Pharmacokinetics of a Testosterone Gel in Healthy Postmenopausal Women

Atam B. Singh, Martin L. Lee, Indrani Sinha-Hikim, Mark Kushnir, Wayne Meikle, Al Rockwood, Sebhat Afework, and Shalender Bhasin

Division of Endocrinology, Metabolism, and Molecular Medicine, Drew-University of California-Los Angeles Reproductive Science Research Center, Charles R. Drew University of Medicine and Science (A.B.S., M.L.L., I.S.-H., S.A., S.B.), Los Angeles, California 90059; and ARUP Institute for Clinical and Experimental Pathology (M.K., W.M., A.R.), Salt Lake City, Utah 84108

Background: The paucity of pharmacokinetic data on androgen formulations in women has hindered clinical trials of testosterone supplementation in women.

Objective: The objective of this study was to determine the time course and profile of serum testosterone concentrations during treatment with different doses of testosterone gel in postmenopausal women and assess whether estrogen treatment affects the pharmacokinetics of testosterone gel.

Methods: Postmenopausal women with total testosterone levels less than 33 ng/dl after baseline 24-h sampling were treated with 4.4, 8.8, or 13.2 mg testosterone gel daily for 7 d each in random order, with a 7-d washout period between doses. We studied 13 women who had not received estrogen therapy (group I) and 13 who had received stable estrogen therapy for 3 months or more (group II). Total and free testosterone concentrations were measured for 48 h on the seventh day of each dose administration.

Results: Twenty-six women were randomized; of these, 24 were evaluable, 13 in group I and 11 in group II. The average steady-state concentrations (Cav) of serum total and free testosterone increased with increasing testosterone dose and were highly correlated with the dose (dose effect, $P < 0.00001$), but were not affected by estrogen therapy ($P = 0.43$). In both groups, the 4.4-mg dose increased Cav total and free testosterone into the mid- to high-normal range, whereas 8.8- and 13.2-mg doses raised total (Cav: 22.3, 51.6, 80.3, and 92.0 ng/dl in group I; 22.7, 59.8, 82.0, and 114.3 ng/dl in group II at 0, 4.4, 8.8, and 13. 2 mg, respectively) and free testosterone (5.9, 8.4, 11.5,12.8 pg/ml in group I and 5.0,7.6,11.1,10.8 in group II, respectively, at the various doses) above the physiological range. The area under the curve, maximum and minimum concentrations, and the change in Cav for total and free testosterone were dose related and significantly higher during administration of the 13.2-mg dose than during the 0- or 4.4-mg dose; estrogen therapy had no significant effect on these measures. Serum estradiol, LH, FSH, and SHBG levels did not change significantly at any dose. Testosterone gel was well tolerated.

Conclusions: Administration of testosterone gel to postmenopausal women raised total and free testosterone concentrations in proportion to the administered dose without affecting estradiol levels. A 4.4-mg dose raised testosterone levels into the mid- to high-normal range. Previous estrogen therapy had no significant effect on testosterone pharmacokinetics over this short duration. (*J Clin Endocrinol Metab* **91:** 136–144, 2006)

I N HEALTHY, MENSTRUATING women, the ovaries and adrenal glands contribute to the production of approximately 300 μg testosterone daily (1–4). However, the physiological role of testosterone in women remains unclear (4–6). It has been speculated that androgen deficiency in older women and women with chronic illness may lead to impairment of sexual function, muscle mass and performance, cognitive function, and bone mass (5–14); however, this premise has not been substantiated. Several methodological issues have hindered efficacy trials of testosterone in women. Most of the available androgen formulations were designed to deliver much higher doses of testosterone to hypogonadal men. In addition, information about the pharmacokinetics of androgen formulations in women has been lacking. The de-

velopment of the testosterone gel and transdermal patches for women has made it possible to provide physiological testosterone replacement in women (15, 16), although these formulations have not been approved for clinical use. Another hurdle in conducting testosterone trials in women has been suboptimal sensitivity, precision, and accuracy of assays for the measurement of total and free testosterone concentrations in women (6). We used a sensitive liquid chromatography tandem mass spectrometry method for the measurement of total testosterone levels in women (17). Using this method, which is considered the gold standard, we performed a dose-finding, pharmacokinetics study with a testosterone gel for women.

The first objective of this pharmacokinetics study was to determine the time course and profile of testosterone concentrations during short-term (7 d) treatment with the testosterone gel in postmenopausal women. The second objective was to determine the relationship between the dose of testosterone gel and the increments in serum total and free testosterone concentrations and find the dose(s) of this gel that would be associated with physiological testosterone levels in postmenopausal women. We also determined whether estrogen therapy

alters the profile of testosterone concentrations after application of testosterone gel. Estrogens increase SHBG concentrations and may affect plasma clearance of testosterone (18).

We studied two groups of healthy, postmenopausal women: those who were not receiving estrogen therapy, and those who were receiving a stable estrogen plus progesterone regimen. We hypothesized that increasing doses of testosterone gel will produce a dose-dependent increase in serum total and free testosterone concentrations in postmenopausal women. We also hypothesized that estrogen administration might alter the plasma clearance of testosterone and the resulting serum total and free testosterone concentrations by increasing the SHBG concentration.

Subjects and Methods

Study design

The institutional review board for the protection of human subjects at Charles R. Drew University approved the protocol.

Participants

We recruited healthy, postmenopausal women, 51 yr of age or older. Menopause was defined as the cessation of menstruation for more than 1 yr and/or an FSH level greater than 30 U/liter in women who were not receiving estrogen therapy. The participants had to have serum testosterone levels (0700–0900 h) below 33 ng/dl, the median for menstruating women in our laboratory, and a normal PAP smear and mammogram in the preceding 12 months. The normative range for menstruating women was established by sampling menstruating women daily for 3 consecutive months (19). We recruited two groups of women: group I included 13 postmenopausal women who had not been receiving hormone therapy for at least 3 months, and group II included 13 postmenopausal women who had been treated for at least 3 months with a stable estrogen regimen.

Women who had undergone oophorectomy were not eligible for the study. We excluded women with any illness, cancer, diabetes mellitus (fasting blood glucose, >126 mg/dl), and blood pressure greater than 160/100 mm Hg. We also excluded women with body mass index above 35 kg/m², current use of illicit drugs, or heavy intake of alcohol (more than three drinks each day) with liver disease or other medical complications of alcohol abuse. Also excluded were women with AST, ALT, or alkaline phosphatase value more than three times the upper limit of normal or bilirubin levels greater than 2 mg/dl. Other exclusion criteria included breast or endometrial cancer and hyperandrogenic disorders, such as hirsutism and polycystic ovary disease. Those who had previ-

ously experienced intolerance to transdermal formulations or had received within the past 3 months drugs known to affect testosterone production or metabolism, such as ketoconazole, megesterol acetate, and androgenic steroids, were excluded.

Testosterone gel for women

Testosterone gel for women is a hydroalcoholic gel that contains 1% testosterone, ethyl alcohol, carbomer, and water (Solvay Pharmaceuticals, Marietta, GA). The gel was supplied in 60-ml bottles with a pump; each actuation of the pump dispensed 0.44 g gel containing 4.4 mg testosterone. The pumps met or exceeded specifications for clinical supplies required by FDA. The gel was applied to the lateral surface of the thigh within the shorts line. The subjects were advised to wash hands after gel application and not to shower for 6 h after gel application. Three doses of testosterone gel were studied: 4.4, 8.8, and 13.2 mg daily.

Study design

The study consisted of a screening period, a 24-h baseline sampling period, and a 5-wk treatment period.

Screening. A medical history was obtained from each subject, and a physical examination was performed. Blood was drawn for complete blood counts, chemistries, and serum total and free testosterone and FSH levels to determine eligibility. Previous menstrual history, the time of cessation of menstruation, and the status of estrogen therapy were determined. All medications were recorded.

Control period On a control day (d 1) when no gel was applied, 7.5-ml blood samples were drawn at 0, 2, 4, 6, 8, and 24 h at the Clinical Research Center, starting between 0700 and 0900 h. Blood counts, chemistries, and lipid and hormones concentrations were measured.

Treatment period. After completion of baseline sampling, each of the three doses of testosterone gel was applied by each subject for 7 consecutive days. The doses were administered in random order, and there was a 1-wk washout period between doses.

Blinding. In this double-blind, pharmacokinetics study, the investigators and participants were unaware of the dose being used.

Blood sampling. Upon admission to the Clinical Study Center on d 8, 22, and 36, medication use was verified, adverse events were recorded, testosterone gel was applied between 0700 and 0900 h, and 7.5 ml blood samples were drawn at 0, 2, 4, 6, 8, 24, 28, 32, 36, and 48 h after gel application. To assure that hormone concentrations had returned to baseline at the end of the washout period, we measured hormone concentrations on d 15 and 29, which coincided with the end of 7-d washout period and represented the first day of administration of the next dose.

TABLE 1. Baseline characteristics of the participants

	Group I	Group II
No. of women	13	13
Age (yr)	57 ± 2	58 ± 2
Height (cm)	156 ± 2	159 ± 2
Body weight (kg)	69 ± 3	70 ± 2
Body mass index (kg/m²)	28 ± 1	27 ± 1
Ethnicity		
White	0	5
Hispanic	6	2
African-American	6	4
Asian	1	2
Duration of HRT (yr)		8 ± 2
Type of HRT		Conjugated equine estrogen (0.625 mg) plus medroxyprogesterone acetate (2.5 mg), n = 11; conjugated equine estrogen (0.625 mg) alone, n = 1;[a] estrogen patch alone;[a] n = 1
Time since menopause (yr)	12 ± 2	12 ± 3

Group I included 13 healthy postmenopausal women who had not been receiving estrogen therapy (HRT) for at least 3 months, and group II included 13 postmenopausal women who had been treated for at least the preceding 3 months with a stable estrogen regimen.

[a] These two women had undergone hysterectomy without the removal of their ovaries. Hence, they were receiving estrogen therapy without a progestational agent.

Outcome measures

The primary outcome measures were serum total and free testosterone levels. In addition, we measured estradiol, LH, FSH, and SHBG levels at selected time points. Local skin reactions at the application site and other adverse events were evaluated.

Hormone measurements. Serum total testosterone levels were measured by liquid chromatography-tandem mass spectrometry (17). We added 10 μl internal standard d3-testosterone to 100-μl aliquots of standards or serum (17). The samples were extracted with methyl-*tert*-butyl ether and redissolved for derivatization in 300 μl hydroxylamine solution (1.5 mol/liter; pH 9). The derivatized testosterone was extracted with solid phase extraction columns (Strata X, Phenomenex, Torrance, CA) and eluted with methyl-*tert*-butyl ether and residue reconstituted in the mobile phase and analyzed on a triple-quadrupole mass spectrometer API 3000 OO (Applied Biosystems/MDS Sciex, Foster City, CA) equipped with a Turboionspray ionization source. The chromatographic separation was performed on 50×2.0-mm Phenomenex Luna C_{18}, 5-μm particle columns. The mobile phase consisted of 70% methanol and 30% water containing 22 mmol/liter formic acid. Mass transitions monitored for testosterone were m/z 304 to 124 and 304 to 112; for d3-testosterone they were m/z 307 to 124 and 307 to 112. The calibration in conjunction with the intensity of the transitions of internal standards was used to calculate testosterone concentrations in unknown samples. The limit of detection was 0.017 nmol/liter (0.5 ng/dl), and intra- and interassay coefficients of variation were less than 11.8% and 5.9% at concentrations below 1.05 nmol/liter and 6.2% and 7.9%, respectively, at higher concentrations.

Free testosterone levels were measured by a sensitive equilibrium dialysis method (19), optimized to measure low concentrations with precision and accuracy. Two hundred microliters of serum in the inner compartment was dialyzed against 2.4 ml dialysis buffer that approximates the composition of a protein-free ultrafiltrate of human serum (18). Dialysis was performed overnight for 16 h at 37 C. The sensitivity of the free testosterone assay was 0.6 pg/ml (2.0 pmol/liter), and intra- and interassay coefficients of variation were 4.2% and 12.3%, respectively. Serum LH, FSH, and SHBG levels were measured by two-site-directed, immunofluorometric assays (Delfia-Wallac, Gaithersburg, MD), with sensitivities of 0.05 U/liter, 0.15 U/liter, and 6.25 nmol/liter, respectively, as previously described (16). The intra- and interassay coefficients of variation were 10.7% and 13.0% for LH, 3.2% and 11.3% for FSH, and 10.0% and 10.2% for SHBG, respectively. Serum estradiol levels were measured by a RIA with a sensitivity of 2.5 pg/ml, and intra- and interassay coefficients of variation were 8% and 10%, respectively.

Pharmacokinetic modeling. Baseline and pharmacokinetic parameters were averaged across subjects within each group to obtain means, SDs, and SEMS. The bioavailability of testosterone was described as the area under the curve (AUC). We evaluated the following pharmacokinetic
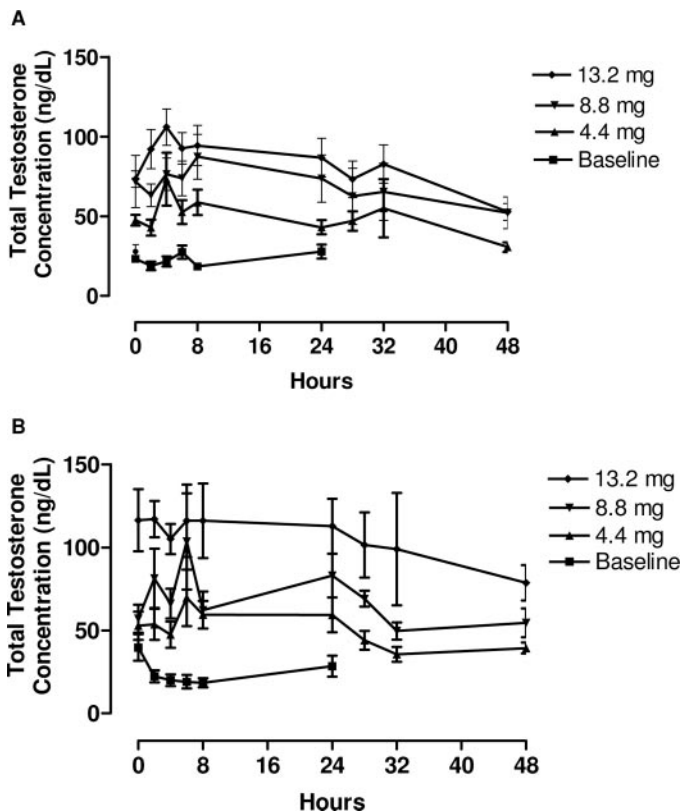

FIG. 1. Serum total testosterone concentrations during baseline sampling or the administration of different doses of testosterone gel in postmenopausal women. After the 24-h baseline sampling, the subjects were asked to apply one of three doses of testosterone gel (4.4, 8.8, or 13.2 mg) in a random order. After daily application of a specific dose of the testosterone gel for 6 d, the subjects were admitted to the Clinical Research Center. Testosterone gel was applied under supervision, and blood samples were obtained over the next 48 h. Data are the mean ± SEM. A, Serum total testosterone concentrations after application of the placebo or different doses of testosterone gel in postmenopausal women who were not receiving estrogen therapy; B, serum total testosterone concentrations after application of the placebo or different doses of testosterone gel in postmenopausal women who were receiving estrogen therapy.
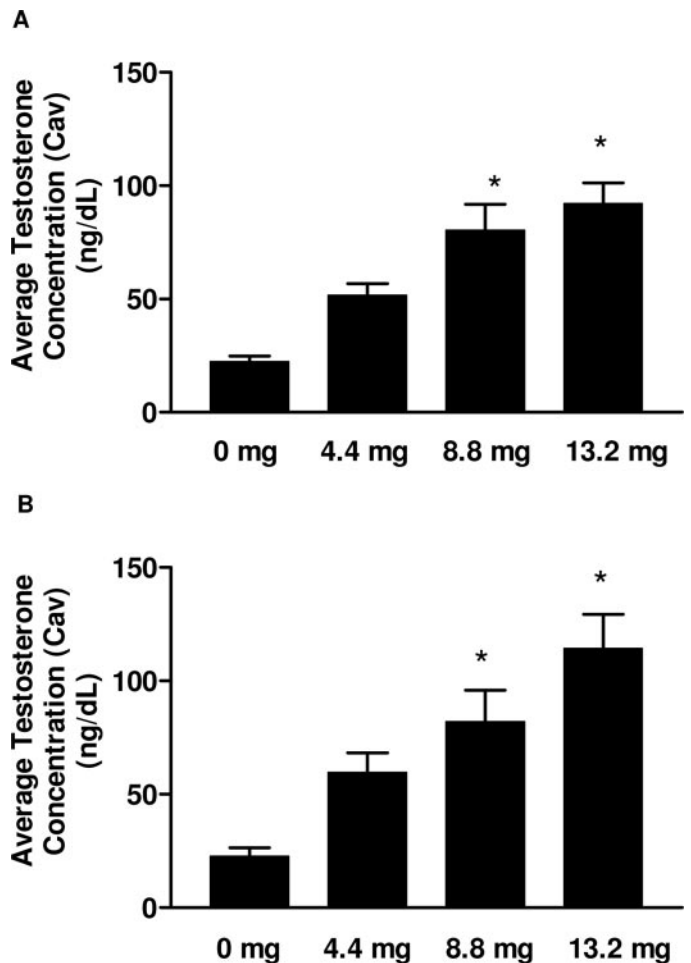

FIG. 2. Serum average total testosterone concentrations in postmenopausal women at baseline and during the administration of different doses of testosterone gel. Data are the mean ± SEM. A, Cav in postmenopausal women who were not receiving estrogen therapy; B, data from women who were receiving estrogen therapy. *, $P < 0.001$ compared with baseline (0-mg dose) or 4.4-mg dose.

parameters from the 24-h profiles of free and total testosterone measured on d 8, 22, and 36: time-average, steady-state concentration (Cav), maximum concentration (Cmax), minimum concentration (Cmin), time of maximum concentration (Tmax), time of minimum concentration (Tmin), and AUC. The Cav was computed by dividing the 24-h AUC by 24 h. For assessment of increments in serum testosterone levels above baseline, we subtracted the baseline 24-h AUC from the AUC on specific treatment days; the change in AUC was divided by 24 h to obtain the increment in average testosterone concentrations (ΔCav).

Statistical analysis

There were two treatment groups: postmenopausal women who received testosterone gel alone and were not receiving estrogen therapy, and postmenopausal women who were receiving a stable estrogen regimen that included premarin and medroxyprogesterone. Different doses of testosterone gel were administered in random order. Therefore, we used a three-way mixed model ANOVA with testosterone dose (0, 4.4, 8.8, or 13.2 mg daily), estrogen (yes and no), and time (hours) after gel application as the three factors. A patient effect (nested within treatment) was included as a covariate in the model. To simplify the estimation process, the three-way interaction among estrogen treatment, dose, and time was assumed to be zero, but all two-way interactions were used in the analysis, including the appropriate interactions with the nested patient effect. If an F test revealed a significant effect, then differences between individual groups were analyzed using the Tukey-Kramer multiple comparison procedure. To meet the distributional assumptions of the ANOVA model, serum hormone concentrations were log-transformed before analyses. The analysis of the pharmacokinetic parameters across dose arms used a two-way, repeated measures ANOVA, where estrogen treatment was the treatment variable, and dose was the repeated factor. The Tukey-Kramer multiple comparison procedure was used for analyzing any significant dose effects.

Results

Baseline characteristics of the subjects

Twenty-six women were enrolled and randomized in the study; all women completed the study. However, in two women, blood samples were contaminated with testosterone

gel during processing; data from these two women were excluded from the analyses. Thus, 24 women were evaluable: 13 in group I and 11 in group II. The two groups of women were not significantly different in terms of age, body weight, height, body mass index (Table 1), and baseline total and free testosterone levels. Serum estradiol and SHBG levels were higher, and LH and FSH levels were lower in women who were receiving estrogen therapy than in those who were not.

Testosterone gel tolerability

Testosterone gel was generally well tolerated. In group I, one subject reported acne, one reported increased hair growth, and one experienced mild leg swelling. In group II, one subject reported mild erythema at the gel application site, one reported oiliness of skin, two reported acne, four reported breast tenderness, and three reported vaginal spotting. There were no significant changes in hemoglobin, aspartate aminotransferase, and alanine aminotransferase during treatment (data not shown).

Hormone levels

Total testosterone. Serum total testosterone concentrations on d 2, 15, and 29 (at the beginning of each treatment period) were not significantly different from baseline values regardless of testosterone dose ($P = 0.343$); thus, the 7-d washout period was adequate to prevent a carryover effect.

The ANOVA revealed a significant testosterone dose effect on total testosterone concentrations ($P = 0.000001$); however, there was no significant effect of estrogen therapy ($P = 0.43$) on Cav (Figs. 1 and 2). In women receiving estrogen as well as in women not receiving estrogen, total testosterone concentrations at all doses were significantly different from one

TABLE 2. Pharmacokinetic parameters derived from total testosterone concentrations after administration of 0, 4.4, 8.8, or 13.2 mg testosterone gel in postmenopausal women who were or were not receiving estrogen replacement therapy

A. Women not receiving estrogen therapy	Testosterone dose (mg)				P
	0	4.4	8.8	13.2	
Cmax (ng/dl)	33.9 ± 4.3	81.7 ± 14.2	111.0 ± 15.8	117.9 ± 11.4	<0.00001
Cmin (ng/dl)	16.6 ± 2.2	35.9 ± 2.7	53.1 ± 9.0	67.1 ± 10.1	<0.000006
Cav (ng/dl)	22.4 ± 2.6	51.7 ± 5.2	80.3 ± 11.5	92.1 ± 9.2	<0.000001
Tmax (h)	9.8 ± 3.1	7.2 ± 2.1	6.9 ± 2.3	7.3 ± 2.4	0.55
Tmin (h)	4.7 ± 0.8	10.5 ± 2.7	13.2 ± 3.0	10.5 ± 3.0	0.046
AUC$_{0-24}$ h (h·ng/dl)	537.3 ± 62.5	1239.6 ± 125.2	1927.7 ± 276.4	2210.0 ± 220.8	<0.000001
ΔCav (ng/dl)		28.0 ± 6.8	50.6 ± 10.6	69.7 ± 10.5	<0.001
FI	0.82 ± 0.20	0.77 ± 0.17	0.72 ± 0.10	0.58 ± 0.08	0.94

B. Women receiving estrogen therapy	Testosterone dose (mg)				P
	0	4.4	8.8	13.2	
Cmax (ng/dl)	30.5 ± 6.6	81.8 ± 14.6	114.8 ± 22.2	150.2 ± 15.3	<0.000001
Cmin (ng/dl)	17.1 ± 2.3	39.3 ± 6.0	60.6 ± 11.1	81.0 ± 12.3	<0.000001
Cav (ng/dl)	22.8 ± 3.7	59.8 ± 8.6	82.0 ± 13.9	114.3 ± 15.1	<0.000001
Tmax (h)	5.6 ± 2.8	10.2 ± 3.1	15.0 ± 3.0	6.5 ± 2.7	0.20
Tmin (h)	6.0 ± 1.9	6.0 ± 2.2	4.4 ± 2.3	8.3 ± 3.6	0.73
AUC$_{0-24}$ h (h·ng/dl)	546.6 ± 89.5	1435.3 ± 207.4	1969.6 ± 334.7	2743.8 ± 361.3	<0.0000001
ΔCav (ng/dl)		38.5 ± 8.0	60.8 ± 12.5	91.0 ± 14.3	0.001
FI	0.48 ± 0.10	0.65 ± 0.11	0.68 ± 0.15	0.67 ± 0.14	0.94

Data are the mean ± SEM. FI, Fluctuation index (the ratio of the lowest to the highest testosterone concentration over the 24-hour period after gel application, a measure of the uniformity of testosterone release over the 24-h treatment interval). *P* values reflect the dose effect.

another at a joint significance level of 0.05 with mean testosterone levels in numerical order by increasing dose level. Thus, testosterone concentrations were higher during treatment with 8.8- and 13.2-mg doses than at baseline or during administration of 4.4 mg testosterone gel (Fig. 2). Administration of the 4.4-mg dose increased total Cav into the mid- to mid-high range for menstruating women, whereas 8.8- and 13.2-mg doses increased Cav above the upper limit of the physiological range. Overall, average total Cav did not change significantly during the 24-h sampling period at any dose (Fig. 1). Total testosterone concentrations started to decline between 24 and 36 h after gel application.

There was no significant effect of estrogen therapy on testosterone concentrations during the 24-h period ($P = 0.11$). There was no significant interaction effect of testosterone dose and estrogen therapy on total testosterone levels ($P = 0.82$).

The Cav, Cmax, and Cmin total testosterone and $AUC_{0-24\ h}$ during the 24-h gel application period were significantly higher during administration of the 13.2-mg dose than at baseline or during administration of the 4.4-mg dose. However, there were no significant differences in Cav, Cmax, Cmin, or $AUC_{0-24\ h}$ between women who were receiving estrogen therapy and those who were not (Table 2). Tmax and Tmin were not significantly different among the three dose groups. The ΔCav was proportional to the administered dose (Table 2).

To determine the average increment in testosterone concentrations after application of each milligram of testosterone transdermally, we performed regression between testosterone dose and ΔCav. This analysis revealed that increments in serum testosterone levels were highly correlated to testosterone dose (group I: $r^2 = 0.47$; $P < 0.0001$; group II: $r^2 = 0.57$; $P < 0.0001$) and increased by an average 5.5 ng/dl (95% confidence interval, 5.50–5.55 ng/dl) for each milligram of testosterone applied transdermally in women who were not receiving estrogen therapy and 6.7 ng/dl (95% confidence interval, 4.78–8.70) in women who were receiving estrogen therapy.

Free testosterone. Free testosterone concentrations were not significantly different from baseline levels at the beginning of each treatment period regardless of the testosterone dose ($P = 0.857$), confirming that the 7-d washout period between doses was adequate to prevent a carryover effect.

After application of the testosterone gel, free testosterone concentrations increased in proportion to the administered dose (Fig. 3; dose effect, $P = 0.0003$). However, there was no significant effect of estrogen therapy ($P = 0.96$) on free testosterone concentrations. The Tukey-Kramer multiple comparison analysis revealed that free testosterone concentrations after administration of the 8.8- and 13.2-mg doses were significantly higher than those at baseline or during administration of the 4.4-mg dose, but were not significantly different from each other. There was no estrogen treatment effect on free testosterone concentrations at any dose. Also, free testosterone concentrations did not differ significantly at different time points over a 24-h period.

The Cav, Cmax, Cmin, and $AUC_{0-24\ h}$ for free testosterone concentrations during the 24-h period increased in proportion to the administered dose and were significantly higher in those receiving the 13.2-mg dose than at baseline or during
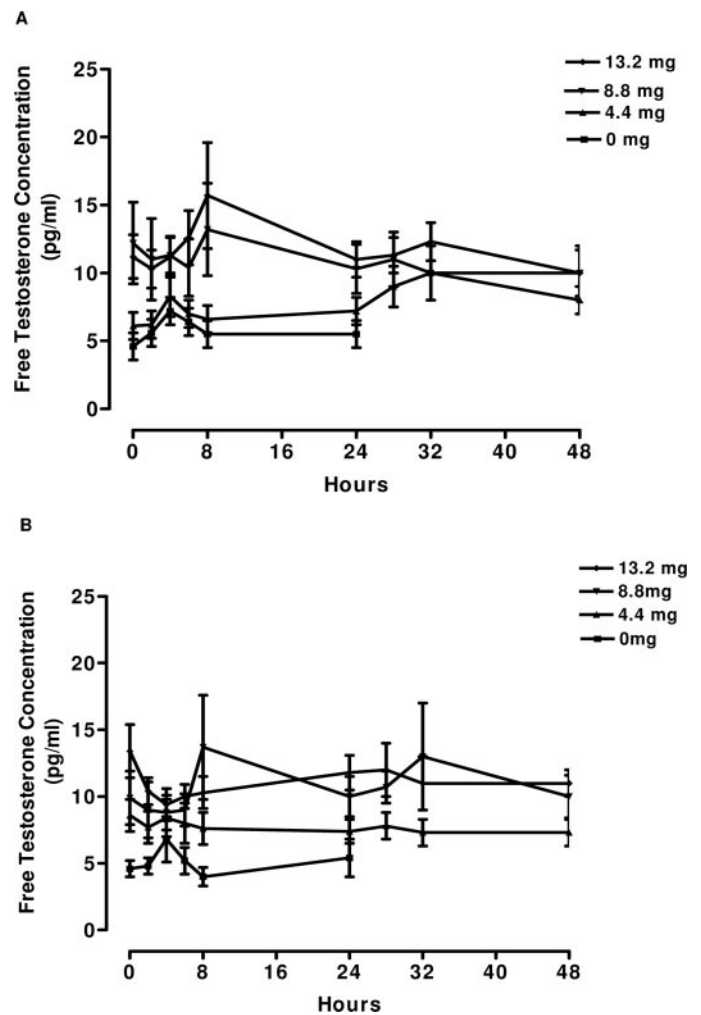

FIG. 3. Serum free testosterone concentrations at baseline or during administration of different doses of testosterone gel in postmenopausal women. After baseline sampling, the subjects were asked to apply one of three doses of testosterone gel (4.4, 8.8, or 13.2 mg) in a random order. After daily application of a specific dose of testosterone gel for 6 d, the subjects were admitted to the Clinical Research Center, testosterone gel was applied, and blood samples were obtained over the next 48 h. Data are the mean ± SEM. A, Serum free testosterone concentrations after application of the placebo or different doses of testosterone gel in postmenopausal women who were not receiving estrogen therapy; B, serum free testosterone concentrations after application of the placebo or different doses of testosterone gel in postmenopausal women who were receiving estrogen therapy.

administration of the 4.4-mg dose (Table 3). The average increments in free testosterone above baseline were related to testosterone dose; thus, ΔCav was greater during administration of the 13.2-mg dose than during baseline sampling or during administration of the 4.4-mg dose. However, Cav, ΔCav, Cmax, Cmin, and $AUC_{0-24\ h}$ were not significantly different at any dose between women who were receiving estrogen therapy and those who were not.

Estradiol. Estradiol levels were higher at baseline (41 ± 3 *vs.* 11 ± 1 pg/ml) and at all time points during testosterone treatment in women who were receiving estrogen therapy than in those who were not ($P < 0.000001$; Fig. 4). Estradiol concentrations did not change significantly in any group

TABLE 3. Pharmacokinetic parameters derived from free testosterone concentrations after administration of 0, 4.4, 8.8, or 13.2 mg testosterone gel in postmenopausal women who were or were not receiving estrogen therapy

A. Women not receiving estrogen therapy					
	Testosterone dose (mg)				*P*
	0	4.4	8.8	13.2	
C_{max} (pg/ml)	9.4 ± 1.5	11.6 ± 1.7	15.6 ± 3.3	19.2 ± 4.0	<0.00002
C_{min} (pg/ml)	4.1 ± 0.7	5.9 ± 1.0	7.6 ± 1.2	8.1 ± 1.1	<0.000001
C_{av} (pg/ml)	5.9 ± 0.9	8.4 ± 1.2	11.5 ± 2.5	12.8 ± 2.1	<0.000001
T_{max} (h)	5.2 ± 1.6	8.9 ± 2.5	7.5 ± 2.2	4.2 ± 0.9	0.14
T_{min} (h)	2.8 ± 1.8	4.3 ± 1.8	7.7 ± 2.6	7.5 ± 2.7	0.29
AUC_{0-24} h (h·pg/ml)	140.0 ± 5.8	201.0 ± 98.4	276.0 ± 58.8	307.0 ± 49.9	<0.000001
ΔC_{av} (pg/ml)		2.5 ± 1.4	5.6 ± 6.0	6.9 ± 2.2	
FI	0.99 ± 0.7	0.7 ± 0.5	0.6 ± 0.3	0.7 ± 0.3	

B. Women receiving estrogen therapy					
	Testosterone dose (mg)				*P*
	0	4.4	8.8	13.2	
C_{max} (pg/ml)	9.3 ± 1.8	11.3 ± 1.6	16.2 ± 3.8*	15.4 ± 1.7	<0.00002
C_{min} (pg/ml)	2.9 ± 0.6	5.2 ± 0.6	5.8 ± 0.8	7.2 ± 0.7	<0.000001
C_{av} (pg/ml)	5.0 ± 0.7	7.6 ± 1.0	11.1 ± 1.9	10.8 ± 1.0	<0.000001
T_{max} (h)	7.8 ± 2.5	7.8 ± 2.6	8.9 ± 2.4	9.8 ± 3.5	0.14
T_{min} (h)	7.5 ± 2.6	8.9 ± 2.3	4.2 ± 2.1	5.1 ± 0.7	0.29
AUC_{0-24} h (h·pg/ml)	119.0 ± 5.5	182.0 ± 22.9	266.0 ± 46.3	258.0 ± 23.7	<0.000001
ΔC_{av} (pg/ml)		2.7 ± 0.7	6.2 ± 1.9	5.8 ± 0.6	

Data are mean ± SEM. FI, Fluctuation index (the ratio of the lowest to the highest testosterone concentration over the 24-h period, a measure of the uniformity of testosterone release over the 24-h treatment interval).

after testosterone application ($P = 0.37$). There was no significant interaction effect of testosterone and estrogen treatment on estradiol levels ($P = 0.59$).

FSH. At baseline, FSH concentrations were significantly lower in women who were receiving estrogen therapy than in those who were not (43 ± 5 *vs.* 74 ± 7 U/liter; $P < 0.00001$; Fig. 5). However, there was no significant effect of testosterone dose in either treatment group ($P = 0.33$).

LH. Serum LH concentrations were significantly lower in women who were receiving estrogen therapy than in those who were not (27 ± 3 *vs.* 35 ± 2 U/liter; $P = 0.001$; Fig. 6). However, there were no significant differences in LH levels during testosterone administration in either group ($P = 0.97$).

SHBG. SHBG concentrations were numerically higher in women who were receiving estrogen therapy than in those who were not; however, the differences in SHBG concentrations between these two groups did not achieve statistical significance ($P = 0.21$; Fig. 7). SHBG levels did not change significantly during the administration of testosterone at any dose in either group ($P = 0.76$).

Discussion

Treatment with testosterone gel in postmenopausal women with low testosterone levels was associated with a dose-dependent increase in total and free testosterone concentrations; each milligram of testosterone applied transdermally raised C_{av} by approximately 5.5–6.7 ng/dl. Thus, transdermal application of 4.4 mg testosterone gel daily raised total and free testosterone levels into the mid- to high-mid range for menstruating women. The flatness of the total and free testosterone concentration profiles is consistent with relatively uniform testosterone delivery during the 24-h

sampling period; testosterone concentrations began to decline between 24 and 36 h after gel application. These data justify once-daily application of the gel. Treatment with conjugated equine estrogen and medroxyprogesterone acetate did not significantly affect total or free testosterone concentrations over this 7-d treatment period.

Baseline total and free testosterone concentrations were lower in postmenopausal women than in menstruating women. This is consistent with data demonstrating an age-related decline in testosterone concentrations (20–27). There is agreement that total and free testosterone concentrations do not change abruptly around the menopausal transition (20–37), but decline gradually starting in the third decade of life.

Many studies of testosterone supplementation have used testosterone esters, testosterone implants, or methyl-testosterone (7–14). Pharmacokinetic data on the use of these androgen formulations have been lacking. Unapproved testosterone formulations compounded by local pharmacies are also being used. A testosterone matrix transdermal system for women increases total testosterone concentrations into the mid- to high-normal range after application of one or two patches twice weekly in surgically menopausal women (15–16). A pilot study of a micronized transdermal testosterone gel reported prolonged elevation of testosterone levels, which were in the hyperandrogenic female range (38). The hydroalcoholic testosterone gel used in this study compares favorably with other androgen formulations in its excellent skin tolerability, ease of application, and ability to provide uniform testosterone delivery. Furthermore, the excellent dose-concentration relationship should facilitate selection of dose regimens for testosterone trials in women. Although the testosterone gel was well tolerated over this short treatment duration, long-term studies are needed to fully assess the safety and efficacy of this formulation.
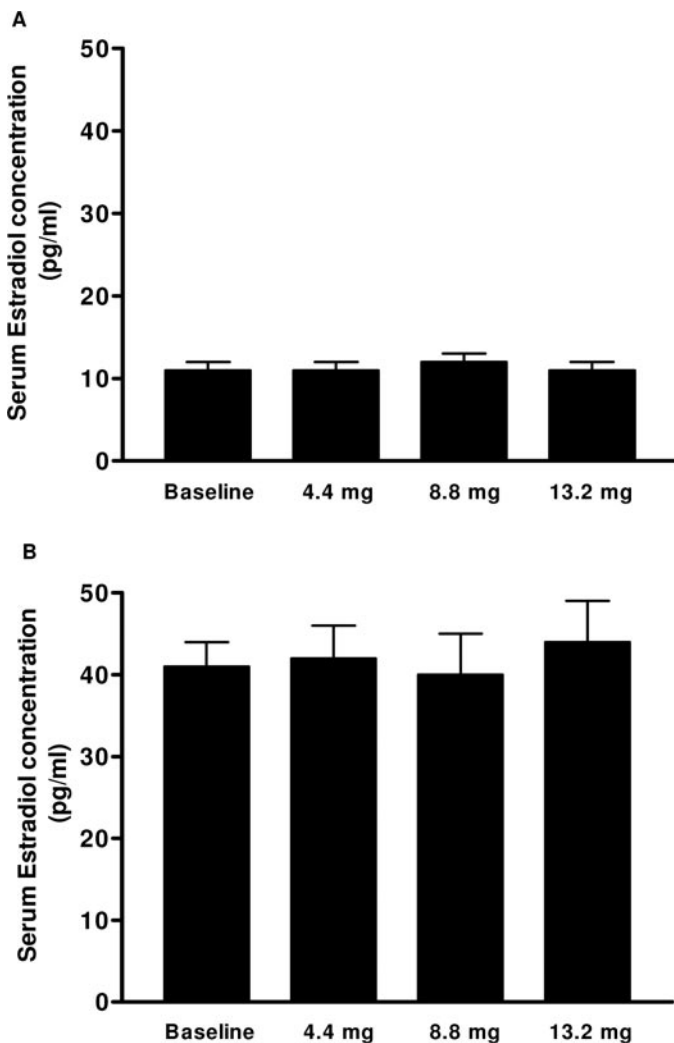
FIG. 4. Serum estradiol concentrations in postmenopausal women at baseline and during treatment with daily doses of 4.4, 8.8, or 13.2 mg testosterone gel. A, Serum estradiol concentrations in women who were not receiving estrogen therapy (group I); B, serum estradiol concentrations in women who were receiving estrogen therapy (group II). Data are the mean ± SEM.

Most of the subjects in group II were receiving conjugated equine estrogen and medroxyprogesterone acetate, which did not significantly affect total or free Cav, testosterone AUC, Cmax, or Cmin during the short treatment duration. Estrogen administration increases SHBG concentrations and might affect plasma clearance of testosterone. Women receiving estrogen therapy have lower free testosterone concentrations than those who are not receiving estrogens, presumably due to the net effects of estrogen treatment on LH secretion and SHBG concentrations that can indirectly affect plasma testosterone clearance (18). However, in this study, neither total nor free testosterone concentrations during treatment were significantly affected by concomitant estrogen plus progesterone administration. It is possible that the duration of testosterone treatment (1 wk) was too short to observe significant interactions of testosterone and estrogen administration. Indeed, serum SHBG concentrations were not significantly affected by testosterone administration at any dose. Additionally, the sample size was
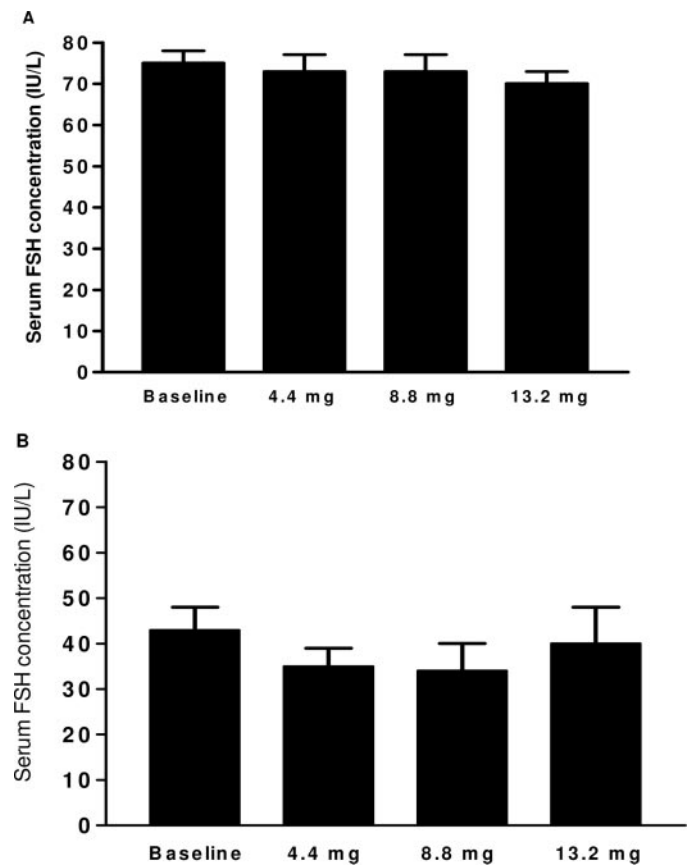

FIG. 5. Serum FSH concentrations in postmenopausal women at baseline and on d 7 of treatment with daily doses of 4.4, 8.8, or 13.2 mg testosterone gel. A, Serum FSH concentrations in women who were not receiving estrogen therapy; B, serum FSH concentrations in women who were receiving estrogen therapy. Data are the mean ± SEM.

relatively small, and small differences between the two groups might have been missed.

Testosterone administration by means of the transdermal gel did not significantly increase serum 17β-estradiol concentrations. Because testosterone is aromatized to 17β-estradiol in many peripheral tissues, increments in serum estradiol levels would have been expected after testosterone administration. In healthy men, graded doses of testosterone are associated with dose-dependent increases in circulating estradiol concentrations (39). However, the peripheral conversion rates of testosterone to estradiol are low, and the increments in testosterone levels in women treated with gel were substantially lower than those observed in hypogonadal men treated with the much higher doses of testosterone than those used in this study. Therefore, it is likely that the changes in estradiol concentrations that might have occurred in women treated with testosterone gel were too small to be statistically or clinically significant.

Consistent with the demographics of the catchment area served by our medical center, a majority of our participants described themselves as either African-American or Hispanic. We do not know whether pharmacokinetics of testosterone gel are different in Caucasians or other ethnic groups. Ethnic differences in the metabolism and pharmacodynamic response to testosterone have been reported in
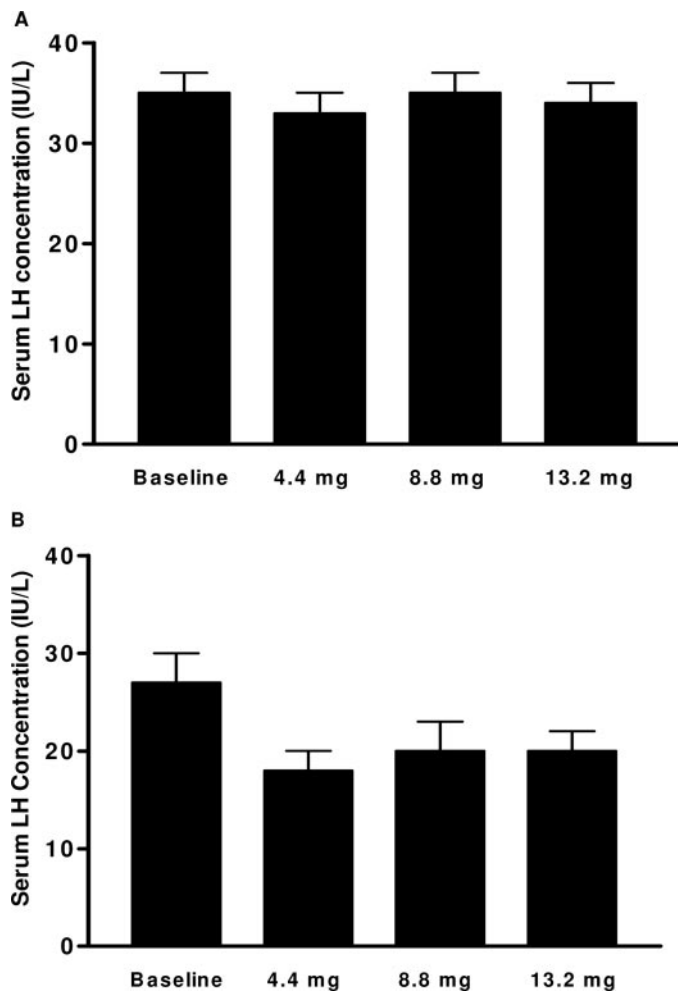
FIG. 6. Serum LH concentrations in postmenopausal women at baseline and on d 7 of treatment with daily doses of 4.4, 8.8, or 13.2 mg testosterone gel. A, Serum LH concentrations in women who were not receiving estrogen therapy; B, serum LH concentrations in women who were receiving estrogen therapy. Data are the mean ± SEM.
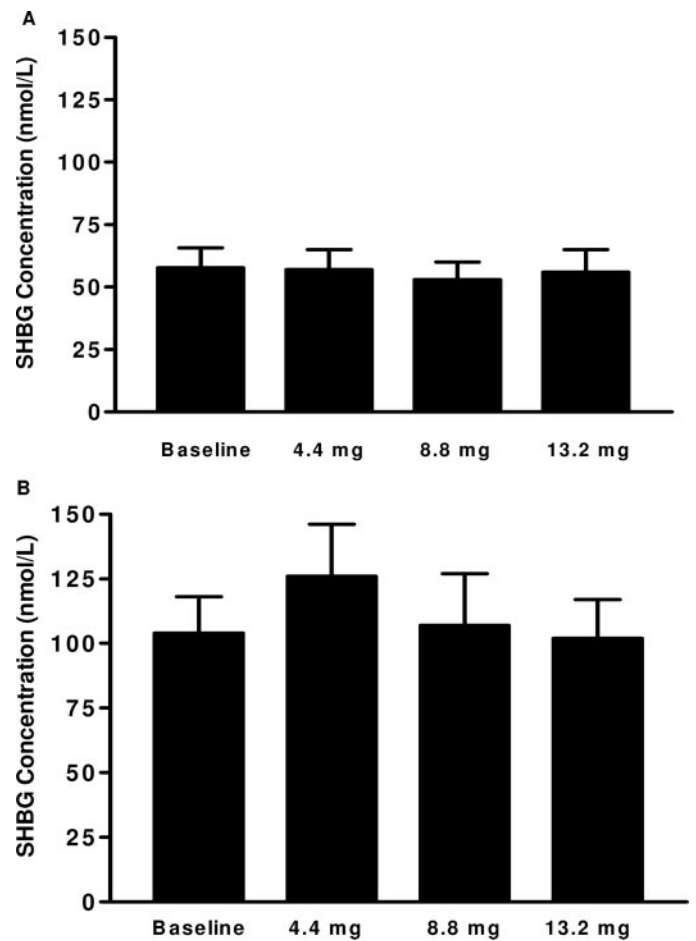
FIG. 7. Serum SHBG concentrations in postmenopausal women at baseline and during administration of daily doses of 4.4, 8.8, or 13.2 mg testosterone gel. A, Serum SHBG concentrations in women who were not receiving estrogen therapy; B, serum SHBG concentrations in women who were receiving estrogen therapy. Data are the mean ± SEM.

men (40, 41). Similarly, ethnogenetic differences in biological response to androgens have also been suggested in women with polycystic ovary syndrome (42, 43).

Some postmenopausal women experience decreased libido, fatigue, and impaired sense of well-being (1–6); cross-sectional studies have reported either no correlation or a weak correlation between testosterone concentrations and measures of sexual function and well-being (25–27). Testosterone supplementation in postmenopausal women with sexual dysfunction has been associated with improvements in these symptoms (1–9, 13, 44–48). The doses of testosterone employed in some studies were relatively high and increased testosterone levels into a range that is supraphysiological for healthy women (6–9, 13). Well-controlled, randomized trials of the transdermal testosterone patch (18, 44, 47, 48) have demonstrated that raising total and free testosterone concentrations into a range that is at the upper end of the normal female range is associated with improvements in several domains of sexual function and well-being. Testosterone supplementation of HIV-infected women with weight loss by 150–300 μg/d testosterone matrix transdermal system

patch does not significantly increase lean body mass (17, 49, 50). We do not know whether testosterone replacement can produce clinically significant improvements in sexual, physical, and neurocognitive functions and whether such benefits can be achieved without virilizing side effects. The pharmacokinetic data reported in this study should facilitate randomized trials to assess the efficacy of testosterone replacement in older women with low testosterone concentrations.

Acknowledgments

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