Andrology

Testosterone gels are being increasingly used throughout the world. There is still some controversy associated with their use, but many of the uncertainties are gradually being overcome and explained. The authors from the USA evaluated the pharmacokinetics and the efficacy of dosing and application sites of another such transdermal gel.

The arrival of oral agents for treating male erectile dysfunction has made it easier for GPs to treat this condition. The authors from Southampton have examined the safety of sildenafil used in general medical practice in England, finding that it was used safely at this level, with no unexpected events.

Transdermal testosterone gel: pharmacokinetics, efficacy of dosing and application site in hypogonadal men

A.W. MEIKLE, D. MATTHIAS* and A.R. HOFFMAN†

*Department of Medicine, University of Utah and ARUP Laboratories, Salt Lake City, UT, *Eastern VA Medical School, Norfolk, VA, and †VA Palo Alto Care System and Stanford University, Palo Alto, CA, USA*

Accepted for publication 7 November 2003

OBJECTIVE

To determine the regimen that would most effectively maintain serum testosterone concentrations in treated hypogonadal men within the normal reference range of 3–11.4 μg/L.

PATIENTS AND METHODS

Eighteen men aged 24–69 years with either primary or secondary hypogonadism participated in and 16 completed a randomized, six-treatment regimen, three-period (phase), three-way matrix-type crossover study. A 1% and 2% testosterone gel (CP601, Cellegy Pharmaceuticals, Inc., San Francisco, USA) was administered either once or twice daily transdermally at different body sites to determine optimal dosing, application sites, and its pharmacokinetics and tolerability in hypogonadal men. Treatments A–F included 1 g of 1% and 2% gel that was equivalent to 10 or 20 mg of testosterone, applied once or twice daily to the skin of either the thigh or the upper arm. Six men also participated in a study of 3 g of 2% gel that was equivalent to 60 mg of testosterone applied once daily, half on each thigh. Pharmacokinetic variables were calculated for testosterone for each man in each treatment period and the results analysed by ANOVA.

RESULTS

In general the higher dose regimens produced higher serum concentrations of testosterone; the 3 g/2% dose was most successful in maintaining serum testosterone within the normal reference range. The average testosterone concentration (C_{avg}) was 6.52 μg/L and all men had a C_{avg} of >3.0 μg/L. The prediction of all men achieving a C_{avg} of >3.0 μg/L was 96%. The mean minimum concentration (C_{min}) was 3.83 μg/L and half the patients had a C_{min} of >3.0 μg/L. Most men had serum testosterone levels within the normal reference range throughout the 24 h, and the treatment was well tolerated.

CONCLUSIONS

The 3 g/2% dose applied to the skin daily resulted in serum testosterone in the normal reference range in most hypogonadal men. Dose adjustments to either a lower or higher dose should shift serum testosterone concentration to the desired range in those who do not achieve this range with this dose.

KEYWORDS

hypogonadism, testosterone, transdermal gel, pharmacokinetics

INTRODUCTION

The Leydig cells of the testes of normal adult men produce ≈95% of the daily output of ≈7 mg of testosterone [1–3]. Testicular, pituitary, and hypothalamic dysfunction, chronic illness and a variety of medications and normal ageing are all associated with subnormal concentrations of serum testosterone [4–6]. Restoring serum testosterone to physiological concentrations in testosterone-deficient men has been a focus of much recent investigation, involving the use of oral and sublingual preparations, depot injections and pellets, and transdermal patches and gels [7–14].

Each route of testosterone administration has favourable and unfavourable features. Oral 17-α alkylated derivatives of testosterone are associated with hepatoxicity [15] and marked reductions of high-density lipoprotein (HDL) cholesterol [16] and vascular lesions [17]; some oral preparations may have poor or variable absorption; injections of testosterone esters may produce undesirable peak and troughs [9,18]; and some testosterone patches result in intolerable skin reactions [19,20], limited testosterone delivery or unphysiologically high dihydrotestosterone concentrations [13,21,22]. The currently available gels have low incidences of skin irritation and deliver sustained testosterone concentrations, but the preparations available make individual dose titration difficult and in some cases expensive [13,23,24].

Numerous clinical studies confirm the efficacy of testosterone for treating testosterone deficiency [13,23,24]. When testosterone deficiency is physiologically replaced several benefits have been reported, including improved sexual function, libido, mood, cognition, bone mineral density and lean muscle mass and strength [10,12,14,25–31]. Adverse consequences of testosterone replacement therapy include gynaecomastia and polycythaemia (particularly with injections of testosterone), weight gain, decreased HDL cholesterol and increased prostate size [25,32,33]. Androgens are contraindicated in men with cancer of the breast or prostate. After decades of research, data supporting an increased risk of testosterone therapy on prostate or breast cancer have never been reported [13,34].

The purpose of this study was to select application sites, doses and dosing intervals for a gel formulation of testosterone that would provide a physiological serum concentration of testosterone over a 24-h period (pharmacokinetics), and to determine the tolerability of this transdermal delivery system.

PATIENTS AND METHODS

The study included 18 men aged 18–75 years with primary or secondary hypogonadism; after withdrawal from any previous testosterone treatment, hypogonadism was defined as a serum testosterone concentration of <3.0 μg/L on two consecutive occasions 7 days apart or <2.5 μg/L in a single sample. However, subsequent serum testosterone measurements during the study confirmed testosterone deficiency. The men were otherwise in good health, based on the results of medical history, physical examination, a 12-lead electrocardiogram and clinical laboratory test results at screening.

The preparations of 1% and 2% testosterone CP601 gel contained 10 and 20 mg testosterone (United States Pharmacopeia/National Formulary, USP/NF) per gram of gel (dispensed from a metered canister), respectively, ethanol, Carbopol 1342 USP (gelling agent), and other approved pharmacologically inert excipients (2-propanol USP, propylene glycol USP, oleic acid USP, butylated hydroxytoluene NF, triethanolamine NF, and purified water USP).

This was an open-label, randomized, six-treatment regimen, three-way, three-period (phase) crossover matrix type, and phase 1/2 study conducted at two sites in the USA. A seventh open-label treatment regimen was added by amending the protocol. The institutional review boards of the University of Utah and Stanford University approved the studies.

Six men participated in each of three treatment regimens, and each was randomly assigned to three treatment phases (I, II and III) of testosterone gel administration. During each treatment phase the man was to follow one of six treatment regimens (A–F). In all treatment regimens testosterone gel was applied at 1 g/100 cm^2 of skin surface area (Table 1). During treatment phase IV in regimen G, 1.5 g of 2% testosterone gel (30 mg testosterone) was applied daily to 150 cm^2 of skin at the same site on each outer thigh (total daily dose 60 mg testosterone).

The order of treatment regimens was randomized, with a minimum 3-day drug washout period between treatment phases. On study days 1 and 7 of treatment phase I, II and III (A–F) and on study day 7 of treatment phase IV (G), venous blood samples were obtained to assay serum concentrations of testosterone before and at specified times after the morning testosterone gel administration. On days 3 and 5 venous blood samples were obtained before (and 1.5 h after, in G) testosterone gel administration to assay serum testosterone. The samples were sent to Endocrine Sciences for analysis (presently Esoterix, Inc., Calabasas Hills, CA).

Serum testosterone concentrations were determined by radioimmunoassay after hexane:ethyl acetate extraction of the serum sample. The intra-assay coefficient of

TABLE 1
The 7-day regimen of testosterone gel application

Regimen	Testosterone			dosing	application site
	g	%	dose, mg		
A	1	2	20	daily	upper arm
B	1	1	10	daily	upper arm
C	1	2	20	days, 1,3,5,7	upper arm
				days, 2,4,6	outer thigh
D	1	1	10	days, 1,3,5,7	upper arm
				days, 2,4,6	outer thigh
E	1	2	20 (40)*	Twice daily	outer thigh, am; upper arm, pm
F	1	1	10 (20)*	Twice daily	outer thigh, am; upper arm, pm
G	1.5	2	30 (60)*	daily each thigh	outer thighs

**Total dose.*

variation was < 9.4% and the interassay coefficient < 8.8%. The sensitivity of the assay was 0.49 μg/L.

PHARMACOKINETICS

To determine a precise pharmacokinetic profile during study days 1 and 7 of treatment phases I–III, and day 7 of treatment phase IV, venous blood samples were obtained to measure testosterone before (time 0) and at 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h after the morning application of the gel. On days 3 and 5 of each treatment phase a venous blood sample was obtained just before the morning dose, and for men in regimen G also at 1.5 h after dosing. Concentration-time profiles were evaluated using a non-compartmental approach.

SAFETY

There were no clinically significant changes except for features of hypogonadism at baseline or during the study in physical examination findings, vital signs (temperature, systolic and diastolic arterial blood pressure, and heart and respiration rates), 12-lead electrocardiogram results, clinical laboratory test results (haematology, blood chemistry, serology and urine analysis), and assessment of treatment-emergent adverse events.

EVALUATION OF SKIN TOLERABILITY

Application-site reactions were graded by the investigator and recorded on the case report form. At 24 h (end of study day 1) and 168 h (end of study day 7) after applying the gel in each treatment phase the site of application was examined for changes. Skin reactions were graded by the investigator as: 1, normal skin with no redness; 2, mild redness; 3, redness and swelling or induration; and 4, blister formation and/or necrosis. The men were instructed to contact the investigator if they noted any inflammation (reddening) or discomfort at the site of application at any time during the study, and to change the application site to the other side of the same anatomical site (arm or thigh) only if inflammation occurred.

STATISTICS

The pharmacokinetic variables C_{max}, C_{min}, C_{avg} and $t_{max,}$ were calculated for serum testosterone concentrations using a noncompartmental approach [35–37]. The areas under the serum testosterone concentration-time curves (AUC) for 0–24 h ($AUC_{0–24}$)on study days 1 and 7 were calculated using the trapezoidal method. For the twice-daily regimens, E and F, C_{max} and t_{max} were calculated for 12–24 h after dosing. Values were compared statistically using ANOVA with regimen sequence, treatment phase (period), regimen, treatment day, and the treatment-by-day interaction as fixed effects. In the ANOVA, residual effects were examined for pattern, outliers and other aspects of fit, such as homogeneous variance and approximate normality.

RESULTS

PHARMACOKINETICS

In phase I the mean (SD, range) pretreatment testosterone concentration, C_0, was 1.76 (0.89, 0.25–3.34) μg/L, and in phase II and III, 1.90 (1.01) and 1.94 (0.96) μg/L, and not significantly different ($P = 0.369$) from phase I, indicating that the 3-day washout between treatment phases was sufficient for serum testosterone to return to baseline levels (Table 2).

TABLE 2 Mean (SD) values (μg/L) of C_0

Regimen	A	B	C	D	E	F	G
N	8	7	8	8	9	8	6
Day							
1	2.01 (0.88)	1.74 (1.05)	1.78 (0.78)	1.88 (0.86)	1.98 (1.00)	1.78 (1.27)	–
3	3.60 (2.11)	2.54 (1.07)	3.89 (2.66)	2.55 (0.84)	4.38 (2.20)	3.04 (0.89)	3.24 (1.32)
5	3.34 (1.39)	2.25 (1.11)	4.94 (5.11)	2.30 (0.89)	4.82 (1.88)	3.14 (1.61)	3.19 (1.20)
7	3.25 (2.06)	2.30 (1.68)	2.09 (0.59)	2.16 (1.10)	3.84 (1.36)	4.21 (1.50)	5.04 (1.65)
8	3.32 (1.68)	2.73 (1.55)	2.81 (0.63)	3.09 (2.16)	4.40 (2.08)	3.85 (1.89)	6.82 (3.13)

Applying the testosterone gel once or twice daily to either the thigh or upper arm resulted in increases from baseline in serum testosterone concentrations (Table 2) with each dose from day 1 before treatment to before dosing on days 3, 5, 7 and 8. An ANOVA of all the pharmacokinetic variables measured was applied for days 1 and 7 using fixed effects of treatment sequence, phase, day, regimen and day-by-regimen (Table 3). Overall, the results of this study showed that there was a significant day effect for all variables except C_{max}, for which it was marginal. Similarly, except for C_0 and C_{min}, there was a significant regimen effect associated with each variable. Some variables had associated phase (AUC_{0-24} and C_{avg}) effects, which were also significant. C_0, day and day-by-regimen were highly significant ($P < 0.001$ and 0.020, respectively). The day effect is easily understood, as the day 7 results were overall higher than those on day 1. The significance of the interaction term is probably partly a result of differences among regimens A, B, C and D from E and F. The increases from study day 1 to 7 were greater in E and F than in A, B, C and D. The mean testosterone AUC_{0-24} increased from day 1 to 7 for all regimens except C. On day 7 the highest mean testosterone AUC_{0-24} was in G (156.63 μg/L/h).

Although the ANOVA identified treatment phase, regimen and day as being statistically significant in the AUC_{0-24} results for A–F ($P = 0.019$, 0.029 and 0.019, respectively; Table 3), which complicates the interpretation of the findings, we conclude that there was an increase in AUC with repeated gel application and that day 7 values were higher than those on day 1.

PHARMACOKINETICS

The higher strength regimens generally resulted in more testosterone being absorbed, as shown in (Fig. 1). The linearity between C_{min} (Fig. 1a) and C_{avg} (Fig. 1b), as a function of the total amount of testosterone applied daily to the skin, was assessed using a random coefficient analysis; the effect of dose on C_{min} ($P = 0.001$) and C_{avg} ($P = 0.002$) was highly significant. This suggests that dosing with this testosterone gel could be adjusted for each patient's dose requirements by compensating for variations in body weight, body mass index, clearance and skin permeability, all

factors that could affect the final serum concentrations.

PHYSIOLOGICAL TESTOSTERONE CONCENTRATIONS

Table 4 summarizes the C_{avg} on days 1 and 7; regimen G best maintained the serum testosterone concentration in hypogonadal men within the physiological range of 3.0–11.4 μg/L. The mean C_{avg} (6.52 μg/L) in G was significantly higher than the lower limit and all men had a C_{avg} of >3.0 μg/L. Based on this study the predicted percentage of patients in this population with C_{avg} values above the lower limit after treatment with 3 g of 2% testosterone gel once daily was calculated to be 96%.

The mean C_{min} (3.83 μg/L, Table 4) was significantly higher than the lower limit in half the patients in G at day 7. The predicted percentage of patients above the lower limit after treatment G was calculated to be 70%. Most men had serum testosterone levels within the reference range throughout the day. Thus we conclude that CP601 testosterone gel is well suited for replacement therapy with a pharmacokinetically driven dose-adjustment scheme.

To determine which regimen significantly increased the C_{avg} within the reference range, a one-sided *P* value was calculated for each regimen (Table 4). The proportion ranged from three of eight for D to all men in G, where the mean C_{avg} was 6.52 μg/L. Regimen G was the only treatment that significantly ($P < 0.001$) increased the C_{avg} to >3.0 μg/L. In a similar analysis for C_{min}, the numbers of patients with a C_{min} of >3.0 μg/L was also calculated (Table 4); regimen G was the only treatment where C_{min} (3.83 μg/L) was significantly ($P < 0.001$) >3.0 μg/L and half the men were above that level. For the other regimen the numbers achieving a C_{min} of >3.0 μg/L were between zero (C) and four of nine men for (E).

In the analyses for C_{avg} and C_{min} only the lower end of the physiological range was considered, as these two variables are more likely to be low than high, as summarized in Table 5. To take into account both ends of the range, the number of men within the reference range at day 7 was computed at all sampling times (Table 5). Regimen G, followed by E and F, were best at maintaining most men within the physiological range throughout the day.

TABLE 3 The results of ANOVA for the pharmacokinetic variables, as P values

Variable	Sequence	Phase	Day	Regimen	Day-by-regimen
C_0	0.129	0.099	<0.001	0.288	0.020
AUC_{0-24}	0.712	0.019	0.029	0.019	0.334
C_{max}	0.747	0.086	0.053	0.010	0.510
t_{max}	0.785	0.568	0.023	0.013	0.853
C_{avg}	0.713	0.018	0.028	0.018	0.325
C_{min}	<0.137	0.061	<0.001	0.730	0.184

SAFETY RESULTS

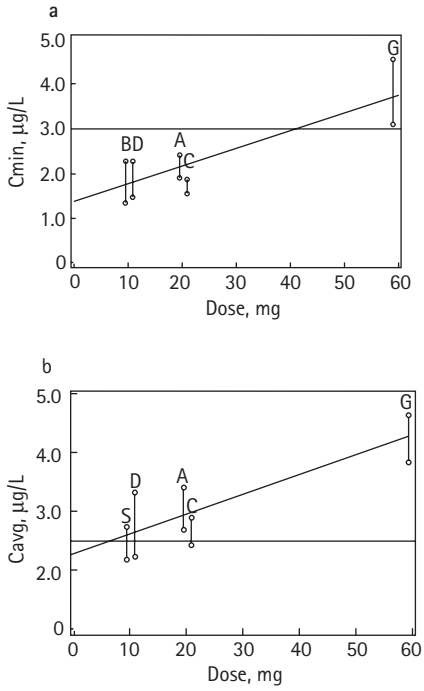
No safety issues were evident from the use of 1% or 2% testosterone gel during the study. Adverse events were reported in 61% of men but most were associated with mild erythema at the application site and all but one was mild or moderate. This profile was consistent with previous clinical experience with topically administered testosterone products [19,23,33]. There was no apparent dose- or regimen-related association with the frequency of adverse events. There were no deaths, serious adverse events, or discontinuations for an adverse event during the study. There were no laboratory, physical examination or other safety-related issues with the administration of this testosterone gel.

DISCUSSION

Applying the testosterone gel once or twice daily to either the thigh or upper arm resulted in significantly higher C_0, AUC_{0-24}, C_{avg}, C_{max} and C_{min} and earlier t_{max} on day 7 than on day 1. Higher-dose regimens generally resulted in more testosterone being absorbed. The results suggested that dosing with testosterone gel could be adjusted to meet a particular patient's dose requirements. Regimen G (60 mg), followed by regimens E and F, was the best at achieving physiological testosterone concentrations throughout the day. Results from regimen G indicated that nearly all men had C_{avg} values below the upper limit of normal and half had C_{min} values of >3.0 μg/L.

While CP601, Androgel® and Androderm® were not compared, regimen G (60 mg) compares favourably with published results

FIG. 1. The linearity with dose of C_{min} (a) and C_{avg} (b), showing the values for a single daily dose (A, B, C, D and G); the effect of dose was highly significant (P = 0.001 and 0.002, respectively).



reported with the Androgel 5 g and Androderm 5 mg doses [9,13,23]. The testosterone profile of CP601 resembles that of Androderm in that both have a distinct peak concentration, whereas Androgel has a less noticeable peak [9,13,23]. The ability of CP601 (60 mg) to achieve physiological concentrations of testosterone throughout the 24-h interval after dosing is as high or higher than those reported with Androgel or Androderm [9,13,23].

As one dose is not appropriate for all men for any of the testosterone preparations, CP601 has an apparent dosing advantage in that the

TABLE 4 Summary statistics for C_{avg} and C_{min} (μg/L) on days 1 and 7; a random coefficient analysis of C_{min} by dose for single dose regimens A, B, C, D and G gave a dose coefficient (SEM) of 3.94 (0.90), an intercept (SEM) of 139 (26.1) and a P for dose of 0.001

	A	B	C	D	E	F	G
No. of men	8	7	8	8	9	8	–
C_{avg}							
Day 1							
Mean (SD)	3.60 (165)	2.19 (1.14)	3.85 (1.53)	3.00 (1.59)	3.52 (1.04)	2.46 (1.01)	–
Median	3.29	2.72	3.74	2.85	3.42	2.20	–
Range	2.08–7.46	0.36–3.11	1.95–6.44	1.35–5.83	1.96–5.45	1.20–4.05	–
Day 7							
Mean (SD)	4.13 (2.08)	2.94 (1.48)	3.38 (1.27)	3.60 (3.17)	4.48 (1.35)	4.01 (1.82)	6.52 (1.98)
Median	3.77	2.64	3.25	2.64	4.36	3.51	5.96
Range	1.92–7.66	0.43–5.06	2.06–6.01	0.91–1.098	2.72–6.80	2.10–7.87	4.34–9.18
*P**	0.085	0.798	0.605	0.823	0.063	0.087	<0.001
C_{min}							
Day 1							
Mean (SD)	1.85 (0.74)	1.56 (0.92)	1.55 (0.70)	1.66 (0.81)	1.73 (0.73)	1.45 (0.75)	–
Median	1.90	1.93	1.50	1.76	1.92	1.41	–
Range	0.25–2.75	0.25–2.35	0.25–2.39	0.25–2.73	0.63–3.02	0.25–2.73	–
Day 7							
Mean (SD)	2.17 (0.72)	1.82 (1.24)	1.72 (0.46)	1.87 (1.11)	2.46 (0.96)	2.47 (1.00)	3.83 (1.63)
Median	2.27	1.72	1.71	1.68	2.75	2.03	3.43
Range	1.24–3.12	0.25–3.26	0.72–2.20	0.25–3.53	1.15–3.85	1.41–4.43	1.99–6.45
Evaluation of C_{avg} and C_{min} on day 7 by regimen, vs 3.0 μg/L							
N	8	7	8	8	9	8	6
*P**	0.115	0.838	0.662	0.857	0.098	0.123	<0.001
N with >3.0 μg/mL	5	3	4	3	8	6	6
%†	70.6	48.5	61.7	57.5	86.5	71.0	96.3
*P**	0.997	1.000	1.000	1.000	0.998	0.971	<0.001
N with >3.0 μg/mL	1	2	0	2	4	2	3
%†	12.5	16.9	0.3	15.6	28.7	29.8	69.5

**One-sided P value testing that the mean is > 3.0 μg/L, based on ANOVA for A–F and on normal approximation for G. †Percentile of normally distributed population > 3.0 μg/L as predicted from the observed mean (SD); –, not done.*

TABLE 5 Numbers of patients with testosterone concentrations of 3.0–11.4 μg/L on study day 7

Regimen	A	B	C	D	E	F	G
Total	8	7	8	8	9	8	6
Nominal sampling time, h							
0	3	2	0	2	7	7	6
0.5	5	3	2	2	6	5	4
1	5	3	5	3	7	6	4
2	6	6	5	5	8	7	3
4	8	5	6	6	8	8	6
6	4	2	3	5	8	3	6
8	4	2	2	4	6	3	5
10	4	2	0	3	4	2	6
12	3	2	0	3	5	2	5
16	4	2	2	2	7	7	4
20	4	3	3	2	7	5	6
24	4	2	2	2	7	4	4

testosterone dose can be adjusted. The gel is delivered from a device which allows for calibrated dosing from the canister. With Androgel and Androderm, additional patches are needed to deliver adequate amounts of testosterone, thereby increasing the cost [9,13,23]. A long-term study using CP601 has been conducted to determine the benefits in improving symptoms and effects on safety related to lipid profiles, bone density changes, PSA and haematological variables.

In conclusion, based on the present results in regimen G (60 mg) the correlation between the testosterone dose applied to the skin and C_{avg} and C_{min}, single daily applications of 3 g CP601 per 300 cm^2 skin should provide an adequate dose of testosterone to maintain the serum testosterone concentration in the physiological range for the large majority of hypogonadal men. The avoidance of supraphysiological levels of testosterone, seen with injection therapy, should provide more physiological hormone replacement therapy, and may prevent unwanted side-effects, e.g. sleep disturbances and an elevated haematocrit [9,13,23,33]. For those hypogonadal men whose serum testosterone concentrations would not be adequately adjusted by the 3 g dose, it is anticipated that adjustment to a lower (e.g. 2 g) or higher (e.g. 4 g) amount of gel will alter their testosterone levels in the desired direction.

The CP601 1% and 2% testosterone gel used in this study was safe and well tolerated by all patients. Adverse events were consistent with the known safety profile of testosterone and

topical application of formulations with a high alcohol content.

ACKNOWLEDGEMENTS

Cellegy Pharmaceuticals, Inc and USPHS Grant M01-RR-00064 supported this work.

CONFLICT OF INTEREST

Source of funding for AW Meikle and AR Hoffman: Cellegy Pharmaceuticals and United States Public Health Grant. Paid consultant for sponsor: AW Meikle, for protocol development. Study investigator funded by sponsor: AW Meikle and AR Hoffman, for evaluation and management of patients in the study.

REFERENCES

1 **Meikle AW, Smith JA, Stringham JD.** Production, clearance, and metabolism of testosterone in men with prostate cancer. *Prostate* 1987; **10**: 25–31
2 **Meikle AW, Stringham JD, Bishop DT, West DW.** Quantitating genetic and nongenetic factors influencing androgen production and clearance rates in men. *J Clin Endocrinol Metab* 1988; **67**: 104–9
3 **Meikle AW, Smith JA, Stringham JD.** Estradiol and testosterone metabolism and production in men with prostate cancer. *J Steroid Biochem* 1989; **33**: 19–24
4 **Odell WD, Swerdloff RS.** Male hypogonadism. *West J Med* 1976; **124**: 446–75
5 **Plymate S.** Hypogonadism. *Endocrinol Metab Clin North Am* 1994; **23**: 749–72
6 **Snyder PJ.** Hypogonadotropic hypogonadism: gonadotropin therapy. *Curr Ther Endocrinol Metab* 1994; **5**: 300–3
7 **Handelsman DJ, Conway AJ, Boylan LM.** Pharmacokinetics and pharmacodynamics of testosterone pellets in man. *J Clin Endocrinol Metab* 1990; **71**: 216–22
8 **Bhasin S, Swerdloff RS, Steiner B *et al.*** A biodegradable testosterone microcapsule formulation provides uniform eugonadal levels of testosterone for 10–11 weeks in hypogonadal men. *J Clin Endocrinol Metab* 1992; **74**: 75–83
9 **Meikle AW, Arver S, Dobs AS *et al.*** Pharmacokinetics and metabolism of a permeation-enhanced testosterone transdermal system in hypogonadal men: influence of application site- -a clinical research center study. *J Clin Endocrinol Metab* 1996; **81**: 1832–40
10 **Wang C, Alexander G, Berman N *et al.*** Testosterone replacement therapy improves mood in hypogonadal men – a clinical research center study. *J Clin Endocrinol Metab* 1996; **81**: 3578–83
11 **Wang C, Eyre DR, Clark R *et al.*** Sublingual testosterone replacement improves muscle mass and strength, decreases bone resorption, and increases bone formation markers in hypogonadal men – a clinical research center study. *J Clin Endocrinol Metab* 1996; **81**: 3654–62
12 **Sih R, Morley J, Kaiser F *et al.*** Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. *J Clin Endocrinol Metab* 1997; **82**: 1661–7
13 **Meikle A, Dobs A, Arver S *et al.*** Androgen replacement in the treatment of Klinefelter's Syndrome: Efficacy and safety of a nonscrotal permeation-enhanced testosterone transdermal system. *Endocrine Prac* 1998; **4**: 17–22
14 **Snyder PJ, Peachey H, Berlin JA *et al.*** Effects of testosterone replacement in hypogonadal men. *J Clin Endocrinol Metab* 2000; **85**: 2670–7
15 **Westaby D, Ogle S, Paradinas F, Randell J, Murray-Lyon I.** Liver damage from long-term methyltestosterone. *Lancet* 1977; **2**: 262–3
16 **Friedl K, Hannan CJJ, Jones R, Plymate S.** High-density lipoprotein cholesterol is not decreased if an aromatizable androgen is administered. *Metabolism* 1990; **39**: 69–74
17 **Nieschlag E.** Is methyltestosterone no longer used therapeutically? *Dtsch Med Wochenschr* 1981; **106**: 1123–5
18 **Snyder PJ, Lawrence DA.** Treatment of male hypogonadism with testosterone enanthate. *J Clin Endocrinol Metab* 1980; **51**: 1335–9
19 **Arver S, Dobs AS, Meikle AW *et al.*** Long-term efficacy and safety of a permeation-enhanced testosterone transdermal system in hypogonadal men. *Clin Endocrinol (Oxf)* 1997; **47**: 727–37
20 **Wilson DE, Kaidbey K, Boike SC, Jorkasky DK.** Use of topical corticosteroid cream in the pretreatment of skin reactions associated with Androderm testosterone transdermal system. *Endocrine Soc* 1997; **79th**: P01–323
21 **Ahmed SR, Boucher AE, Manni A *et al.*** Transdermal testosterone therapy in the treatment of male hypogonadism. *J Clin Endocrinol Metab* 1988; **66**: 546–51
22 **Cunningham GR, Cordero E, Thornby JI.** Testosterone replacement with transdermal therapeutic systems. Physiological serum testosterone and elevated dihydrotestosterone levels. *JAMA* 1989; **261**: 2525–30
23 **Wang C, Berman N, Longstreth JA *et al.*** Pharmacokinetics of transdermal testosterone gel in hypogonadal men: application of gel at one site versus four sites: a General Clinical Research Center Study. *J Clin Endocrinol Metab* 2000; **85**: 964–9
24 **Wang C, Swerdloff RS, Iranmanesh A *et al.*** Effects of transdermal testosterone gel on bone turnover markers and bone mineral density in hypogonadal men. *Clin Endocrinol (Oxf)* 2001; **54**: 739–50
25 **Arver S, Dobs AS, Meikle AW *et al.*** Improvement of sexual function in testosterone deficient men treated for 1 year with a permeation enhanced testosterone transdermal system. *J Urol* 1996; **155**: 1604–8
26 **Brodsky IG, Balagopal P, Nair KS.** Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men – a clinical research center study. *J Clin Endocrinol Metab* 1996; **81**: 3469–75
27 **Katznelson L, Finkelstein JS, Schoenfeld DA *et al.*** Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. *J Clin Endocrinol Metab* 1996; **81**: 4358–65
28 **Bhasin S, Storer TW, Berman N *et al.*** Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. *J Clin Endocrinol Metab* 1997; **82**: 407–13
29 **Tenover JL.** Testosterone and the aging male. *J Androl* 1997; **18**: 103–6
30 **Jain P, Rademaker AW, McVary KT.** Testosterone supplementation for erectile dysfunction. results of a meta-analysis. *J Urol* 2000; **164**: 371–5
31 **Dobs AS, Bachorik PS, Arver S *et al.*** Interrelationships among lipoprotein levels, sex hormones, anthropometric parameters, and age in hypogonadal men

treated for 1 year with a permeation-enhanced testosterone transdermal system. *J Clin Endocrinol Metab* 2001; **86**: 1026–33
32 **Meikle AW, Arver S, Dobs AS *et al.*** Prostate size in hypogonadal men treated with a nonscrotal permeation- enhanced testosterone transdermal system. *Urology* 1997; **49**: 191–6
33 **Dobs AS, Meikle AW, Arver S *et al.*** Pharmacokinetics, efficacy, and safety of a permeation-enhanced testosterone transdermal system in comparison with bi-weekly injections of testosterone enanthate for the treatment of hypogonadal men. *J Clin Endocrinol Metab* 1999; **84**: 3469–78
34 **Meikle AW.** Androgen replacement therapy of male hypogonadism. In Meikle AW ed. *Endocrine Replacement Therapy in Clinical Practice*. Chapt 19. Totowa, NJ: Humana Press, 2003: 333–68
35 **Chiou WL.** Critical evaluation of the potential error in pharmacokinetic studies of using the linear trapezoidal rule method for the calculation of the area under the plasma level–time curve. *J Pharmacokinet Biopharm* 1978; **6**: 539–46
36 **Schuirmann DJ.** A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *J Pharmacokinet Biopharm* 1987; **15**: 657–80
37 **Steinijans VW, Diletti E.** Statistical analysis of bioavailability studies: parametric and nonparametric confidence intervals. *Eur J Clin Pharmacol* 1983; **24**: 127–36

Correspondence: A. Wayne Meikle, MD, University of Utah School of Medicine, Division of Endocrinology, 615 Arapeen Drive, Ste100, Salt Lake City, Utah 84108, USA.
e-mail: Wayne.Meikle@hsc.utah.edu

Abbreviations: **HDL**, high-density lipoprotein; **USP/NF**, United States Pharmacopeia/National Formulary; **AUC**, area under the serum testosterone concentration vs time curve; $\mathbf{C_0}$, serum testosterone concentration before treatment, i.e. immediately before gel application; $\mathbf{C_{avg}}$, average serum testosterone concentration, determined as $AUC_{0\text{-}24}$ divided by 24 h; $\mathbf{C_{max}}$, maximum serum testosterone concentration at 0–24 h after dose; $\mathbf{C_{min}}$, minimum serum testosterone concentration; $\mathbf{C_{max12–24}}$, maximum serum testosterone concentration at 12–24 h after dose; $\mathbf{AUC_{0–24}}$, AUC from time 0–24 h; $\mathbf{t_{max}}$, time of maximum serum testosterone concentration at 0–24 h after dose.