Pharmacokinetics and Degree of Aromatization Rather Than Total Dose of Different Preparations Determine the Effects of Testosterone: A Nonhuman Primate Study in *Macaca fascicularis*

GERHARD F. WEINBAUER,* CARL-JOACHIM PARTSCH,† MICHAEL ZITZMANN, STEFAN SCHLATT, AND EBERHARD NIESCHLAG

From the Institute of Reproductive Medicine of the University, Domagkstrasse 11, D-48129 Münster, Germany

ABSTRACT: Currently available testosterone (T) preparations differ substantially in their pharmacokinetic profile that might influence their androgenic properties in terms of suppression of the gonadal axis, effects on anabolic parameters, lipid metabolism, and erythropoiesis. The present work was undertaken to determine the physiological effects of three T preparations with different serum kinetics. Twenty adult male cynomolgus monkeys (*Macaca fascicularis*) were randomly assigned to receive treatment for 28 weeks with either T enanthate (TE) every 4 weeks, T buciclate (TB) every 7 weeks, or T undecanoate (TU) every 10 weeks or remaining untreated (controls). Each injection delivered 20 mg pure T per kilogram body weight. Pharmacokinetic profiles demonstrated higher peak levels of T for TE-treated animals; serum half-lives were longer for TU or TB. Estradiol levels (area under the curve) were significantly higher in TB vs TU or TE. All T regimens suppressed serum luteinizing hormone bioactivity and testicular volumes declined (all $P <$ .001 vs controls). Sperm counts were markedly lowered in all animals but least in TE ($P <$ .01 vs TB or TU). During recovery phase,

return to normal for all three parameters occurred significantly earlier in TE-treated animals, followed by those given TU, compared with TB (all $P <$ .001 between groups). Body weight increased significantly during T exposure. This effect was stronger and more sustained in TB vs TU or TE (both $P <$ .001). Serum creatinine and hemoglobin increased with high significance in all T-treated animals (all $P <$ .001 vs controls). The lowering impact of T on serum lipids was markedly stronger in the longer-acting T preparations in comparison with TE, as were effects on purine metabolism (all $P <$ .001). The pattern of exposure and degree of aromatization rather than overall exposure to T determine its effects in the preclinical primate model. Both fluctuations of androgen concentrations and the conversion rate to estradiol influence gonadal suppression as well as metabolism. These results have to be considered in men receiving treatment for hypogonadism or regimens for hormonal contraception.

Key words: Spermatogenesis, testis, male contraception, clinical chemistry, primates.

J Androl 2003;24:765–774

Androgen substitution should provide physiological androgen levels over prolonged periods of time, and supraphysiological peaks should be avoided (WHO et al, 1992). Various injectable testosterone (T) preparations differ substantially with regard to the elicited serum pattern of T levels. Whereas T enanthate (TE) produces short-lived but supraphysiologically high serum T peaks, T buciclate (TB) and T undecanoate (TU) provide more favorable T kinetic profiles (Behre and Nieschlag, 1998).

Serum half-lives of TB and TU are severalfold higher than those of TE, and TB and TU produce rather smooth serum T levels (Behre and Nieschlag, 1992; Partsch et al, 1995; von Eckardstein and Nieschlag, 2002). Both TB and TU exhibit pronounced androgenic effects in java monkeys (Partsch et al, 1995; Weinbauer et al, 2001).

It can be speculated that the substantially different pharmacokinetic properties of various T esters exert differential androgenic effects on physiological parameters known to be affected by T, such as lipid metabolism (eg, Whitsel et al, 2001), erythropoiesis (eg, Wang et al, 2000), and anabolic properties (eg, Bhasin et al, 2001). Also, the suppression of luteinizing hormone (LH) release from the pituitary gland and subsequent inhibition of spermatogenesis can serve as clinical marker. Therefore, the present work was undertaken to compare directly the androgenic potency of a short and 2 long-acting T esters, TE, TB, and TU. The cynomolgus monkey was chosen as the preclinical model because its endocrine control of testicular function is similar to that of men (Weinbauer and Korte, 1999). We refrained from including transder-

mal or oral preparations in the monkey model because this procedure would have involved using not only various substances but also different administration pathways (which can cause problems of reliable absorption in an animal model); androgenic potency in relation to pharmacokinetic properties would be more difficult, if not impossible, to differentiate.

Material and Methods

Animals

Twenty adult, male, noncastrated cynomolgus monkeys (*Macaca fasicularis*) (4.3–6.8 kg body weight) were maintained under a 12:12 hour day:night regimen in a temperature-controlled environment. All animals were fed species-specific pelleted food, had unlimited access to tap water, and monkey chow supplemented daily with fresh fruit. All experimental studies were undertaken in accordance with the German Federal Law on the Care and Use of Laboratory Animals.

Androgen Preparations

TE—The substance is dissolved in castor oil and marketed worldwide. Ampoules with a concentration of 250 mg/mL TE sesame oil were obtained from Schering AG (Berlin, Germany).

TU—Ampoules with a concentration of 125 mg/mL TU tea seed oil were obtained from Zhejiang Xian Ju Pharmaceutical Corp. (Zhejiang, People′s Republic of China).

TB—This experimental substance (T 178 trans4-n-butylcyclohexylcarboxylate 20 Aet-I) in aqueous solution was obtained from the World Health Organization (WHO)/National Institutes of Health. It was prepared by Palmer Research (Holywell, Great Britain) under WHO auspices under good manufacturing practice conditions. After wet milling of crystalline TB to a particle size of at least 75% in the range of l0–50 μm, the drug was sterilized by γ-irradiation and suspended in sterile aqueous solution. The TB suspension was shaken vigorously for 1 minute before injection. The batch used for all injections had a concentration of 250 g/L TB, which equals a concentration of 158.6 g/L unesterified T (0.55 mol/L).

Experimental Design and Sample Collection

Animals were randomly assigned to 4 groups of 5 animals. Following baseline investigations, the experimental protocol consisted of injections of TE, TB, or TU. The fourth group did not receive injections and served as control. Injections (intramuscular) of TE were given in 4-week intervals (weeks 0, 4, 8, 12, 16, 20, 24); TB in 7-week intervals (weeks 0, 7, 14, 21); and TU in 10-week intervals (weeks 0, 10, 20). The entire observation period lasted for 65 weeks. Animals were dosed to receive 20 mg/kg of pure T per each injection (the calculation accounted for the differences in molecular weight among the 3 esters). Injection volumes ranged between 1.0 and 1.9 mL. During the 28-week period, the total dose administered to each monkey was 140 mg/kg T for TE, 80 mg/kg T for TB, and 60 mg/kg T for TU. Because the application frequency was different for each T preparation and a sham injection scheme would have been different for each preparation, we refrained from giving sham injections.

Ejaculates were collected by an established procedure using rectal probe electrostimulation of ketamine hydrochloride (10–15 mg/kg) in sedated animals. Serum samples were collected from sedated animals by venipuncture of the cubital or saphenous vein, and cooled blood was allowed to clot overnight, centrifuged twice, and stored at −20°C until analysis of serum bioactive LH, T, inhibin B, and estradiol levels. Blood samples were collected on days 1, 2, 4, 6, 8, 10, 12, 14, 17, 21, 24, 28, and 35 and in weekly intervals thereafter until day 450. During the treatment interval, samples to determine nadir levels were obtained immediately before T injections. Analysis of inhibin B was limited to samples collected in week 0 and biweekly intervals until week 28. In weeks 0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 40, 48, and 68, blood was also processed for analysis of hematocrit and hemoglobin, alkaline phosphatase, triglycerides, glutamic-oxaloacetic transaminase, glutamate pyruvate transaminase, γ-glutamyl transpeptidase (GGT), lipid fractions, electrolytes, and total protein. Lipid measurements were performed from fasting animals.

Determinations

Bioactivity of LH was measured using an established mouse Leydig cell-based bioassay (Wickings et al, 1979). Assay sensitivity was 2.7 IU/L and intra- and inter-assay variation was below 5% and 10%, respectively. Testosterone was determined from ether-extracted serum using an established radioimmunoassay (Chandolia et al, 1991). Detection limit was 0.67 nmol/L and intra- and interassay variation was 6.9% and 13.3%, respectively. Estradiol levels were assessed by a commercial radioimmunoassay kit (Sorin Biomedica, Puchheim, Germany). The limit of detection was 18.5 pmol/L and intra- and interassay coefficients of variation were 5.6% and 11.1%. Serum concentrations of inhibin B were determined using a commercially available assay as described earlier (Foppiani et al, 1999). Detection limit was <8 ng/L and intra- and interassay variations were 3.1% and 11%, respectively.

Testicular volume was determined from caliper measurements of testicular width and length using the formula for a regular ellipsoid for volume calculation. Data are expressed as combined volume of left plus right testis. Sperm numbers were counted in the liquid and solid portions of the ejaculate and numbers are expressed per whole ejaculate sample.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) and multivariate analysis of variance procedures and Duncan multiple range test for post hoc comparisons. Areas under curve (AUC) data were analyzed by ANOVA followed by least significant differences test. Sperm concentrations showed a skewed distribution; respective data are expressed as median levels and analyzed as percent changes from baseline values by a nonparametric test for repeated measurements (FRIEDMAN test). For comparison of clinical chemistry, data between groups, ANOVA for repeated measurements was used. To allow post hoc tests between various groups according to Scheffé, which is not possible using covariates, data were transformed within each group

to homogenous dimensionless baseline values. Because post hoc tests between time points and groups were numerous, corrections of *P* levels according to Bonferroni were performed. Computations were performed using the statistical software package SPSS (Chicago, IL, release 10.0.7).

Results

Hormones

Under TE, serum T levels, on average, attained interinjection peaks of 200–300 nmol/L within 1 week and dropped thereafter (Figure 1). In groups exposed to TB and TU, peak T levels were clearly lower (100 and 200 nmol/L) but were maintained for several weeks. Control group T levels were < 50 nmol/L. Serum estradiol levels followed a similar pattern, compared with T concentrations.

AUCs were calculated for weeks 0–28 of the study for the T-exposed groups. Values for AUC were similar for T ($P > .70$) and estradiol ($P > .20$) (Figure 2). Response areas under curve (RAUCs) were also calculated following subtraction of the baseline levels: T levels during T exposure were similar for TE and TU groups but were about twofold higher, compared with the TB group (Figure 2). Conversely, RAUC estradiol levels were highest in the TB group and similar for the TE- and TU-exposed groups (Figure 2). Ratios for T_{RAUC} over estradiol$_{RAUC}$ were significantly lower in the TB group (0.23 ± 0.04), compared with TE (0.62 ± 0.11, $P < .001$) and TU (0.77 ± 0.09, $P < .001$).

Pharmacokinetic data were determined from averaged T measurements during the first injection interval, ie, days 0–49 for TB, days 0–28 for TE, and days 0–70 for TU (Table 1). Half-lives were shortest for TE and longest for TU. Conversely, peak T levels were highest and occurred earliest under TE.

All T regimens suppressed serum LH bioactivity (Figure 3) to levels close to or below the detection limit of the assay. During treatment, the reduction of LH activity was similar for all T-treated groups ($P < .001$ vs controls). Recovery of LH secretion occurred earliest in the TE group; differences of LH concentration in these animals in comparison with those treated with TU or TB became significant after week 48 (both $P < .001$). The longest suppression was seen in TB-treated monkeys, also in comparison with those given TU ($P < .01$). Similarly, all regimens markedly lowered inhibin B concentrations during the period of exposure to T esters (respective $P < .001$ in comparison with the control group).

Body Weight

Baseline body weights were 5.9 ± 0.2 kg, 6.0 ± 0.3 kg, 5.9 ± 0.3 kg, and 6.0 ±0 .5 kg in the control, TB-, TE-,

and TU-exposed groups, respectively. Body weight started to increase in all T-treated groups within 3 weeks of exposure (Figure 4, *P* for every group <.001 in comparison with controls). Highest gain of body weight was seen in the TB group with an increase of about 25% (1.5 kg) by weeks 25–30 of the study. Body weights in the TE- and TU-treated groups were similar, and increments were around 10% over baseline during the latter period of androgen exposure. The differences in gain of body weight were not only significant in comparison with the control group but also between the TE- as well as the TU-treated group vs the TB-treated animals ($P < .001$ starting from week 9–10, respectively). The TE- and TU-treated monkeys did not differ in their gain of body mass. Weight returned to baseline significantly earlier in these animals in comparison with TB-treated ones (both $P < .001$, Figure 4).

Testis Size

Baseline testicular dimensions were 21 ± 1.5 mL, 23 ± 1.0 mL, 27 ± 3 mL, and 24 ± 2 mL kg in the control, TB-, TE-, and TU-exposed groups, respectively. Testicular volume was significantly ($P < .001$) reduced to 55–60% of baseline in all T-exposed groups vs controls (Figure 5). Recovery of testis size (50%) was most rapid in the TE-treated (7 weeks) animals followed by TU (10 weeks) and TB (20 weeks). The differences between treatment groups during recovery started to occur in week 34 and were significantly different for TE vs TB, TE vs TU, and TB vs TU (all $P < .001$).

Sperm Numbers

Sperm numbers were suppressed significantly ($P < .001$) in all T-treated groups (Figure 6). Data for the control group were 49 million/ejaculate (median). Overall, during T exposure, sperm numbers were higher in the TE group (median 26 million/ejaculate), compared with TU (median 7.5 million/ejaculate) and TB (median 15 million/ejaculate) (significant differences between TE vs TU or TE vs TB for changes from baseline: both $P < .01$, no significant difference between TU and TB during the treatment phase) (Figure 6). Average suppression in comparison with baseline during the last 10 weeks of medication was 14.3 ± 6.0% for TE, 7.9 ± 3.1% for TB and 8.4 ± 5.3% for TU (Figure 6). Sustained azoospermia could not be achieved except for 1 animal in the TE group. This animal also had very low baseline sperm counts (26 million/ejaculate). Recovery of sperm numbers (50%) occurred significantly earlier following TE (12 weeks), compared with TU (16 weeks) and TB (23 weeks) (TE vs TB, TE vs TU, and TU vs TB: all $P < .01$).

Blood Count, Clinical Chemistry

Testosterone-treated animals showed significantly higher hemoglobin production and higher levels of serum cre-
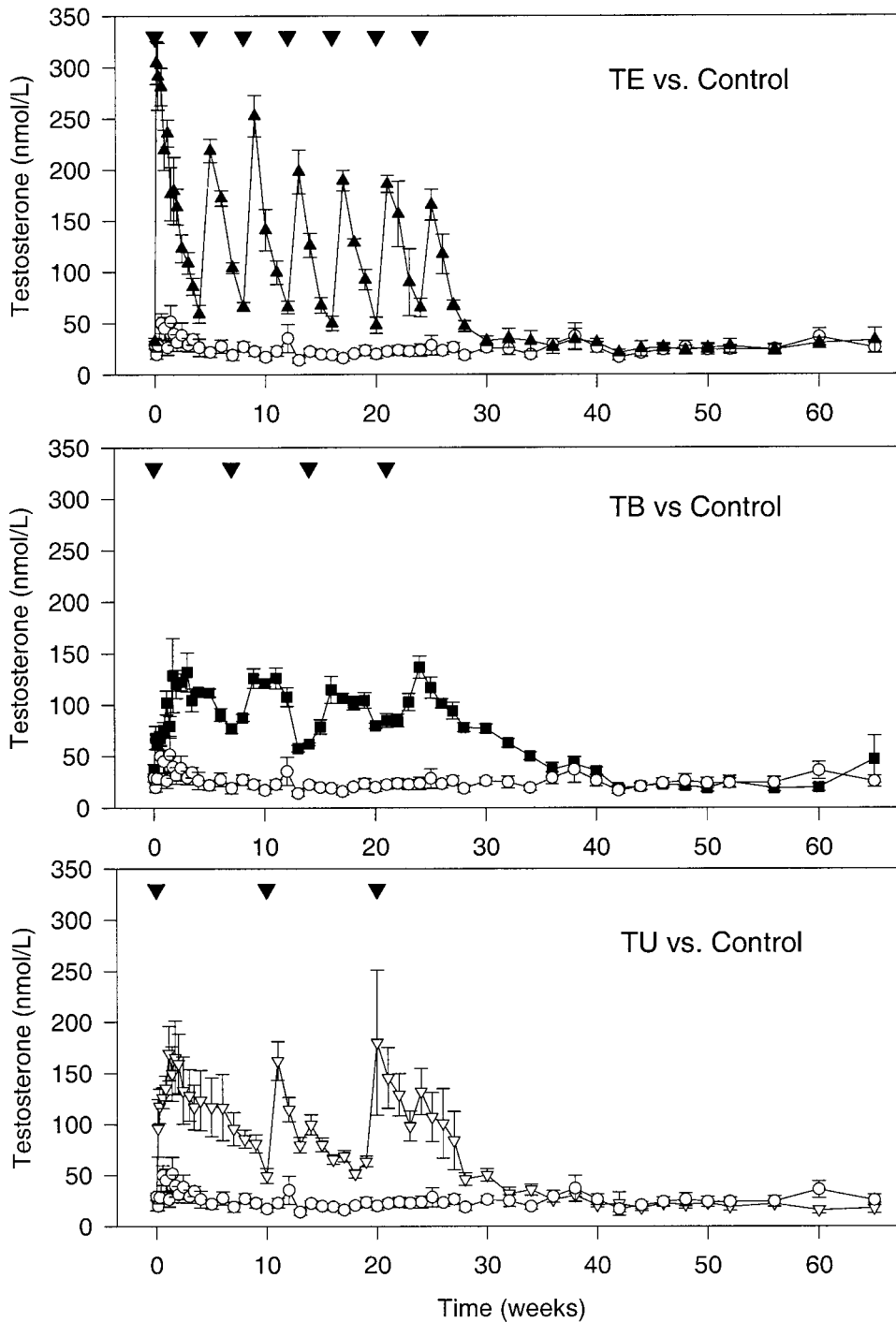
Figure 1. Serum testosterone concentrations in male cynomolgus monkeys treated (arrowheads) with testosterone enanthate **(TE**, solid triangles), testosterone buciclate **(TB**, solid squares), or testosterone undecanoate **(TU**, open triangles), or vehicle (control group, open circles). Mean values ± SEM (n = 5/group).

atinine ($P < .001$). Significant differences were also measurable for lipid levels and purine metabolism; groups treated with long-acting testosterone-esters showed a more pronounced decrease of lipids and uric acid levels in comparison with control animals or those treated with TE (Tables 2 and 3). Differences between androgen-sub-

stituted animals and controls were also measurable concerning levels of alkaline phosphatase and GGT (lower levels in androgen-exposed animals; data not shown). No differences from the control group were seen in regard to other liver parameters, blood urea nitrogen, and electrolytes.
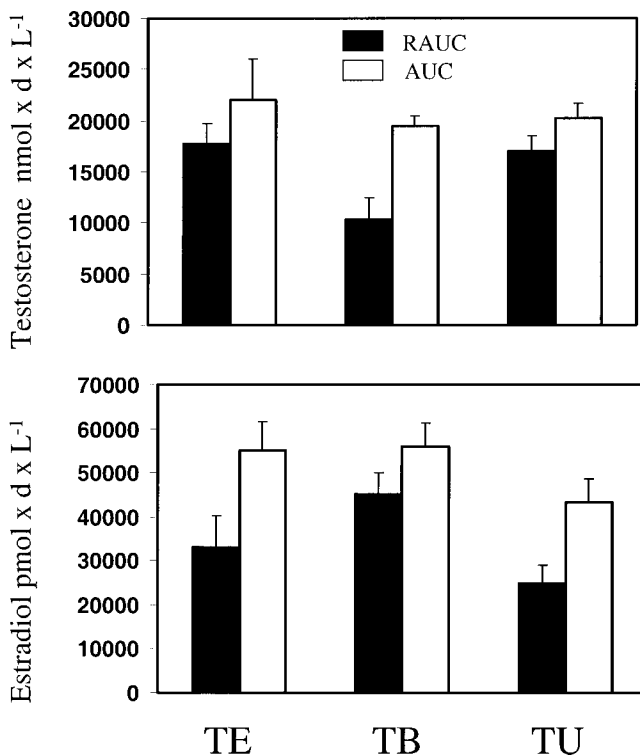
Figure 2. Values for area under testosterone and estradiol curves (AUC) and response area under curve (AUC minus baseline values) in male cynomolgus monkeys treated (arrowheads) with testosterone enanthate (TE), testosterone buciclate (TB), or testosterone undecanoate (TU), or vehicle (control group, open circles). Hormone data for weeks 0–28 of the study period were used for calculation. Mean values ± SEM (n = 5/group).

Differences between treatment groups in parameters influenced by T administration are summarized in Table 3.

Discussion

The purpose of the present investigation was to compare the androgenic potency of 3 T esters with substantially different pharmacokinetic profiles. The esters selected were TE, TB, and TU. Doses and injection intervals were based on previous experience with the compounds in men and nonhuman primates (eg, Monder et al, 1994; Behre and Nieschlag, 1998; Weinbauer et al, 2001). By week 4 after the last injection (week 24), T levels had returned to baseline. Therefore, the period of 0–28 weeks was chosen for direct comparison of the pharmacodynamic effects of the T esters, but also effects during recovery were considered (Figure 1).

Injected doses were standardized on the basis of pure T to administer 20 mg/kg pure T on each occasion. During the 28-week period, the total dose administered to each monkey was 140 mg/kg T for TE, 80 mg/kg T for TB, and 60 mg/kg T for TU. As expected, TE exhibited

Table 1. *Testosterone pharmacokinetic data during the first injection interval, ie, days 0–28 for TE, days 0–49 for TB, and days 0–70 for TU**

Treatment	MRT (d)	$T_{1/2}$ (d)	Cmax (nmol/L)	Tmax (d)
TE	10.6	8.5	305	1.07
TB	24.8	32.5	120	19.5
TU	30.2	42.7	152	10.4

  * Calculations are based on the group mean testosterone levels during the first injection interval: testosterone enanthate (TE), testosterone buciclate (TB), and testosterone undecanoate (TU). or details of the sampling frequency, the reader is referred to the ''Materials and Methods'' section. MRT indicates mean residence time; Cmax, maximal concentration; Tmax, time to reach Cmax; and $T_{1/2}$, half-life. It has to be noted that serum half-life and the other parameters are most likely not determined by metabolization but by the release rate from the intramuscular depot.

the shortest and TU the longest period of provision of T, and peak T levels were far higher under TE as compared with TB and TU. Although TE-group animals were exposed to 2.5–3 times higher T doses than animals in TB or TU groups, analysis of AUC for T revealed comparable values for all T preparations during the entire observation period. This is due mainly to the blood sampling intervals that were less frequent from the second injection on; therefore, postinjection high T levels caused by TE were obviously missed. Because sampling was performed on a weekly basis, low T levels in the TE group were not missed (Figure 2).

Analysis of AUC and RAUC for estradiol revealed a higher amount of aromatization in TB-treated animals in comparison with TE- or TU-treated ones (Figure 2). Because estradiol has significant effects on pituitary secretion patterns and metabolism, putative differences between the 2 long-acting esters TU and TB were also investigated.

Suppression of spermatogenesis as physiological response to exogenous androgens was least pronounced in animals exposed to TE, although this group had received a much higher total dose of T (Figure 6). Hence, merely increasing the dose of T failed to improve the antispermatogenic effects of T. This suggests that the pattern of exposure (for the effect of aromatization see below) rather than the total dose administered, determines the antispermatogenic effect of T esters, at least in this nonhuman primate model. Thus, not only from the obvious viewpoint of clinical safety, but also for the purpose of spermatogenic suppression, provision of constant rather than fluctuating T levels appears advantageous.

In the TB and TU groups, suppression of LH levels (Figure 3) as well as sperm density was demonstrated also when T levels had dropped into the normal range. It can be assumed that the animals were exposed to—albeit within in the normal range—rather constant levels of testosterone, also during the washout phase. In this circum-
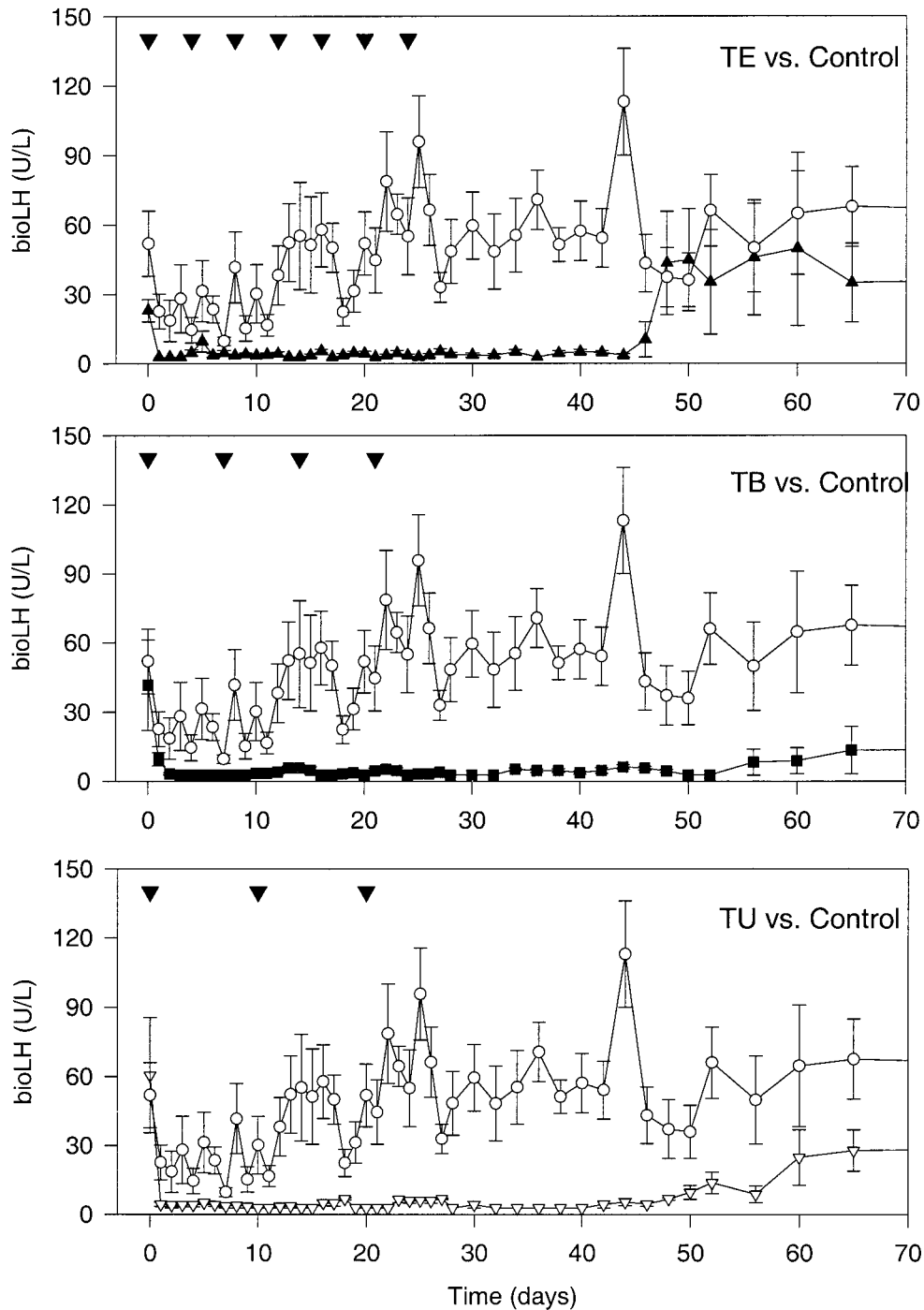
Figure 3. Serum luteinizing hormone bioactivity in male cynomolgus monkeys treated (arrowheads) with testosterone enanthate **(TE)**, testosterone buciclate **(TB)**, or testosterone undecanoate **(TU)** or vehicle (control group, open circles). Mean values ± SEM (n = 5/group).

stance, circadian fluctuations of T levels are not restored and negative feedback on the pituitary persists. Because frequent sampling was not conducted during washout and circadian T levels are not available, this remains speculative.

None of the T preparations induced sustained azoospermia. With regard to TB, this finding is similar to that

observed in a recent report in the adult cynomolgus monkey (Weinbauer et al, 2001). In a recent study in the cynomolgus monkey, administration of pure T via silastic implants was reported to induce azoospermia in 4 of 9 animals (O'Donnell et al, 2001). The effects of administration of T alone on spermatogenesis have also been studied in macaques with seasonal reproductive activity
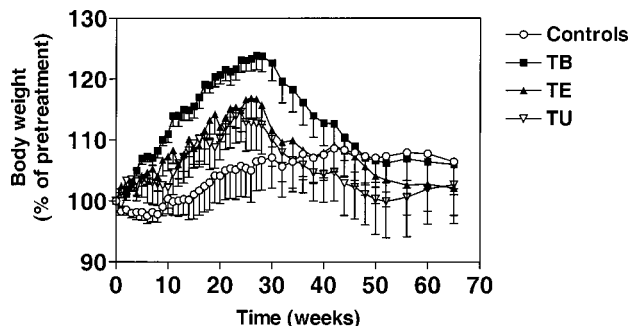
Figure 4. Body weights of male cynomolgus monkeys treated with testosterone buciclate (TB), testosterone undecanoate (TU), or testosterone enanthate (TE) or vehicle (control group, open circles). Mean values ± SEM (n = 5/group).
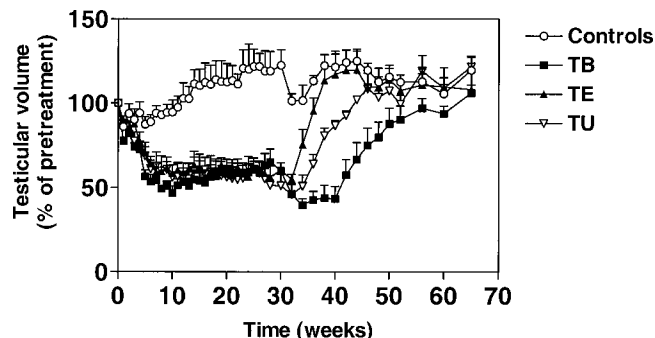

Figure 5. Testicular volume (left plus right testis) of cynomolgus monkeys treated with testosterone buciclate (TB), testosterone undecanoate (TU), or testosterone enanthate (TE) or vehicle (control group, open circles). Mean values ± SEM (n = 5/group).

(Tyagi et al, 1999). In the bonnet monkey, administration of TB induced a reduction of sperm numbers but failed to cause azoospermia (Kinger et al, 1995). It must be taken into account, however, that seasonal macaques might represent a special model because it has been claimed—in the case of the bonnet monkey—that merely blocking the nocturnal T surge provokes suppression of spermatogenesis (Suresh et al, 1995).

It is striking that the decrease of testis size from baseline (Figure 5) was far less pronounced during T treatment, compared with hypophysectomy or treatment with potent gonadotropin-releasing hormone (GnRH) antagonists (Weinbauer et al, 1991, 1994, 2001). For the latter, testicular involution by 70–80% has been observed, whereas under T regimens, only a 40–50% reduction was achieved. Although direct comparative studies are not available, the factor explaining the different effects is follicle-stimulating hormone (FSH) (Narula et al, 2002). The extent of suppression of bioactive LH secretion was comparable under T or GnRH antagonist treatment, thus ruling out interference by this gonadotropin. FSH is the other key regulator and particularly important for primate spermatogenesis (Nieschlag et al, 1999). At least twofold higher doses of T than normal are required to lower FSH secretion in *Macaca fascicularis* (Weinbauer et al, 2001). It is conceivable that even higher doses might be needed to completely abolish FSH secretion and spermatogenesis in primates. In men, this problem is currently overcome by adding gestagenic compounds (eg, norethisterone) to T preparations for the purpose of endocrine male contraception (Kamischke et al, 2001, 2002). Recovery of testis size during washout was most rapid in the TE-treated group. The groups treated with the longer-acting T preparations TU and TB showed prolonged suppression of testicular volume (Figure 5). This effect was more pronounced in the TB-treated animals, which were exposed to relatively higher estradiol levels than the TU-treated monkeys; an additional effect on gonadotropin secretion can be assumed.

Summarizing the effects on gonadal-pituitary functions, suppression of LH secretion and testicular size was similar in all treatment groups, but the decrement of sperm numbers was more pronounced in the animals treated with long-acting T esters. During recovery, differences between treatment groups became more obvious, showing the shortest impact on LH secretion, testicular size, and sperm count in TE-treated animals, which were followed by those given TU after a significantly longer time period. Treatment with TB suppressed LH secretion, testicular volume, and sperm counts longer than in the other groups including TU-treated animals. Hence, not only serum kinetics but also the degree of aromatization to estradiol represent important characteristics for a T preparation (see Table 3 and Figures 3, 5, and 6).

Administration of T induced a sustained and significant elevation of body weight (Figure 4). Body weight increment was significantly more pronounced for TB (25%),
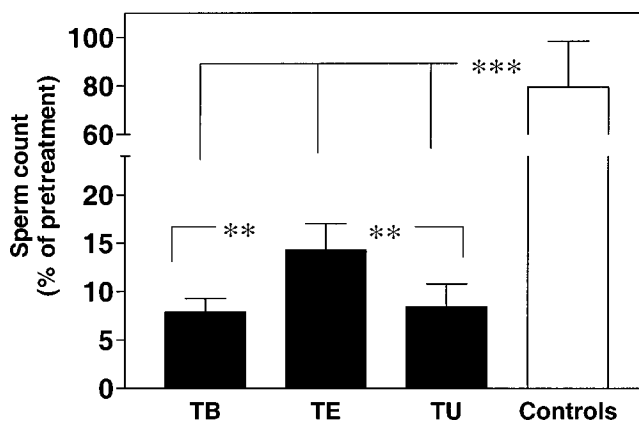

Figure 6. Mean suppression of sperm counts from baseline during the last 10 weeks of treatment (weeks 19–28) in the ejaculates of cynomolgus monkeys treated with testosterone buciclate (TB), testosterone undecanoate (TU), or testosterone enanthate (TE) or vehicle (control group, open circles). Mean values ± SEM (n = 5/group). Note that for analysis, all data points were used in a nonparametric test. Significant differences for all treatment groups vs controls ($P < .001$) and for TE vs TU or TB ($P < .01$).

Table 2. *Clinical chemistry and blood count*

Parameter	Absolute Changes During Treatment†			
	Controls	TE	TB	TU
Δ Hemoglobin g/dL	−0.3 ± 0.5	1.5 ± 0.4***	2.3 ± 0.6***	2.1 ± 0.8***
Δ Serum creatinine μmol/L	−2.3 ± 3.8	5.4 ± 2.7***	5.9 ± 4.0***	8.7 ± 3.6***
Δ Uric acid μmol/L	1.2 ± 3.6	1.1 ± 1.1	−6.2 ± 3.6***	−5.8 ± 3.5***
Δ Total cholesterol mmol/L	0.2 ± 0.2	−0.2 ± 0.1	−0.7 ± 0.1**	−0.7 ± 0.2***
Δ Triglycerides mmol/L	−0.1 ± 0.2	−0.2 ± 0.1	0.1 ± 0.1	−0.6 ± 0.2**
Δ HDL cholesterol mmol/L	0.1 ± 0.1	−0.3 ± 0.1***	−0.3 ± 0.1***	−0.2 ± 0.1**

† Absolute changes during treatment as difference of baseline to end of treatment levels are given as mean ± SEM. Note that for analysis of variance for repeated measurements, all data points were used (see ''Sampling Methods and Statistics''). According to this analysis, differences to the controls group are indicated as asterisks (*, **, *** representing p values below .05, .01, and .001, respectively). Differences between T-treated groups are summarized in Table 3.

compared with TE or TU (10–15%); in agreement, the stimulatory effects on weight gain lasted longer in TB-treated monkeys than in the others. Such an observation concerning TB and body weight has been previously reported for this macaque species (Weinbauer et al, 2001). The mechanism behind the relative greater potency of TB could be the higher estradiol levels found in TB-treated animals; when considering the RAUC values for the present study, RAUC for T was lower and RAUC for estradiol was higher for TB, compared with TE and TU. Although T mainly causes increases in fat-free mass (Sinha-Hikim et al, 2002), estradiol could be responsible for a gain of body fat (Vermeulen et al, 2002). This hypothesis is in agreement with comparable serum levels of creatinine as marker of muscle tissue (Navarro et al, 2002) among all treatment groups (Table 2).

Lipid-lowering effects of T substitution are well described in hypogonadal men (Whitsel et al, 2001). In cynomolgus monkeys, long-acting T esters such as TB and TU seem to have a higher impact than the short-acting enanthate ester (Table 2). The effect on triglyceride suppression was significantly less pronounced in the TB-treated animals, compared with those treated with TU (Tables 2 and 3). This is in agreement with the hypothesis of higher estrogenic activity (Kuller, 2003) in TB-treated monkeys. A difference between the short- and long-acting

esters can also be discerned with regard to levels of uric acid (Tables 2 and 3). According to current knowledge concerning androgens and purine metabolism, this reflects increased protein synthesis and decreased protein degradation, hence a stronger anabolic influence of TU and TB (Pizzichini et al, 1995; Hussein et al, 1999; Mukhin et al, 2002). Anabolic effects of androgens are also reflected by significantly higher levels of serum creatinine in the T-treated groups, indicating higher muscle mass (Navarro et al, 2002) (Table 2). The erythropoietic influence of testosterone is expressed by higher levels of hemoglobin in the T-treated animals (Table 2).

This first study directly comparing the effects of different T esters on gonadal and metabolic functions clearly shows a differential impact between short- and long-acting androgen preparations as well as the importance of the variable degree of aromatization (Table 3). Even a two- to threefold higher dose of the short-acting ester TE cannot fully achieve the effects of long-acting T-esters such as TU or TB concerning gonadal and metabolic functions. This suggests why these substances are preferable for the purpose of hormonal male contraception and substitution therapy in male hypogonadism. In addition, the higher degree of aromatization in TB, compared with TU, which was accompanied by measurable effects on weight gain, triglyceride levels, and the duration of

Table 3. *Summary of differences between treatment groups*

Parameter*	Effect During Treatment†	Duration of Effect During Recovery
Luteinizing hormone suppression	TE = TU = TB	TE < TU < TB
Suppression of testicular volume	TE = TU = TU	TE < TU < TB
Suppression of sperm count	TE < TU = TB	TE < TU < TB
Gain of body weight	TE = TU < TB	TE = TU < TB
Decrement of serum uric aid	TE < TU = TB	TE = TU = TB
Increment of serum creatinine	TE = TU = TB	TE = TU = TB
Suppression of serum lipids	TE < TB < TU	TE = TU = TB
Stimulation of erythropoeisis	TE = TU = TB	TE = TU = TB

* All parameters were changed by testosterone application in comparison with controls (see Table 2, Figures 3–6, ''Results''). Differences between treatment groups are symbolized according to results of analysis of variance for repeated measurements and post hoc tests.
† TE indicates testosterone enanthate; TU, testosterone undecanoate; and TB, testosterone buciclate.

influence on gonadal functions demonstrates why estradiol-related effects should be considered when comparing various T preparations.

In conclusion, we suggest that the pattern of exposure to T and the degree of its aromatization rather than the dose determine the effect of T preparations in nonhuman male primates. These results are most likely transferable to men and should be considered in further studies comparing different androgen preparations.

Acknowledgments

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