

Treatment of Male Hypogonadism With Testosterone Undecanoate Injected at Extended Intervals of 12 Weeks: A Phase II Study

SIGRID VON ECKARDSTEIN AND EBERHARD NIESCHLAG

From the Institute of Reproductive Medicine of the University, D-48129 Münster, Germany

ABSTRACT: This paper reports the result of an open-label, non-randomized clinical trial investigating the efficacy and safety of an injectable preparation of testosterone undecanoate (TU) dissolved in castor oil and given over a 3.2-year period. In a previous study we demonstrated that injections of TU every 6 weeks resulted in satisfactory substitution but a tendency toward testosterone accumulation. Here we investigate prolonged TU treatment at extended injection intervals in 7 hypogonadal men. Injections were given at gradually increasing intervals between the fifth and 10th injection, and from then on every 12 weeks. Steady state kinetics were obtained after the 13th injection. Well-being, sexual activity, clinical chemistry, prostate volume, and prostate-specific antigen (PSA) and serum hormone levels were monitored. Patients were clinically well-adjusted throughout the study. Before the next injection, testosterone, dihydrotestosterone, and estradiol levels were mostly within the

normal range and showed a tendency to decrease with increasing injection intervals. Body weight, hemoglobin, serum lipids, PSA, and prostate volume did not change significantly during the 3.2 years of treatment. PSA levels were always within the normal limit. Maximal testosterone levels during steady state kinetics were measured after 1 week with 32.0 ± 11.7 nmol/L (mean \pm SD). Before the last injection, mean testosterone concentrations were 12.6 ± 3.7 nmol/L. Compared with conventional testosterone enanthate or cypionate treatment requiring injection intervals of 2–3 weeks and resulting in supraphysiological serum testosterone levels, injections of TU at intervals of up to 3 months offer an excellent alternative for substitution therapy of male hypogonadism.

Key words: Injectable testosterone undecanoate, substitution of male hypogonadism.

J Androl 2002;23:419–425

Testosterone preparations have been in clinical use for substitution of male hypogonadism for more than a half century. However, only within recent years has the choice of different delivery forms increased. The newly developed transdermal preparations having short half-lives are predominantly tailored for therapy of senescent men (Nieschlag, 1998). For substitution of younger hypogonadal men and for hormonal male contraception, long-acting substances are more desirable. Therefore, the development and first clinical uses of injectable testosterone buciclate (Behre et al, 1995) and testosterone undecanoate (TU; Partsch et al, 1995; Zhang et al, 1998; Behre et al, 1999a) attracted much attention.

Six-week injections of 1000 mg TU in 4 mL castor oil resulted in well-maintained androgen-dependent functions without serious side effects (Nieschlag et al, 1999). However, after 4 injections, a tendency toward a gradual increase in testosterone levels was observed, suggesting that prolongation of application intervals should be possible.

In the present paper we report the results of continued substitution therapy with TU in 7 hypogonadal men, and explore the efficacy and safety of injection intervals of up to 12 weeks, for a total period of 3.2 years.

Materials and Methods

Patients

Seven men with primary or secondary hypogonadism aged 20 to 57 years, who had already participated in the first trial with 6-week injections of TU agreed to receive continuing treatment. Inclusion and exclusion criteria to enroll patients for TU treatment have been described previously (Nieschlag et al, 1999). Prolongation of the initial study protocol was approved by the ethics committee of the university and the State Medical Board, Münster. Written informed consent was obtained from subjects. Rules for clinical studies as provided by the Declaration of Helsinki and the standards of good clinical practice were followed.

Five patients entering the follow-up phase had primary hypogonadism, and 2 had secondary hypogonadism. The limit of serum testosterone for establishing the diagnosis of hypogonadism in our institute is 12 nmol/L. Diagnosis and the previous mode of substitution are given in Table 1. Two men had previously participated in the initial study comparing the pharmacokinetics of TU dissolved in either tea seed (“Chinese preparation”) or castor oil (Behre et al, 1999a).

Correspondence to: Dr E. Nieschlag, FRCP, Institute of Reproductive Medicine of the University, Domagkstrasse 11, D-48129 Münster, Germany (e-mail: nieschl@uni-muenster.de).

We are grateful to Jenapharm GmbH for supplying the TU injections (Prof Dr M. Oettel, Dr D. Hübler, and Dr Saad).

Received for publication August 30, 2001; accepted for publication December 19, 2001.

Table 1. Clinical characteristics of patients entering the follow-up phase of substitution therapy with 1000 mg TU

Patient	Diagnosis	Age (y)†	Treatment (before TU)†
1	Bilateral orchidectomy due to seminoma	37	TE 250 mg/4 weeks
2	Bilateral testicular atrophy due to cryptorchidism	19	None
3	Bilateral orchidectomy due to seminoma	49	TE 250 mg/2.5 weeks
4	Bilateral orchidectomy due to seminoma	37	None
5	Hypopituitarism (postcraniopharyngeoma)	57	TE 250 mg/3 to 4 weeks
6	Bilateral orchidectomy due to seminoma	31	TE 250 mg/2 to 4 weeks
7	Hypogonadotropic hypogonadism (ectopic neurohypophysis)	29	TE 250 mg/4 weeks

* Patients who had already participated in the study on comparative pharmacokinetics with TU in castor oil or tea seed oil.

† Age and previous treatment modalities refer to the date before the first TU application. TE indicates testosterone enanthate.

Testosterone Preparation

TU was obtained from Jenapharm GmbH & Co. KG, Jena, Germany. Each ampule contained 1000 mg TU dissolved in 4 ml castor oil. Single injections were administered with the total volume at one site intramuscularly into the musculus gluteus medius, taking care to perform injections slowly to avoid pain.

Study Design

The study was a clinical, open label, nonrandomized trial. Screening examinations had been completed before the first injection with TU as described previously (Nieschlag et al, 1999). Before the first TU application, all men under current treatment completed a washout phase of at least 4 weeks. An overview of studies evaluating TU, including the current design, is given in Table 2. Before entering the follow-up phase, all patients underwent another complete physical and genital investigation and assessment of clinical chemistry, hematology, and lipids, as well as sonography of testes and prostate. Well-being and sexuality were investigated by standardized questionnaires immediately before and at half-time between TU injections. Before each application, blood samples for measurements of hormones, sex hormone binding globulin (SHBG), albumin, PSA, clinical chemistry, lipidology, and hematology were obtained. Prostate size was determined sonographically before every second injection.

After 4 injections had been given at 6-week intervals, the intervals were gradually extended between the 5th and 10th injections. Intervals were extended by 1 to 2 weeks if serum testosterone levels were above 12 nmol/L before the next injection, and if subjective impairment of well-being was absent. From the 10th injection onward, TU was applied every 12 weeks. After the 13th application, steady state kinetics were obtained as evidenced by weekly determinations of testosterone serum concen-

trations for 12 weeks. Six weeks after the 18th application, the study was finished with a detailed final investigation, including a physical and genital examination, sonography of prostate and scrotal contents, and all blood values that had been monitored during the study.

Hormone Assays

Analysis was performed from venous blood samples that were centrifuged at $800 \times g$ for 10 minutes and then stored at -20°C until measurements were performed at the end of the study. Care was taken so that samples of one subject were measured within one assay.

Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, SHBG, prolactin, and PSA were analyzed by highly specific time-resolved immunofluorometric assays (Autodelphia; Wallace, Freiburg, Germany). The lower detection limits were 0.12 IU/L and 0.25 IU/L for FSH and LH, respectively; and 25 pmol/L, 6.3 nmol/L, and 0.5 $\mu\text{g/L}$ for estradiol, SHBG, and PSA, respectively. The normal range in our laboratory is 1–7 IU/L and 2–10 IU/L for FSH and LH, respectively, and 11–71 nmol/L for SHBG. The upper limits of normal for estradiol and PSA are 250 pmol/L and 4 $\mu\text{g/L}$, respectively. The intraassay and interassay coefficients of variation were 0.5 and 1.9 for FSH, 1.7 and 2.2 for LH, 1.9 and 5.0 for estradiol, 1.0 and 7.2 for SHBG, and 3.4 and 4.9 for PSA. Serum testosterone was measured by an enzyme-linked immunosorbent assay (Biocam Immunosystems; DRG Instruments, Marburg, Germany). The lower limit of normal is 12 nmol/L. Dihydrotestosterone (DHT) was analyzed by radioimmunoassay (DSL 9600; Diagnostic System Laboratories, Sinsheim, Germany). Intraassay and interassay coefficients of variation for testosterone and DHT were 3.4 and 5.6, and 4.8 and 9.2, respectively. Free

Table 2. Overview of studies evaluating pharmacology and effectiveness of injectable TU dissolved in castor oil

Study	Design	Patients	Publication
I	Pharmacokinetics after a single injection	$n = 14$	Behre et al, 1999a
II	Four injections at 6-week intervals	$n = 14$ ($n = 2$ continued from study I)	Nieschlag et al, 1999
III	a) Nine injections at increasing intervals from 6 to 12 weeks b) Steady state pharmacokinetics c) Five injections at 12-week intervals	$n = 7$ ($n = 2$ continued from studies I and II) ($n = 5$ continued from study II)	

testosterone was calculated using the formula suggested by Vermeulen et al (1999).

Clinical Chemistry, Hematology, and Lipids

Biochemical and hematological parameters were determined at the Institute of Laboratory Medicine, University of Münster using, standard techniques. Quality control was performed according to the standards provided by the German Society of Clinical Chemistry.

Evaluation of Well-Being and Sexuality

During treatment patients were asked to complete standardized questionnaires to assess mood and sexual performance. Completed questionnaires were obtained before injections and at the halfway point of the respective injection interval.

Evaluation of Prostate

Prostate volume was monitored by transrectal ultrasound using a 7.5 MHz probe (The Panther; B&K Medical, Norderstedt, Germany). Prostate examinations included planimetric determination of volume (Behre et al, 2000) and assessment of sonographic texture.

Statistics

Statistical analysis was performed using the SPSS statistical package for Windows (version 10.0). All variables were checked for normal distribution by the Kolmogorov-Smirnov one-sample test for goodness-of-fit. Descriptive statistics are given as either means \pm SD or median, and the 2.5 to 97.5 percentiles. For analysis of variance (ANOVA) over time, one-way analysis of variance was calculated, which was followed by the Dunnett post-hoc test for intergroup comparison if an overall level of significance of $P < .05$ was reached. When necessary, analysis was performed after logarithmic transformation of data.

Results

General Effects, Well-Being, and Sexual Function

During TU applications, patients reported stable values for all parameters of well-being and sexual function (numbers of erections and ejaculations per week and satisfaction with sex life). At the end of the injection interval, when questionnaires were compared with those at half-time, no statistically significant differences were found.

Injections were well tolerated by all men except one, who requested extremely slow injections to avoid discomfort. No local side effects or impaired well-being occurred, except for one occasion when, during prostate sonography, a patient had short-term circulatory problems after the injection. One patient complained initially of mild acne within 2 weeks following injection. However, these problems disappeared during the 12-week intervals. In addition, the same patient developed slight gynecomastia during the first part of the study (6 weekly injections), which remained unchanged during the follow-up period.

General adverse events related to the treatment were not observed. One patient experienced an episode of herpes zoster, which required antiviral therapy after severe psychological trauma.

Body Weight

During TU applications, body weight increased slightly from 83.5 ± 9.5 kg to 85.7 ± 9.1 kg without reaching the level for statistical significance. Compared with the baseline, the maximum mean body weight was observed at the end of the study period.

Testosterone and Free Testosterone

Testosterone serum levels and calculated free testosterone levels obtained before injections are shown in Figure 1. During the 6-week injection interval, testosterone levels increased initially from 5.2 ± 3.1 nmol/L to 23.8 ± 7.8 nmol/L after patients had received 4 injections in 6 weeks. With extended injection intervals, preapplication testosterone levels decreased and were just at the lower limit of normal, with 12.6 ± 3.7 nmol/L before the last injection. A comparable pattern was observed for calculated free testosterone levels, which rose to 573 ± 202 pmol/L after the 6-week period, and then returned to the lower limit of normal (291 ± 93 pmol/L) after 8 injections had been performed at 12-week intervals.

Maximum steady state kinetics for levels of testosterone and free testosterone were reached after 1 week. The mean maximum concentration for testosterone was 32 nmol/L, ranging from a minimum of 15.6 to a maximum of 44.3 nmol/L. A comparable pattern was observed for free testosterone levels, with a mean of 787 pmol/L (Table 3). Initial kinetics obtained in 14 subjects after the first injection of TU and steady state kinetics in the current trial are shown in Figure 2.

Estradiol and DHT

DHT and estradiol concentrations essentially followed the pattern of that for testosterone and free testosterone. During the short injection intervals, DHT levels occasionally exceeded the upper normal limit but returned to the lower limit of normal after 5 injections over 12 weeks had been applied (Figure 1). Estradiol levels always stayed within normal limits.

LH and FSH

LH and FSH values decreased significantly during the study, from initial values of 18.7 ± 7.1 IU/L (LH) and 30.5 ± 27.3 IU/L (FSH) to 0.4 ± 0.8 IU/L (LH) and 1.5 ± 2.9 IU/L (FSH) after 24 weeks. Before the last injection, LH values of 3.0 ± 5.0 IU/L and FSH values of 7.7 ± 13.9 IU/L were measured (Figure 3). Complete sup-

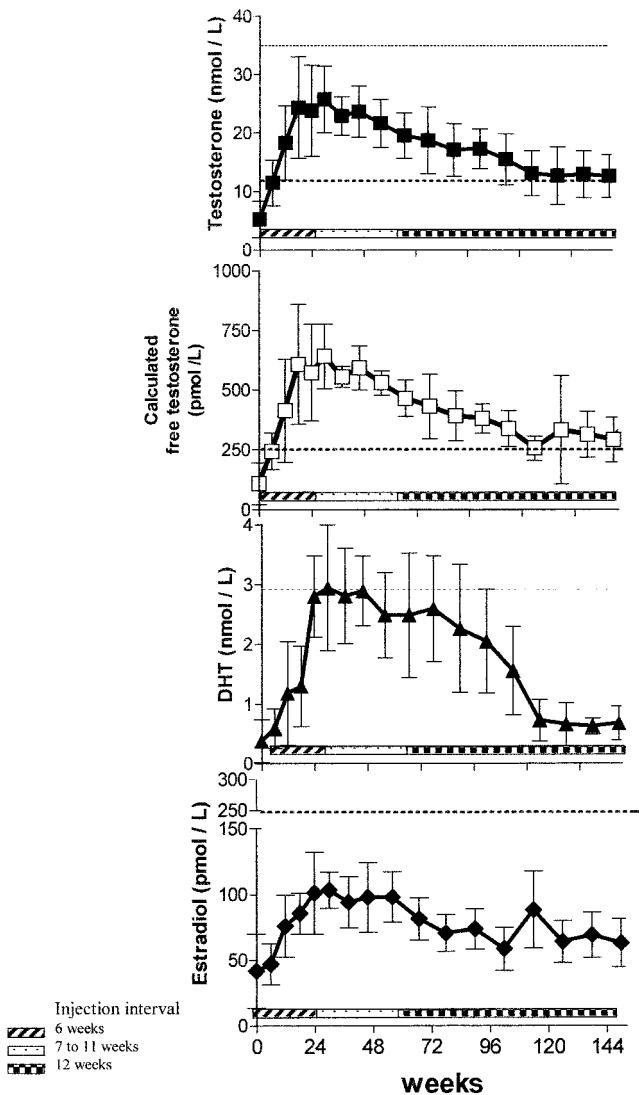


Figure 1. Serum testosterone, free testosterone, DHT, and estradiol in 7 hypogonadal men treated with 1000 mg TU in increasing injection intervals. Values were obtained before injections and are given as means \pm SD. Dotted lines indicate normal limits.

Table 3. Serum testosterone and calculated free testosterone steady state pharmacokinetics obtained after 102 weeks (13th injection) of treatment with 1000 mg TU i.m.

	Testosterone (nmol/L)*	Free testosterone (pmol/L)*
C _{max}	32.0 \pm 11.7	844 \pm 272
C _{min}	14.3 \pm 4.1	293 \pm 71
C _{avg}	20.7 \pm 7.9	481 \pm 215
AUC _(102-114 weeks)	1617 \pm 372	Not determined
T _{max} (days)	9 \pm 4.9	9 \pm 4.9
T _{1/2} (days)	70.2 \pm 21.2	Not determined

* Values are means \pm SD.

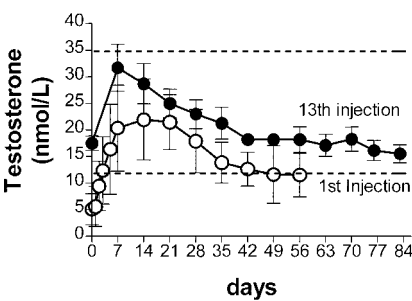


Figure 2. Serum testosterone after a single injection of 1000 mg TU in 14 untreated hypogonadal men (Behre et al, 1999) (open circles) and during treatment with TU for 102 weeks (closed circles). Values are given as means \pm SD. Dotted lines indicate normal limits.

pression of LH and FSH was seen in 3 out of 5 hypergonadotropic subjects, whereas suppression was incomplete with undetectable LH but still detectable FSH levels in 2 out of 5 men.

Clinical Chemistry, Hematology, and Lipids

Biochemical parameters (sodium, potassium, chloride, total protein, glucose, alkaline phosphatase, creatinine, uric acid, total bilirubin, gamma glutamyl transferase [γ GT], aspartate amino transferase [ASAT], alanine amino transferase [ALAT], prothrombin time, and Quick) remained unchanged during treatment.

Figure 4A shows hematocrit values as an example of hematological parameters during TU application. During treatment, no statistically significant changes (using ANOVA) were observed for hemoglobin, hematocrit, or erythrocytes. Compared with the baseline, a small increase in hemoglobin, hematocrit, and erythrocytes oc-

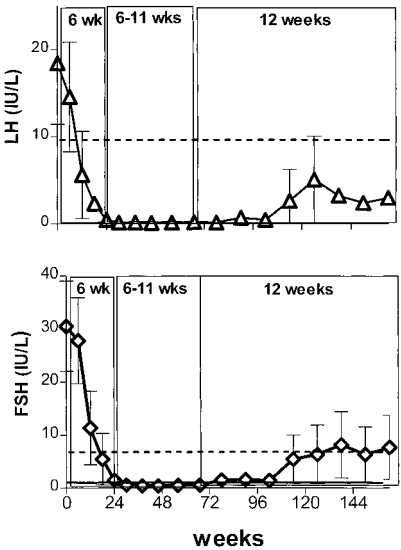


Figure 3. Serum LH and FSH in 7 hypogonadal men treated with 1000 mg TU in increasing injection intervals. Values were obtained before injections and are given as means \pm SD. Dotted lines indicate normal limits. Injection intervals are marked with boxes.

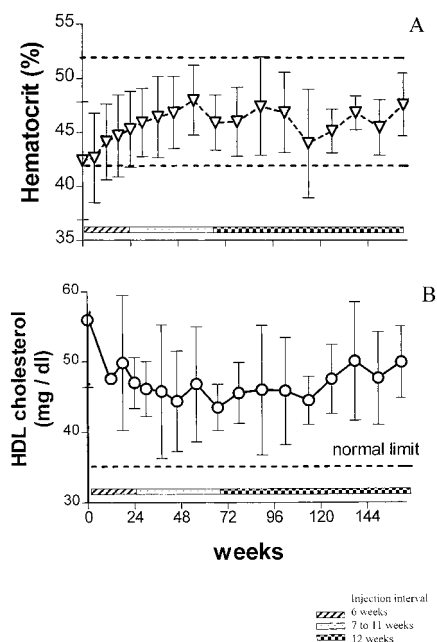


Figure 4. Hematocrit (A) and HDL cholesterol (B) concentrations in 7 hypogonadal men treated with 1000 mg TU in increasing injection intervals. Values were obtained before injections and are given as means \pm SD. Dotted lines indicate normal limits.

curred, which ranged between 11.3% and 14.6%. Only on a single occasion was a hemoglobin level measured above the upper limit of normal (18.0 g/dL), and in 8.2% of all values measured, a hematocrit level above the upper limit of normal was measured. All these values were observed in a single subject who was age 47 at the beginning of the study.

During treatment, lipid parameters did not change significantly. Compared with baseline levels, a slight decrease in high-density lipoprotein (HDL; -13.8%) (Figure 4B) as well as low-density lipoprotein levels (LDL; -10.4%) was observed. However, of all measurements performed, on only one occasion was an HDL level outside the recommended range for the primary prevention of cardiovascular disease, 11.3% of LDL levels were outside the range, and 14.3% of triglyceride levels were outside the range.

Prostate and PSA

When the entire treatment period was evaluated, no statistically significant changes in prostate volume or PSA were observed. However, there was an increase in prostate volumes from an initial size of 13.6 ± 6 mL to 23 ± 6.1 mL at the final examination. In none of the cases were PSA values measured beyond the normal range. During the 6-week injection phase, a slight but insignificant increase in PSA values was observed, which decreased steadily with prolongation of injection intervals (Figure 5). Initial PSA values were 0.6 ± 1.3 $\mu\text{g/L}$ compared with

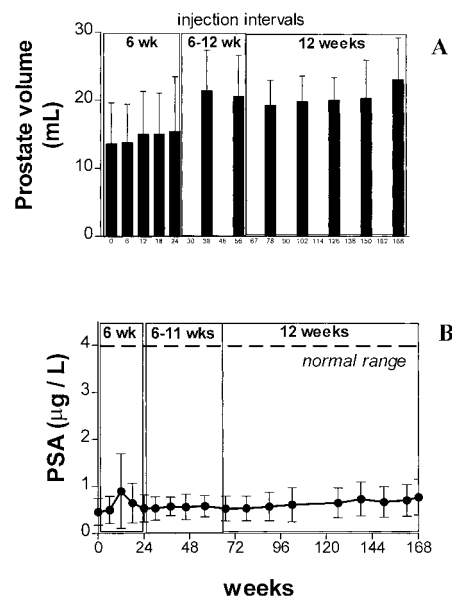


Figure 5. Prostate volumes (A) and serum PSA (B) in 7 hypogonadal men treated with 1000 mg TU at increasing injection intervals. Values were obtained before injections (PSA) or before every second injection (prostate volume) and are given as means \pm SD. Dotted lines indicate normal limits. Injection intervals are marked with boxes.

1.1 ± 2.4 $\mu\text{g/L}$ after 3 injections, and 0.8 ± 0.4 $\mu\text{g/L}$ at the end of the trial.

Discussion

The current paper summarizes a 3.2-year period of treating male hypogonadism with injectable TU. Apart from three other studies for oral TU (Gooren et al, 1994), transscrotal testosterone substitution (Behre et al, 1999b), and the body patch (Snyder et al, 2000), this is one of the longest reported observation periods with a single testosterone preparation.

Based on our initial pharmacokinetic study we postulated that injections will maintain normal testosterone levels for 6 to 10 weeks (Behre et al, 1999a). Choosing the shortest injection interval, it turned out that TU had a tendency to accumulate when given at 6-week periods (Nieschlag et al, 1999). The current trial confirmed that the schedule of application can be extended up to 12 weeks once normal testosterone levels have been achieved. It is difficult to speculate on testosterone profiles if treatment would have been based on 12-week intervals from the beginning, because all men participating in this trial had already participated in the earlier 6-week schedule. Withdrawal of therapy is accompanied by severe disturbances in well-being and, as a result, it is disliked by patients. For this reason we did not include a second washout phase.

The half-life of injectable TU in our patients is some-

what longer than that reported from Chinese men receiving a single injection of 1000 mg TU (Zhang et al, 1998). However, data from the initial comparison of pharmacokinetics of both preparations in white men had suggested that the dissolvent not only affected maximum concentrations, but also exerted an effect on duration of action (Behre et al, 1999a). Data obtained in men of different ethnic origins may not be comparable. For example, maximum testosterone concentrations achieved with TU in tea seed oil are considerably higher in Chinese men than in white men (Zhang et al, 1998; Behre et al, 1999a). Whether these differences result from differences in body composition or whether they are due to inherited traits of metabolism is still being debated (van Houten and Gooren, 2000). In addition, it has to be considered that due to the weekly sampling strategy in our study, there is a small risk of missing higher peak levels before Day 7, although this seems unlikely based on pharmacokinetic data obtained after a single injection (Behre et al, 1999a).

Steady state pharmacokinetics differed considerably from those obtained after a single injection. The longer half-life of the substance is probably due to the structural differences between TU and testosterone enanthate (TE). TU has a longer aliphatic and thus more hydrophobic side chain (Behre et al, 1999a). C_{max} is higher during steady state kinetics and is reached earlier. Comparable observations have been reported for transdermal testosterone preparations (Swerdlow et al, 2000). The maximum concentrations seen in steady state kinetics with TU are lower than those reported for the higher dose of testosterone gel, Androgel (Unimed, Buffalo Grove, Ill) (100 mg/day, 37.5 nmol/L) and only slightly higher than those reported for the body patch, Androderm (SKB, Collegeville, Pa) (26.5 nmol/L) or the lower dose of testosterone gel (50 mg, 28.8 nmol/L) (Dobs et al, 1999; Swerdlow et al, 2000). Maximum as well as preinjection free testosterone levels in our trial were even lower than those reported following a dose of 100 mg of testosterone gel given daily. However, it has to be considered that we used calculation of free testosterone based on testosterone and SHBG determinations, and Swerdlow et al (2000) used equilibrium dialysis to determine free testosterone. In addition, with increasing use of testosterone preparations, it may become important that resorption rates of all transdermal preparations are comparably low. With each application of, for example, 100 mg of testosterone gel, 85 mg of testosterone escapes resorption and enters the environment.

Improvement and stabilization of mood is one of the prime effects of testosterone substitution in male hypogonadism (Wang et al, 1996). In this connection, the fluctuations in serum testosterone levels arising during treatment with TE or testosterone cypionate (TC) are not well tolerated by patients. Even though TU injections initially result in slightly supraphysiological levels, depending on

the injection intervals and decrease steadily thereafter, no clinically apparent changes in mood occurred. Features of well-being did not differ when they were examined at the end or at the half point of injection intervals. These data also confirm that although mood and sexual function are normalized, they are unaffected by actual testosterone levels as long as they are within normal limits (Zitzmann and Nieschlag, 2001). However, it must be considered that the number of men who could be included in the follow-up part of the study is relatively small. Therefore, statistical power is probably insufficient to detect smaller changes in parameters with a high baseline variability. Direct comparisons between short-acting and long-acting testosterone preparations or the larger phase III trials may be better suited for evaluating such physiological effects.

One of the arguments put forward against injectable testosterone preparations is the risk of polycythemia, especially in older men (Hajar et al, 1997). When TE is compared with testosterone patches, the frequency of increased hemoglobin or hematocrit is 2.8-fold higher with TE treatment (Dobs et al, 1999). It is interesting that in our study, the occasions of elevated hematological parameters were as low with transdermal testosterone, although our population included two men older than age 50. Therefore, the relative time period of supraphysiological testosterone concentrations rather than the total testosterone values achieved may increase the risk for polycythemia. A comparable observation was made for changes in lipoproteins. After the expected initial decrease in HDL and increase in total cholesterol, values remained generally stable, and at the end of the 3-year treatment phase, the increase of 10.4% in LDL and the decrease of 13.8% in HDL were much smaller than those previously reported for TE or testosterone implant application (Jockenhövel et al, 1999). It must be considered that the composition of the study group may explain these effects, because some men may be more susceptible to testosterone-induced changes in lipid parameters (Zitzmann et al, 2001) than others. However, it is unclear how pharmacological testosterone effects on lipoproteins translate to adverse cardiovascular events (von Eckardstein, 1998), and it is therefore difficult to judge whether the observed lipid profile after TU injections is more desirable than those of TE or TC.

PSA levels are especially sensitive to changes in testosterone concentrations because the pattern of PSA very much mimics that of testosterone in serum. As shown by Jin et al (2001), prostate volumes depend on testosterone levels to a lesser extent. The gradual increase in prostate volume during TU treatment occurring after the initial increase in prostate volume resulting from correction of hypogonadism most likely reflects age-related changes. There is convincing evidence that prostate volumes in tes-

tosterone-treated men do not exceed those of eugonadal controls (Behre et al, 1994; Jin et al, 2001).

In summary, results of this trial show that in an injectable form, TU is a highly interesting alternative to the currently most widely used injectable preparations, TE and TC. When applied at appropriate intervals of 10 to 12 weeks, TU injections by and large avoid supraphysiological testosterone levels, and their unwanted side effects. In addition, the study is one of the few trials reporting long-term treatment extending over more than three years with a single preparation. As recent advances in hormonal male contraception (Kamischke et al, 2000 and 2001) and substitution of male senescence indicate an increased demand for testosterone preparations, such information on the long-term safety of testosterone application is timely and crucial.

Acknowledgments

We thank M. Ernst for monitoring the study, N. Terwort for technical assistance, and Susan Nieschlag for language assistance.

References

- Behre HM, Bohmeyer J, Nieschlag E. Prostate volume in testosterone-treated and untreated hypogonadal men in comparison to age-matched normal controls. *Clin Endocrinol*. 1994;40:341–349.
- Behre HM, Baus S, Kliesch S, Keck C, Simoni M, Nieschlag E. Potential of testosterone buciclate for male contraception: endocrine differences between responders and nonresponders. *J Clin Endocrinol Metab*. 1995;80:2394–2403.
- Behre HM, Abshagen K, Oettel M, Huebler D, Nieschlag E. Intramuscular injection of testosterone undecanoate for the treatment of male hypogonadism: phase I studies. *Eur J Endocrinol*. 1999a;140:414–419.
- Behre HM, von Eckardstein S, Kliesch S, Nieschlag E. Long-term substitution therapy of hypogonadal men with transscrotal testosterone over 7–10 years. *Clin Endocrinol*. 1999b;50:629–635.
- Behre HM, Yeung CH, Holstein AF, Weinbauer GF, Gassner P, Nieschlag E. Diagnosis of male infertility and hypogonadism. In: Nieschlag E, Behre HM, eds. *Andrology: Male Reproductive Health and Dysfunction*. 2nd edition. Heidelberg: Springer; 2000:89–124.
- Dobs AS, Meikle AW, Arver S, Sanders SW, Caramelli KE, Mazer NA. 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;84:3469–3478.
- Gooren LJ. A ten-year safety study of the oral androgen testosterone undecanoate. *J Androl*. 1994;15:212–215.
- Hajar RR, Kaiser FE, Morley JE. Outcomes of long-term testosterone replacement in older hypogonadal males: a retrospective analysis. *J Clin Endocrinol Metab*. 1997;82:3793–3796.
- Jin B, Conway AJ, Handelsman DJ. Effects of androgen deficiency and replacement on prostate zonal volumes. *Clin Endocrinol*. 2001;54:437–445.
- Jockenhövel F, Bullmann C, Schubert M, Vogel E, Reinhardt W, et al. Influence of various modes of androgen substitution on serum lipids and lipoproteins in hypogonadal men. *Metabolism*. 1999;48:590–596.
- Kamischke A, Plöger D, Venherm S, von Eckardstein S, von Eckardstein A, Nieschlag E. Intramuscular testosterone undecanoate with or without oral levonorgestrel: a randomized placebo-controlled feasibility study for male contraception. *Clin Endocrinol*. 2000;53:43–52.
- Kamischke A, Venherm S, Plöger D, von Eckardstein S, Nieschlag E. Intramuscular testosterone undecanoate and norethisterone enanthate in a clinical trial for male contraception. *J Clin Endocrinol Metab*. 2001;86:303–309.
- Nieschlag E. Therapeutic controversy VII: If T, which T? Which androgen regimen should be used for supplementation in older men? Formulation, dosing and monitoring issues. *J Clin Endocrinol Metab*. 1998;83:3443–3445.
- Nieschlag E, Büchter D, von Eckardstein S, Abshagen K, Simoni M, Behre HM. Repeated intramuscular injections of testosterone undecanoate for substitution therapy of male hypogonadism. *Clin Endocrinol*. 1999;51:757–763.
- Partsch CJ, Weinbauer GF, Fang R, Nieschlag E. Injectable testosterone undecanoate has more favourable pharmacokinetics and pharmacodynamics than testosterone enanthate. *Eur J Endocrinol*. 1995;132:514–519.
- Snyder PJ, Peachey H, Berlin JA, et al. Effects of testosterone replacement in hypogonadal men. *J Clin Endocrinol Metab*. 2000;85:2670–2677.
- Swerdlow RS, Wang C, Cunningham G, et al. Long-term pharmacokinetics of transdermal testosterone gel in hypogonadal men. *J Clin Endocrinol Metab*. 2000;85:4500–4510.
- van Houten ME, Gooren LJ. Differences in reproductive endocrinology between Asian men and Caucasian men—a literature review. *Asian J Androl*. 2000;2:13–20.
- Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab*. 1999;84:3666–3672.
- von Eckardstein A. Androgens, cardiovascular risk factors and atherosclerosis. In: Nieschlag E, Behre HM, eds. *Testosterone: Action, Deficiency, Substitution*. 2nd edition. Heidelberg: Springer; 1998:229–258.
- 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;81:3578–3583.
- Zhang GY, Gu YQ, Wang XH, Cui YG, Bremner WJ. A pharmacokinetic study of injectable testosterone undecanoate in hypogonadal men. *J Androl*. 1998;19:761–768.
- Zitzmann M, Nieschlag E. Testosterone levels in healthy men and the relation to behavioural and physical characteristics: facts and constructs. *Eur J Endocrinol*. 2001;144:183–197.
- Zitzmann M, Brune M, Kornmann B, Gromoll J, von Eckardstein S, von Eckardstein A, Nieschlag E. The CAG repeat polymorphism in the androgen receptor gene affects high density lipoprotein cholesterol and arterial vasoreactivity. *J Clin Endocrinol Metab*. 2001;86:4867–4873.