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Pharmacokinetics of a transdermal testosterone cream in healthy postmenopausal women

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Abstract

Objective: The steady-state pharmacokinetics of two doses of a transdermal testosterone cream (TTC) was investigated after daily application for 21 days.

Methods: This was a two-way cross-over study conducted for 6 weeks. Seven healthy postmenopausal women (mean age, 59.3 y) were randomly allocated to 5 or 10 mg of TTC applied daily to the upper arm. Serum total testosterone (TT), free testosterone (fT), sex hormone–binding globulin, and metabolite concentrations were measured. Baseline-corrected and uncorrected serum TT and fT pharmacokinetic parameters ($AUC_{0\text{-}24}$, C_{avg}, C_{max}, and T_{max}) were calculated using a standard model-independent approach.

Results: After the single-dose application of 5 mg of TTC on day 22, the median uncorrected TT C_{avg} was found to be 0.54 ng/mL (range, 0.43-1.31), and the median uncorrected fT C_{avg} was found to be 4.14 pg/mL (range, 2.41-9.72). Doubling of the dose only resulted in a 30% increase in baseline-corrected TT C_{avg} (0.52 vs 0.69 ng/mL for 5 and 10 mg, respectively) and a 31% increase in baseline-corrected fT C_{avg} (4.75 vs 6.24 pg/mL for 5 and 10 mg, respectively). Neither dose resulted in any meaningful variation in dihydrotestosterone, estrone, estradiol, or sex hormone–binding globulin across the postdose sampling period.

Conclusions: The 5-mg TTC dose restores TT and fT levels to levels above and within the reference range, respectively, for premenopausal women.

Key Words: Testosterone replacement – Postmenopausal androgen therapy.

Several studies have shown that judicious testosterone therapy improves sexual desire and satisfaction and decreases distress in women with low sexual desire.[1-8] Improved general psychological well-being in women with low libido has also been reported in some studies.[5,8,9] Testosterone therapy may also have favorable effects on body composition,[10] bone,[11] cardiovascular health,[12] and cognitive performance[13] in postmenopausal women.

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Transdermal delivery of testosterone therapy is preferred because it avoids first-pass hepatic metabolism and has the potential to produce sustained and relatively constant plasma testosterone levels during a 24-hour period.[14] As a lipophilic steroid hormone, testosterone can readily permeate the stratum corneum barrier of the skin, transit the epidermis, and reach the dermal capillaries, where it is absorbed.[15] The testosterone patch, which has been shown to be effective in improving sexual function and in reducing distress, received restricted approval from the European Medicines Agency, but not from the Food and Drug Administration. However, it is no longer available in the market. Subcutaneous testosterone implants, which were the mainstay of therapy for women in the UK and Australia, are also no longer available. As a consequence, there is extensive prescribing of compounded testosterone and offlabel male testosterone formulations to women.[16]

A 1% transdermal testosterone cream (TTC; AndroFeme; Lawley Pharmaceuticals, Subiaco, Western Australia, Australia) has been approved for the treatment of testosterone deficiency in women in Western Australia. Pretreatment and steady-state testosterone levels in postmenopausal women treated with this preparation have been reported,[17] but a formal pharmacokinetic evaluation of the recommended treatment doses (5-10 mg/d) is lacking. In this study, we documented the steady-state pharmacokinetics of two doses of this TTC in healthy postmenopausal women. Besides profiling total testosterone (TT) and

free testosterone (fT), we also report the effects of treatment on dihydrotestosterone (DHT), estrone (E_1), estradiol (E_2), androstane-3α,17β-diol (3α-diol), androstane-3β,17β-diol (3β-diol), and sex hormone–binding globulin (SHBG) levels.

METHODS

This was a single-center, open-label, randomized, two-way cross-over pharmacokinetic study conducted at the Women's Health Research Program, Monash University (Melbourne, Australia).

Study population

We recruited healthy naturally postmenopausal women (aged 50-65 y) who were not using systemic hormone therapy. Vaginal estrogen use was permitted. Menopause was defined as the cessation of menstruation for more than 2 years and/or a follicle-stimulating hormone level greater than 30 IU/L. Participants were required to have a body mass index between 18 and 35 kg/m^2 and clinically acceptable mammogram and Papanicolaou test results within the preceding 2 years.

Women were excluded if they had had bilateral oophorectomy or if they had known allergy to almonds (a constituent of the cream), a chronic skin disorder (such as eczema or psoriasis) that is likely to interfere with transdermal drug absorption, intolerance to transdermal testosterone, a clinically significant infection requiring systemic antibiotics, undiagnosed vaginal bleeding, or evidence of clinical hyperandrogenism (hirsutism, androgenic alopecia, or acne, as assessed by the recruiting clinician). We also excluded women with a history of testosterone, tibolone, or dehydroepiandrosterone treatment or who were taking herbal remedies, grapefruit juice, or medications known to be strong inducers/inhibitors of cytochrome *P*450 3A4 or known to interact with testosterone metabolism or reproductive hormone levels. History of smoking within the past year, active substance abuse, or alcohol consumption of more than two standard drinks per day resulted in exclusion. Those who had donated blood within the last 6 weeks or plasma within 7 days of the first dosing in this study were also excluded. The study was approved by the Monash University Human Research Ethics Committee and was conducted in accordance with the Declaration of Helsinki. Participants were fully informed of the study procedures and provided a written informed consent form before study initiation.

Study design

The study involved a screening period of up to 4 weeks followed, in succession, by a 21-day treatment period for each dose with no washout. Each participant was randomized to apply 5 mg of TTC to the upper arm in one period and 10 mg of TTC daily in the other period. Blood was drawn before commencement of treatment at 8 AM. On day 22 of each period, the participants were admitted to the study center and had blood samples drawn before the morning dose (0 hour) and at 2, 4, 6, 8, 10, and 24 hours after the dose application.

Participants were asked not to ingest alcohol, caffeinated beverages, or chocolate, and not to engage in vigorous physical activity from 24 hours before admission to, and including, the time of discharge from the center. Blood samples were drawn from the arm that had not been the site of the TTC application. Serum samples were stored at −20°C until analyzed.

Study treatment

The TTC (AndroFeme; Lawley Pharmaceuticals) contains testosterone British Pharmacopoeia 10 mg/mL, dl-α-tocopherol acetate (vitamin E), and almond oil. The cream was dispensed in 50-mL tubes with an applicator marked with 0.5-mL graduations. Participants were instructed to measure the assigned dose using the applicator and to only apply the TTC to the upper arm and to massage it onto the skin until absorbed (approximately 30-60 s). The application of the first dose for each treatment period was administered under supervision. At this time, the women were instructed to apply the larger dose on a larger surface area of the upper arm. They were asked to refrain from washing the area for 8 hours after each application. The participants were instructed to record the time of the cream application in daily diary logs. On day 22 of each treatment period, the remaining cream was weighed. A participant was considered to be compliant with the study medication if more than 90% of the expected amount of cream was used during each study period.

Hormone measurements

Serum levels of TT, E_1, E_2, DHT, 3α-diol, and 3β-diol were measured by liquid chromatography–tandem mass spectrometry (LC-MS/MS) in the Andrology Laboratory of Professor David Handelsman at the ANZAC Research Institute (Sydney, Australia), as previously described.[18] The assay limits of detection, limits of quantification (LOQ), within-run coefficients of variation, and between-run coefficients of variation are as follows: TT, 0.01 ng/mL, 0.025 ng/mL, 2.0%, and 3.9% to 6.5%; DHT, 0.05 ng/mL, 0.1 ng/mL, 8%, and 6.7% to 13.4%; E_2, 2.5 pg/mL, 5 pg/mL, 6.6%, and 4.8% to 8.6%; E_1, 1.25 pg/mL, 2.5 pg/mL, 4.7%, and 4.6% to 7.5%. The limits of detection and LOQ for 3α-diol and 3β-diol are 0.05 and 0.1 ng/mL, respectively. SHBG was measured with a two-site directed immunofluorometric assay with 0.5 nm sensitivity and less than 0.1% cross-reactivity with other circulating proteins (Delfia-Wallac Inc, Turku, Finland). fT was calculated from TT, SHBG concentrations, and a fixed albumin concentration (43 g/L). Calculating fT using this method is a reliable index of bioavailable testosterone when the albumin concentration in individuals varies by as much as 25%.[19]

Pharmacokinetic analysis

Descriptive statistics, including means, medians, SDs, and coefficients of variation (%), were calculated for serum TT and fT concentrations at each nominal sample collection time point for each study treatment and dose-normalized (to a dose of 5 mg) for the 10-mg dose data.

The pharmacokinetics of 5 and 10 mg of TTC was based on serum TT, fT, baseline-corrected TT, and baseline-corrected fT data. Serum concentration data below the LOQ of the assay were assigned a value of 1/2 LOQ for the calculation of descriptive statistics and pharmacokinetic parameters and for the

graphical representation of concentration-time data. All times used in the pharmacokinetic analysis were actual times, with the exception of predose pharmacokinetic samples (0 h), which were assigned a nominal time of 0.000 hour.

Serum concentration-time data for each treatment were used in the calculation of pharmacokinetic parameters: area under the serum concentration-time curve ($AUC_{0\text{-}24}$), calculated using the linear-trapezoidal method; mean serum concentration during the dosing interval (C_{avg}), calculated as $AUC_{0\text{-}24}/24$; maximal observed (postdose) concentration (C_{max}), taken directly from the data without interpolation; and time of maximal serum concentration (T_{max}).

To assess the comparative pharmacokinetics of 5 and 10 mg of testosterone cream, we used a linear mixed-effects analysis of variance model to perform statistical comparisons on log-transformed, dose-normalized $AUC_{0\text{-}24}$, C_{avg}, and C_{max} data. Residual error (error mean square) was used to construct 90% CIs for the ratio of treatment means. Dose proportionality was concluded if the 90% CIs were within the limits of 80% to 125%. Two-sample Kruskal-Wallis test was used to assess treatment differences in untransformed T_{max} data. The level of significance was set at an α error level of 0.05.

Pharmacokinetic analysis was conducted by S.E.R. using Phoenix WinNonlin version 6.3 (Pharsight; a Certara company, Mountain View, CA). Statistical analyses were performed using Phoenix WinNonlin version 6.3 and MYSTAT 12 for Windows (SYSTAT Software Inc; San Jose, CA).

TABLE 1. *Baseline characteristics of the study participants (n = 7)*

Participant characteristics	Values
Age, mean (SD) [range], y	59.3 (2.9) [56-64]
Height, mean (SD) [range], cm	163 (6.0) [155-172]
Weight, mean (SD) [range], kg	67.3 (9.9) [52-78]
Body mass index, mean (SD) [range], kg/m^2	25.4 (4.3) [18-29.7]
Time since menopause, mean (SD) [range], y	6.9 (4.3) [3-16]
Hysterectomy, n	2
Vaginal estrogen use, n	3
Total testosterone, median [range], ng/mL	0.18[a] [0.04-0.29]
Free testosterone, median [range], pg/mL	1.3[a] [0.22-2.61]
3α,17β-Androstanediol, median [range], ng/mL	0.05[a] [ND-0.07]
3β,17β-Androstanediol, median [range], ng/mL	0.05[a] [ND-0.25]
Dihydrotestosterone, median [range], ng/mL[b]	0.05[a] [ND]
Estrone, median [range], pg/mL	9[a] [6-19]
Estradiol, median [range], pg/mL	2.5[a] [ND-3]
Sex hormone–binding globulin, median [range], nmol/L	132[a] [90-178]

To convert ng/mL into nmol/L (or pg/mL into pmol/L), multiply by 3.467. To convert dihydrotestosterone levels from ng/mL into nmol/L, multiply by 3.44. To convert estrone or estradiol levels from pg/mL into pmol/L, multiply by 3.671. ND, nondetectable.

[a]Plasma concentrations below the assay's limits of quantification (LOQ) were assigned a value of 1/2 LOQ. The LOQ for total testosterone, 3α,17β-androstanediol, 3β,17β-androstanediol, dihydrotestosterone, estrone, and estradiol were 0.025 ng/mL, 0.1 ng/mL, 0.1 ng/mL, 0.1 ng/mL, 2.5 pg/mL, and 5 pg/mL, respectively.

[b]All values were undetectable.

RESULTS

Of nine women screened, seven were enrolled and randomized. Two women did not provide 24-hour data for the 5-mg dose and another woman did not provide data for the 10-mg dose, such that five women provided 24-hour pharmacokinetic data for the 5-mg dose and six women provided 24-hour pharmacokinetic data for the 10-mg dose. The mean (SD) age of participants was 59.3 (2.9) years, and their mean (SD) body mass index was 25.4 (4.3) kg/m^2. The median baseline concentration of TT was 0.18 ng/mL (range, 0.04-0.29), and the median baseline concentration of fT was 1.3 pg/mL (range, 0.22-2.61). Further baseline characteristics of the study participants are presented in Table 1.

Pharmacokinetic outcomes

fT and TT

Serum concentration-time profiles for uncorrected serum TT and fT levels are displayed in Figures 1 and 2, respectively. After the single-dose application of 5 mg of TTC to the upper arm on day 22, the median peak level (C_{max}) of TT was found to be 0.75 ng/mL (range, 0.66-1.85) and that of fT was found to be 4.70 pg/mL (range, 3.64-12.80). For the 10-mg dose, the median C_{max} for TT and fT were 1.54 ng/mL (range, 0.33-3.08) and 12.6 pg/mL (range, 1.87-21.8), respectively.

Across the 24-hour blood sampling period, the median C_{avg} for TT were 0.54 ng/mL (range, 0.43-1.31) after the 5-mg dose and 0.91 ng/mL (range, 0.22-1.44) after the 10-mg dose. The median C_{avg} for fT observed across 24 hours after the 5- and 10-mg doses were 4.14 pg/mL (range, 2.41-9.72) and 7.24 pg/mL (range, 1.30-9.82), respectively.

We compared the pharmacokinetics of TT and fT between the 5-mg dose and the 10-mg dose (normalized to 5 mg; Table 2). For the 10-mg dose to result in twice the exposure of that of the 5-mg dose, the ratio of dose-normalized 10 mg to 5 mg should equal 1. Although there were no statistical differences in outcomes between the 5-mg dose and the dose-normalized 10-mg dose, the ratio of treatment means in all cases was approximately 0.6 to 0.8. Examination of mean baseline-corrected concentrations for each of the 5- and 10-mg doses (not dose-normalized) indicates that a doubling of dose only resulted in a 30% increase in TT C_{avg} (0.52 vs 0.68 ng/mL for 5 and 10 mg, respectively) and a 31% increase in fT C_{avg} (4.75 vs 6.24 pg/mL for 5 and 10 mg, respectively).

Other outcomes measures

All women had undetectable pretreatment DHT levels that became measurable with treatment. E_2 levels were undetectable at baseline in all women, except for one woman. Neither dose resulted in any meaningful variation in E_1, E_2, DHT, SHBG, or 3β-diol levels across the 24-hour postdose sampling period (data not shown). After administration of the 10-mg dose, median concentrations of 3α-diol increased from a median predose level of 0.05 ng/mL (range, 0.05-0.17) to a peak of 0.17 ng/mL

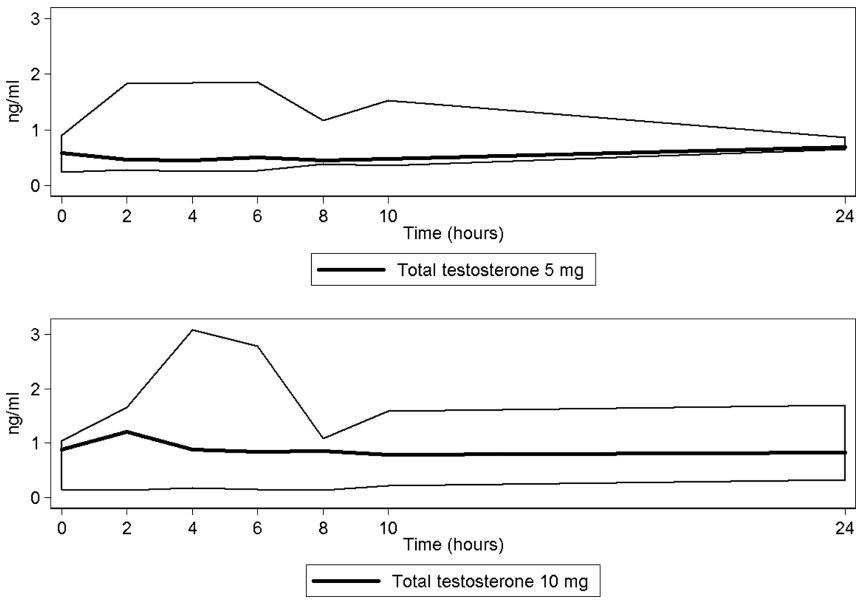

FIG. 1. Serum total testosterone concentrations (not baseline-corrected) at time 0 (pretreatment) and at 2, 4, 6, 8, 10, and 24 hours posttreatment with 5 and 10 mg of testosterone cream on day 22. The thick black line represents uncorrected median values, and the thin black lines depict minimal and maximal levels measured. To convert ng/mL to nmol/L multiply by 3.467.

(range, 0.05-0.25), coinciding with the increase in fT at this dose (data not shown).

Adverse events

The TTC was well-tolerated, with no androgenic adverse events or application site reactions. Three adverse events (headache, abdominal bloating, and constipation) were reported after treatment with the 10-mg dose, and one adverse event (headache) was reported after treatment with the 5-mg dose. Only headache was considered to be possibly related to the administration of testosterone. There were no clinically relevant changes in any vital sign measurements. The compliance rate was 100%.

DISCUSSION

Treatment of naturally postmenopausal women not taking concurrent systemic hormone therapy with 5 mg of TTC (formulated as AndroFeme) results in a median uncorrected C_{avg} for TT across 24 hours that is just above the upper limit of the range measured with LC-MS/MS in other studies of healthy premenopausal women (0.09-0.49 ng/mL) and in a median C_{avg} for fT that is within the reported range of 2.22 to

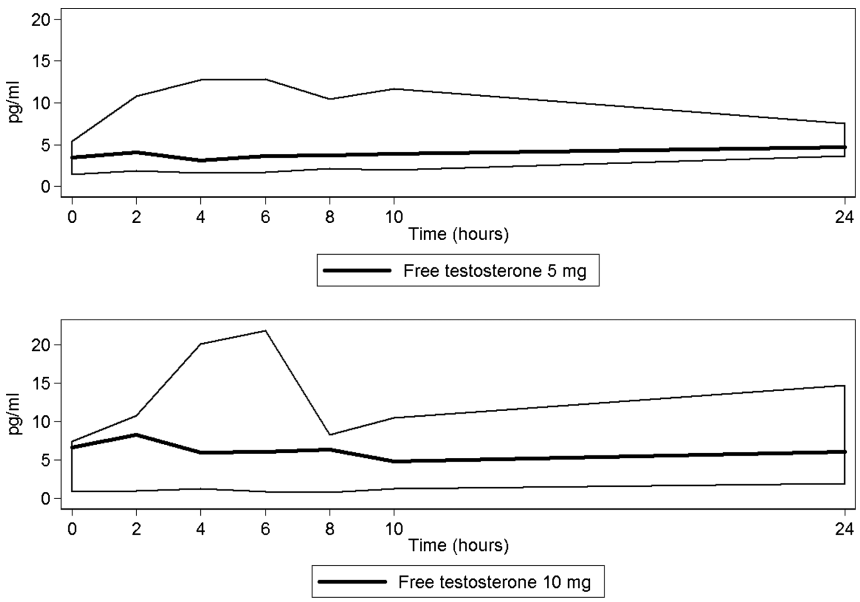

FIG. 2. Serum free testosterone concentrations at time 0 (pretreatment) and at 2, 4, 6, 8, 10, and 24 hours posttreatment with 5 and 10 mg of testosterone cream on day 22. The thick black line represents uncorrected median values, and the thin black lines depict minimal and maximal levels measured. To convert pg/mL to pmol/L multiply by 3.467.

TABLE 2. *Summary of baseline-corrected pharmacokinetic parameters*

Baseline-corrected parameters	Treatment[a]			
	5 mg (n = 5)	10 mg (dose-normalized 5 mg; n = 6)	*P*	Dose-normalized 10 mg/5 mg ratio[b]
Serum total testosterone[c]				
$AUC_{0\text{-}24,}$ ng h/mL	12.58 (7.76) [9.16; 8.77-26.46]	8.20 (4.21) [8.32; 2.12-14.83]	0.256	0.66 [0.34-1.27]
C_{avg}, ng/mL	0.52 (0.32) [0.38; 0.37-1.10]	0.34 (0.18) [0.35; 0.09-0.62]	0.256	0.70 [0.34-1.27]
C_{max}, ng/mL	0.80 (0.48) [0.63; 0.49-1.65]	0.69 (0.43) [0.66; 0.14-1.4]	0.563	0.79 [0.36-1.73]
T_{max}, h	20.4 (8.04) [24.0; 6.00-24.0]	10.3 (10.7) [5.04; 2.00-24.0]	0.316	
Serum free testosterone[c]				
$AUC_{0\text{-}24,}$ pg h/mL	113.94 (68.30) [95.21; 56.70-228.44]	74.89 (35.01) [84.78; 15.19-115.38]	0.195	0.64 [0.34-1.18]
C_{avg}, pg/mL	4.75 (2.85) [3.97; 2.36-9.52]	3.12 (1.46) [3.53; 0.63-4.81]	0.195	0.64 [0.34-1.18]
$C_{max,}$ pg/mL	6.50 (3.68) [4.58; 3.60-12.60]	5.97 (3.45) [6.19; 0.91-10.80]	0.544	0.77 [0.33-1.78]
T_{max}, h	20.4 (8.04) [24.0; 6.00-24.0]	10.7 (10.5) [6.00; 2.00-24.0]	0.316	

[a]Values are presented as mean (SD) [median; range].
[b]Values are presented as mean [90% CI].
[c]To convert ng/mL into nmol/L (or pg/mL into pmol/L), multiply by 3.467.

5.42 pg/mL.[20] Doubling of the study dose to 10 mg/day resulted in a median TT C_{avg} that was almost double the upper limit of reference levels in premenopausal women and in a median fT C_{avg} that was slightly above the upper limit of the reported premenopausal reference range. These data suggest that daily application of 5 mg of TTC is likely to restore fT levels to the premenopausal reference range but that higher doses will result in supraphysiological TT and fT levels.

The study participants' SHBG levels were within the middle to upper ranges for healthy women. About 66% of TT binds to SHBG with high affinity, and SHBG circulates with most of its binding sites unoccupied.[21] Therefore, although the C_{avg} for TT was elevated after TTC treatment, much of this reflects uptake of the administered testosterone by SHBG. The more appropriate clinical measure is fT concentration for which the C_{avg} after the 5-mg dose approximated that in premenopausal women. The flatness of TT and fT concentration profiles is consistent with a relatively uniform testosterone delivery during the 24-hour sampling period.

Although there was considerable variability in the data, doubling the dose of testosterone cream from 5 to 10 mg did not result in a baseline-adjusted doubling of serum TT and fT concentrations, indicating nonproportionality of the doses. The difference in serum TT and fT between the 5-mg dose and the 10-mg dose in this study was similar to the difference observed between transdermal testosterone patches delivering 150 and 300 μg/day.[16]

An important finding was the lack of impact of either dose of TTC on E_1 and E_2 levels, which remained within the LC-MS/MS ranges reported by Labrie et al[22] for postmenopausal women. DHT levels became detectable with both TTC doses but did not fluctuate in the 24-hour period after dosing and also remained within the reported postmenopausal range.[22] DHT is metabolized to 3α-diol and 3β-diol. The metabolite of 3α-diol (3α-diol-17-glucuronide) is believed to be a marker of peripheral androgen action and is significantly elevated in idiopathic hirsutism. In contrast, 3β-diol is thought to be only weakly androgenic.[23] Despite the absence of change in DHT across the 24-hour postdose sampling period, median 3α-diol levels increased after the 10-mg dose, with the profile parallel to that of fT. This suggests that the supraphysiological testosterone levels achieved with the 10-mg dose resulted in rapid metabolism of some of the administered testosterone, through DHT, to its 3α-diol metabolite. SHBG levels did not show any specific pattern of change across the study. It is noteworthy that there were random within-individual variations in SHBG levels during the study, which may reflect normal physiological variation in what is usually considered a stable plasma protein.

In line with previous studies of this testosterone cream,[8,24] application of the transdermal cream was well tolerated, and no participant withdrew from the study because of treatment-related adverse effects.

Major strengths of our study include quantification of the sex steroids with LC-MS/MS and our ability to measure androgenic and estrogenic metabolites of testosterone simultaneously. LC-MS/MS is considered to be superior to conventional immunoassays[25] in assessing low sex hormone concentrations, especially in women.[18,26,27]

The main study limitation is our small sample size.

In studies that are highly demanding of participants, the sample size is often small.[28] All participants in our study were fully compliant (based on weighing of the returned study drug). We do not believe that the fundamental findings would have been different had the sample size been slightly greater (eg, 10 women rather than 7 women).

CONCLUSIONS

One percent TTC (formulated as AndroFeme), when applied daily as a 5-mg dose to the upper arm of naturally postmenopausal women not using concurrent systemic hormone therapy, restores TT and fT levels to levels above and within the reference range, respectively, for premenopausal women.

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