bioavailable testosterone levels [22, 23]. Measurements were performed in the same laboratory, using commercial kits for immunoassays (testosterone: Orion Diagnostica Oy, Espoo, Finland, reference ranges 12.9–27.7 nmol/L, accuracy 0.1 nmol/L, intra-assay coefficient of variation (CV) 6%, inter-assay CV 6%;

SHBG: Orion Diagnostica Oy, Espoo, Finland, reference ranges 14–50 nmol/L, accuracy 1.3 nmol/L, intra-assay CV 3%, inter-assay CV 7%; albumin: Assaypro LLC, St. Charles, Missouri, USA, reference ranges 3.5–5.5 g/dl, accuracy 1.5 µg/mL, intra-assay CV 5.9%, inter-assay CV 9.5%); testosterone measurements were later converted to ng/ml (10 nmol/l = 2.88 ng/ml). All testosterone measurements were blinded until the end of the study. Hematocrit and serum PSA were also assessed at all time points.

Statistical analysis

All statistical analyses were performed using R version 3.5.0; data cleaning was performed with the *dplyr* package [24]. Data were tested for normality using a Kolmogorov–Smirnov test; a paired Wilcoxon signed-rank test with continuity correction was used for analysis of non-normally distributed data. A repeated-measures mixed effects model was drawn for multivariate analysis using the *nlme* package [25]. Additionally, a Chi square test was used to assess whether the starting dose would be equally efficacious at the two time points. Plots were drawn using the *ggplot2* package [26].

Results

General results

All recruited subjects completed the study. None of the subjects developed any significant side effects from testosterone gel. Mean age of the recruited patients was 51.75 ± 16.33 years; mean BMI was 26.12 ± 4.2 kg/m^2.

Intra-individual variability

Results from the four samples are reported in Table 1. Total, free and bioavailable testosterone did not show any significant changes between the two samples taken at the same time points (A vs A′ and B vs B′). Additionally, to prevent bias resulting from repeated statistical tests, a repeated-measures mixed effects regression model was applied to measure the differences between serum androgen levels at all time points (Table 2). As expected, serum levels of total, free and bioavailable testosterone were significantly lower at + 23 h compared to + 2 h (total testosterone, $\beta = -3.050 \pm 0.704$, $p < 0.001$; free testosterone, $\beta = -85.187 \pm 22.746$,

Table 2 Repeated-measures mixed effects regression models for multivariate assessment of serum androgens in 30 male subjects undergoing testosterone replacement therapy with 40 mg/day transdermal 2% testosterone gel

	β	SD	*p*
Total testosterone (ng/ml)			
(Intercept)	6.389	0.595	< 0.001
Time (+ 2 h vs + 23 h)	− 3.050	0.704	**< 0.001**
Sample (A–B vs A′–B′)	− 0.041	0.704	0.954
Interaction time: sample	− 0.504	0.995	0.614
Free testosterone (pg/ml)			
(Intercept)	156.006	18.725	< 0.001
Time (+ 2 h vs + 23 h)	− 85.187	22.746	**< 0.001**
Sample (A–B vs A′–B′)	− 6.765	22.505	0.764
Interaction time: sample	− 5.497	31.941	0.864
Bioavailable testosterone (ng/ml)			
(Intercept)	3.224	0.420	< 0.001
Time (+ 2 h vs + 23 h)	− 1.519	0.497	**0.003**
Sample (A–B vs A′–B′)	− 0.036	0.492	0.943
Interaction time: sample	− 0.323	0.698	0.644

Significant effects are highlighted in bold

Table 1 Intra-individual fluctuations in serum T in 30 male subjects undergoing testosterone replacement therapy with 40 mg/day transdermal 2% testosterone gel. Analysis performed using paired Wilcoxon signed-rank test with continuity correction

	+ 2 h		+ 23 h	
	Sample A	Sample A′	Sample B	Sample B′
Total testosterone (ng/ml)				
Mean ± SD	6.39 ± 4.15	6.35 ± 3.93	3.34 ± 2.53	2.79 ± 1.85
Median [IQR]	5.07 [3.54–9.35]	5.70 [2.61–9.64]	2.47 [1.56–4.22]	2.09 [1.70–3.79]
p	0.5716		0.2993	
Free testosterone (pg/ml)				
Mean ± SD	156.0 ± 139.0	149.0 ± 120.0	71.3 ± 60.3	59.1 ± 47.0
Median [IQR]	116 [73.7–187]	123 [52.7–178]	53.2 [31.4–110]	47.6 [27.6–75.3]
p	0.5387		0.5769	
Bioavailable testosterone (ng/ml)				
Mean ± SD	3.22 ± 3.11	3.19 ± 2.60	1.71 ± 1.45	1.35 ± 1.12
Median [IQR]	2.46 [1.07–4.44]	2.5 [0.896–4.09]	1.33 [0.74–2.60]	1.09 [0.62–1.88]
p	0.9019		0.1858	

$p < 0.001$; bioavailable testosterone, $\beta = -1.519 \pm 0.497$, $p = 0.003$); however, neither the between-sample variability, nor the interaction terms reached statistical significance. These findings are plotted in Fig. 1.

Inter-individual variability

21/30 patients (70%) reached average serum total testosterone > 3.5 ng/ml at + 2 h while undergoing treatment with transdermal 2% testosterone gel, 40 mg/day. At + 23 h, only 11 patients (36.7%) still had adequate serum total testosterone (Fig. 2). A Chi squared test was calculated comparing the frequency of low average serum total testosterone between the two time points: a statistically significant difference was found (X^2 (1) = 5.4241, $p = 0.0199$), consistent with our hypothesis that only a minority of subjects maintain adequate serum testosterone at + 23 h.

Discussion

Our results prove that a single measurement might be adequately representative of the efficacy of the TRT at any given time point. While two measurements are required for diagnosis of hypogonadism, the non-significant changes between the two samples taken at each of the two time points allows us to conclude that the absorption rate of transdermal 2% testosterone gel is almost constant for each individual. The same findings can be applied to both free and bioavailable testosterone. As suggested by existing guidelines, the assessment should be performed in a dedicated setting, as different commercial immunoassays kits could provide different results [27].

When using the minimum recommended dose, a significant percentage of patients failed to achieve an adequate

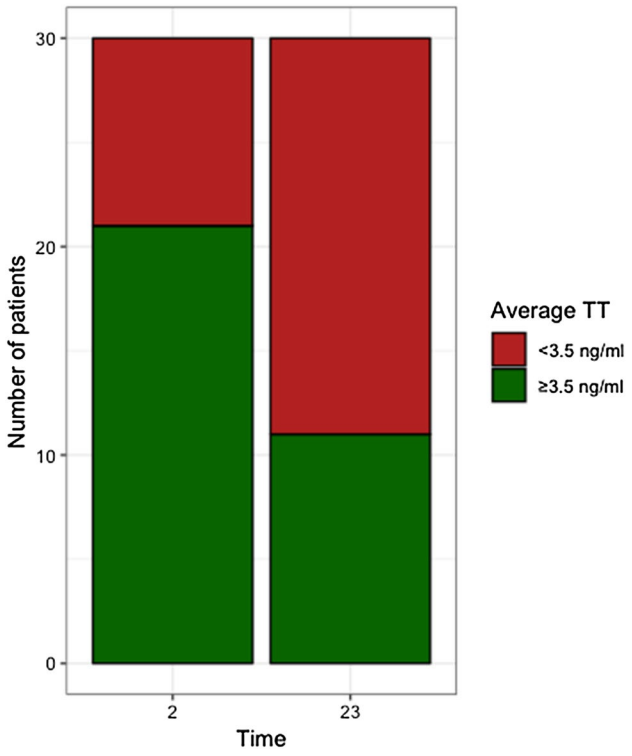

Fig. 2 Decreasing concentrations of average serum testosterone between + 2 and + 23 h

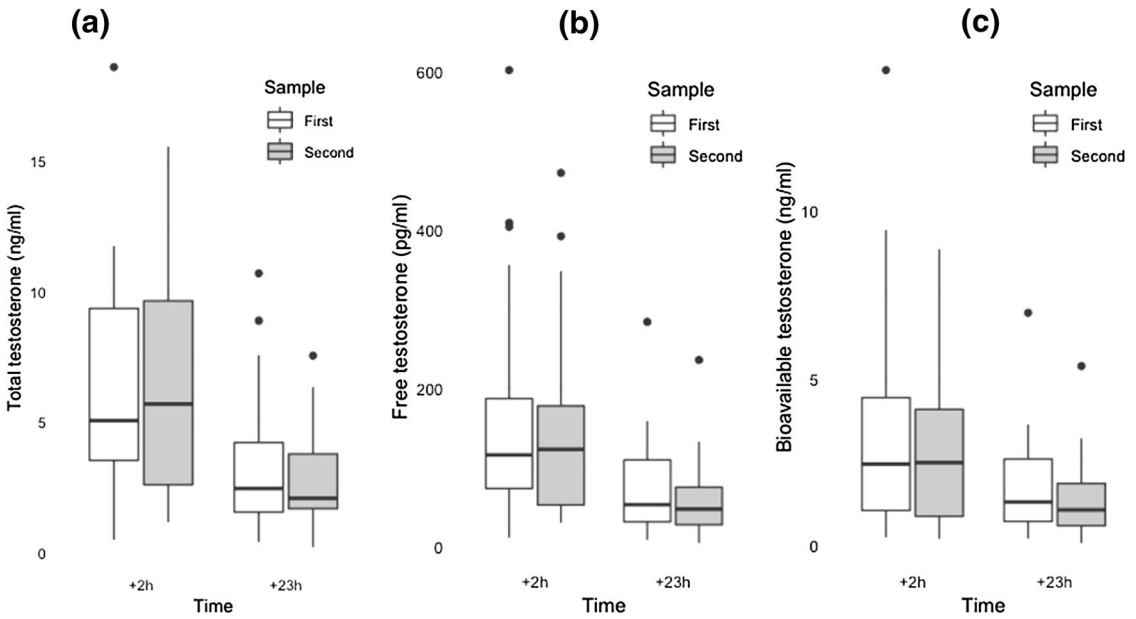

Fig. 1 Measurement of total (**a**), free (**b**) and bioavailable (**c**) testosterone concentrations at all time points

testosterone concentration. A higher dose of treatment is often required in obese or overweight subjects [28]; however, several other parameters, such as skin hydration, compliance and aromatase activity should be considered as well. Sexual activity has a bidirectional relationship with serum androgens: it has long been established that recovery of a "healthy" sexual activity, even with the support of pro-erectile drugs, can improve serum T even in the absence of TRT [29, 30]. In our study, we used the same dosage for all patients, despite differences in BMI, skin hydration and sexual activity, to have a "real-life" assessment of the efficacy of TRT. Additionally, we identified a significant inter-individual difference concerning the duration of TRT efficacy. A significant percentage of those patients who achieved an adequate serum testosterone concentration at + 2 h did not maintain their serum testosterone levels over the threshold of 3.5 ng/ml at + 23 h. While a decrease in serum testosterone was expected given the short duration of each gel application, the extent of this decrease could explain the reason for lack of improvement for some patients undergoing TRT. Guidelines suggest treatment discontinuation in subjects whose signs and symptoms do not improve while undergoing TRT [31]; however, our findings suggest that patients who are biochemically "normal" at + 2 h might have significantly lower serum testosterone concentration at a later assessment, therefore proving that persistence of symptoms might be a consequence of inadequate treatment. Additionally, while milder forms of erectile dysfunction could be treated by restoration to the eugonadal state [32, 33], it is well established that improvements in body composition, bone density and other signs and symptoms require weeks or months of treatment before being clinically appreciated [34, 35]: the frequent up and downs resulting from inadequate treatment might be a reason for impaired clinical response.

Strengths and limitations

Our study is, to our knowledge, the first one depicting in a real-life setting the daily changes in serum androgens following administration of a fixed dose of transdermal gel for TRT. Additionally, a post hoc power analysis has reported a statistical power of 0.942, which proves the relevance of our findings concerning the difference between the two time points. However, several confounding factors, such as BMI, skin hydration, sexual activity and aromatase activity were not considered in the analysis. While the "real-life" setting improves the reliability of our results, studies with larger population samples are required to adequately correct for these confounding variables.

Conclusions

The treatment of male hypogonadism is associated with improvements in several health outcomes, ranging from sexual symptoms to metabolic profile. Most patients undergo TRT; transdermal testosterone gels are often preferred by patients due to their ease of use and allow for quick treatment discontinuation if needed. Monitoring of hypogonadism is necessary, as TRT should increase serum testosterone without reaching supra-physiological levels; guidelines suggest assessing "peak" concentration, although we hypothesize that assessment of serum T at its lowest point—just before a new gel application—could be useful in assessing the correctness of the treatment dosage. In the authors' opinion, if only one measurement is feasible, the "lowest-point" measurement could be helpful in patients with no improvement despite treatment, whereas the "peak" measurement providing useful safety information remains a priority in all other patients. While two measurements are necessary for diagnosis, the little intra-individual variation could spare the necessity of a second measurement for subjects undergoing treatment with testosterone transdermal gels.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional ethics committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent All patients provided written informed consent for their participation.

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