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Intramuscular Testosterone Undecanoate and Norethisterone Enanthate in a Clinical Trial for Male Contraception*

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ABSTRACT

Recent trials for hormonal male contraception are based on gestagens or GnRH antagonists combined with oral or injectable testosterone substitution. However, the efficacy of most trials remained disappointing. Norethisterone enanthate (NETE) has been used as a long-acting injectable female contraceptive and has shown sustained suppression of spermatogenesis in male monkeys and prolonged suppression of gonadotropins in men. This study was designed to prove the efficacy of the long-acting testosterone undecanoate ester (TU) alone or in combination with NETE in a phase II clinical trial. Fourteen healthy men received injections of 1000 mg TU in combination with injections of 200 mg NETE every 6 weeks over a period of 24 weeks, followed by a control period of 28 weeks. Another 14 volunteers received TU alone. During the study semen variables, reproductive hormones, clinical chemistry and lipid parameters, well-being, and sexual function were monitored. Scrotal content and prostates were checked sonographically. During the entire treatment period mean testosterone serum concentrations remained within the normal limits. Marked suppression of gonadotropins in both treatment groups resulted in azoospermia in 7 of 14 and 13 of 14 volunteers and in oligozoospermia in 7 of 14 and 1 of 14 in the groups given TU only or TU/NETE, respectively. However, the highest azoospermia rate in the TU/NETE group was achieved 8 weeks after the end of the treatment period, and 1 volunteer with very high initial sperm counts (mean, 190 million/mL at baseline) remained oligozoospermic (10.2 million/mL). From week 20 to week 24 there was a significant, fully reversible maximum weight gain of 3.7 kg, on the average, in the NETE group. In the NETE and TU alone groups there were significant 26.6% and 11.5% maximum decreases in high density lipoprotein cholesterol compared with baseline values during the treatment period. A significant elevation of low density lipoprotein and a decrease in lipoprotein(a) were detected in the TU/NETE group. In conclusion, combination treatment with NETE showed suppression of spermatogenesis comparable with results using testosterone esters in combination with GnRH antagonists or cyproterone acetate, but had more favorable injection intervals and better efficacy. Because of its long-lasting, profound suppression of spermatogenesis and the absence of serious side-effects, the combination of TU and NETE can be considered a first choice for further studies of hormonal male contraception. (*J Clin Endocrinol Metab* **86:** 303–309, 2000)

THE GOAL OF hormonal male contraception is the suppression of spermatogenesis to azoospermia. Initial studies were based on weekly injections of testosterone enanthate, and they achieved suppression of spermatogenesis in about two thirds of Caucasian and almost all Chinese volunteers (1, 2). For better efficacy, testosterone was combined with different gestagens (for a review, see Ref. 3). However, despite better gonadotropin suppression, suppression of spermatogenesis remained unsatisfactory in most of these studies (4–6) or, as in the case of cyproterone acetate, produced an unwanted decline of red blood cell production (7, 8). Therefore, the search for more appropriate gestagens continues. In addition, most regimens tested to date are based on impractical weekly or biweekly im injections of testosterone enanthate, as injection-free approaches, such as oral or transdermal testosterone application, were not successful (6, 9, 10). Apart from testosterone implants (11), to date only the injectable testosterone esters, testosterone undecanoate (TU) (12–17) and testosterone buciclate (18), have half-lives long enough to warrant long injection intervals and, hence, long-term acceptance.

In a phase II clinical trial for hormonal male contraception we evaluated injectable TU in combination with norethisterone enanthate (NETE). NETE has proven useful in female contraception and showed rapid and sustained suppression of serum FSH and testosterone levels in males (19, 20). NETE was also chosen because it produces partial androgenic effects in women, which might be of advantage in male contraception.

Subjects and Methods

Subjects

The study consisted of 3 arms, each comprising 14 volunteers. All volunteers received im injections of TU. In addition, 1 group received oral levonorgestrel, the second was given oral placebo, and the third group received im NETE injections. While results from the first 2 groups have been published previously (17), we report here the results from the NETE group and again include the placebo group for comparison. The study was approved by the ethics committee of the University and the State Medical Board (Münster, Germany). All volunteers gave written informed consent to participate in the study.

Caucasian men, aged 18–45 yr, were recruited by local press advertisement and were examined for normal general medical history; normal physical condition; normal blood values for routine clinical chemistry, lipids, hematology, and reproductive hormones; and normal semen

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parameters. Volunteers with clinically relevant abnormalities of the above-mentioned parameters were excluded, and the remaining volunteers were again screened for fulfillment of the inclusion criteria. All 28 volunteers finished the study.

Study design

After the 2 screening visits, all volunteers received im injections of 1000 mg TU dissolved in 4 mL castor oil in study weeks 0, 6, 12, and 18. In addition, at the same intervals 14 volunteers received 200-mg NETE (dissolved in 1 mL castor oil) injections or daily oral placebo treatment over 24 weeks. Examinations consisting of general and genital examination; evaluation of adverse events; measurements of blood biochemistry, lipid profile, hematology, sex hormone-binding globulin (SHBG), prostate-specific antigen (PSA), and reproductive hormones (FSH, LH, PRL, estradiol, testosterone); and semen analysis were performed every 4 weeks. In addition, at every visit all volunteers answered a questionnaire about their condition and sexual behavior. During the recovery period volunteers were seen at study weeks 28, 32, 36, 44, and 52. If semen parameters had not normalized within this period, the recovery period was extended until the volunteer provided a semen sample with normal sperm counts and motility.

In addition to these regular examinations, blood for reproductive hormone analysis was drawn immediately before the following injection in study weeks 6 and 18. In the second pretreatment examination and in study weeks 12, 24, 36, and 52, sonography of scrotal content and transrectal sonography of the prostate were additionally performed. Basal and augmented glucose (oral challenge with 75 g glucose) were determined during the second pretreatment examination and in weeks 12, 24, and week 52.

Blood samples

Venous blood was sampled between 0800–1200 h at every visit after a 10-h fasting period. Blood samples for endocrine determinations were separated at 800 × *g* and stored at −20 C until evaluation. All other blood parameters were analyzed on the same day.

Assays

Serum levels of LH, FSH, PRL, SHBG, and PSA were determined by highly specific time-resolved fluoroimmunoassays (Autodelfia, Wallac, Inc., Turku, Finland). The lower detection limits for FSH, LH, SHBG, and PSA were 0.25 IU/L, 0.12 IU/L, 6.3 nmol/L, and 0.5 μg/L, respectively. The normal range in our laboratory for LH is 2–10 IU/L, that for FSH is 1–7 U/L, that for PRL is less than 500 mU/L, that for SHBG is 11–71 nmol/L, and that for PSA is less than 4 μg/L. Mean intra- and interassay coefficients of variation during the hormone analysis period were 1.5% and 2.9% for LH, 1.7% and 4.5% for FSH, 0.8% and 4.5% for PRL, 1.0% and 9.6% for SHBG, and 3.2% and 4.3% for PSA, respectively.

Testosterone was determined either with a commercial fluoroimmunoassay (Autodelfia, Wallac, Inc.) or with a commercial enzyme-linked immunosorbent assay (DRG Instruments GmbH, Marburg, Germany). Consecutive samples of every proband were measured with one assay. The lower detection limit for testosterone in the fluoroimmunoassay was 0.28 nmol/L; mean intra- and interassay coefficients of variation were 2.1% and 6.1%, respectively. The lower detection limit in the enzyme-linked immunosorbent assay was 0.24 nmol/L, with mean intra- and interassay coefficients of variation of 3.4% and 9.2%, respectively. Estradiol was measured by highly specific time-resolved fluoroimmunoassays (Autodelfia, Wallac, Inc.), with a lower detection limit of 37 pmol/L and mean intra- and interassay coefficients of variation of 3.7% and 6.4%, respectively. The normal serum level for testosterone is above 12 nmol/L, and the upper normal limit for estradiol is 250 pmol/L.

Clinical chemistry and hematology parameters were analyzed with a Hitachi 947 autoanalyzer (Roche Diagnostics, Mannheim, Germany) and an H3 autoanalyzer (Bayer Corp., Leverkusen, Germany), respectively. A Hitachi 917 autoanalyzer (Roche Diagnostics) was used to quantify serum concentrations of glucose, cholesterol, and triglycerides with enzymatic tests (all from Roche Diagnostics), high density lipoprotein (HDL) cholesterol with a homogenous enzymatic assay (Roche Diagnostics), and apolipoprotein A-I (apoA-I), apoB, and lipoprotein(a) [Lp(a)] with immunoturbidimetric tests (Roche Diagnostics).

Semen analysis

Semen samples were analyzed according to the WHO laboratory manual (21) and were subjected to rigid internal (22) and external quality control (23). In cases of extremely low sperm counts or azoospermia, the ejaculates were centrifuged, and analysis was performed on the sediment. Azoospermia was defined as no sperm found after centrifugation and analysis of the pellet. Severe oligozoospermia was defined as a sperm count of 3 million/mL or less. The volunteers were requested to abstain from sexual activity for 48 h to 7 days before the investigation.

Evaluation of well-being and sexual function

For evaluation of possible psychosexual effects of the treatment, a standardized questionnaire was used. Intensity of sexual thoughts and fantasies, sexual interest and desire, and satisfaction with sexuality during the last week before investigation were rated by the volunteer on an unscaled line ranging from 0–10 cm, with 0 cm reflecting low and 10 cm reflecting high intensity. The intensity was measured using a ruler and was given in centimeters. For evaluation of frequency of erections and ejaculations and morning erections during the last week before investigation, the number of events were estimated by the volunteers. This previously described questionnaire (24) was completed by the volunteers at every visit.

TABLE 1. Hormone parameters and testes and prostate volumes at baseline and in weeks 12, 24, 36, and 52

Parameters	Group	Mean baseline	Week 12	Week 24	Week 36	Week 52
PRL (mU/L)	TU/NETE	237 ± 24	360 ± 43	433 ± 56[a]	302 ± 49	245 ± 28
	TU alone	214 ± 12	261 ± 28	238 ± 21	243 ± 16	292 ± 29
E_2 (pmol/L)	TU/NETE	68 ± 5	63 ± 6	76 ± 6	55 ± 4	59 ± 4
	TU alone	80 ± 6	74 ± 6	83 ± 7	71 ± 6	76 ± 5
DHT (nmol/L)	TU/NETE	2.26 ± 0.15[b]	1.60 ± 0.08	2.14 ± 0.15	1.95 ± 0.21	1.93 ± 0.13
	TU alone	1.12 ± 0.13	1.15 ± 0.17	1.59 ± 0.28	1.00 ± 0.10	1.12 ± 0.16
PSA (μg/L)	TU/NETE	0.63 ± 0.08	0.79 ± 0.10	0.83 ± 0.11	0.72 ± 0.09	0.84 ± 0.13
	TU alone	0.48 ± 0.04	1.0 ± 0.4	0.52 ± 0.06	0.48 ± 0.04	0.64 ± 0.16
Paired testicular vol (mL)	TU/NETE	60 ± 3	43 ± 3[a]	39 ± 4[a]	42 ± 5[a]	56 ± 3
	TU alone	54 ± 2	43 ± 3[a]	37 ± 2[a]	47 ± 3[a]	55 ± 4
Prostate vol (mL)	TU/NETE	20 ± 1	22 ± 2	22 ± 1	20 ± 1	19 ± 1
	TU alone	17 ± 1	18 ± 1	18 ± 1	18 ± 2	19 ± 2
BW (kg)	TU/NETE	79.3 ± 3.1	82.7 ± 2.3	85.1 ± 2.3[a]	83.2 ± 2.3	82.5 ± 2.3
	TU alone	80.3 ± 3.0	81.0 ± 3.2	82.0 ± 3.5	81.7 ± 3.9	81.9 ± 3.7

Data are given as the mean ± SEM.
[a] $P < 0.05$ compared with baseline.
[b] $P < 0.05$ between the groups.

Ultrasonography of testicular volume/transrectal ultrasonography of the prostate

Sonographic (Sonoline Versa Pro, Siemens, Erlangen, Germany) measurements of testes and prostate volumes were performed applying a high frequency 7.5-MHz sector scanner (25). All measurements of prostate volume were performed by transrectal ultrasonography with a mechanical biplanar 7.5-MHz sector scanner (Endo-P, Siemens). Prostate volume was calculated using the ellipsoid method (26).

Statistics

All variables were checked for normal distribution in the Kolmogorov-Smirnov one-sample test for goodness of fit. Variations between study groups were evaluated by two-way ANOVA for repeated measurements. Variations over time within the study group were evaluated by one-way ANOVA for repeated measurements. In the case of an overall $P < 0.05$ in the ANOVA, differences between baseline values and the following time points were tested by Tukey's *post-hoc* test. If data were not normally distributed, Friedman ANOVA for repeated measurements followed by Dunn's multiple comparison test were used instead. In the case of a single missing value per time point, the appropriate mean was inserted to allow ANOVA for repeated measurements. In the case of more than one missing value per time point, ANOVA was performed. Proportions were analyzed using the χ^2 test. Two-sided P values of 0.05 were considered significant. All analyses were performed using the statistical software GraphPad Prism for Windows, version 2.01 (GraphPad Software, Inc., San Diego, CA). In general, results are given as the mean ± SEM.

Results

General well-being and sexual function

In general, treatment was well tolerated by all volunteers. Three volunteers given only TU injections and three volunteers in the NETE group complained about mild acne during treatment. Three volunteers in the NETE group experienced increased mild nocturnal sweating. The significant weight increase from week 20 to week 24 in the NETE group was reversible, whereas no significant changes could be observed in the TU alone group or between the groups (Table 1). No significant changes in physical symptoms, mood ratings, individual well-being, or frequency of erections and sexual intercourse were observed at any investigated time point compared with baseline.

Semen parameters

Ejaculate volumes in both treatment groups remained unchanged throughout the study period. Treatment with only TU and with TU/NETE resulted in a significant suppression of sperm counts in all participants (Fig. 1). In the overall ANOVA, suppression of sperm counts was significantly more pronounced ($P = 0.004$) in the NETE group, although differences between the groups could not be detected at any single time point. Compared with baseline, within the groups suppression of sperm counts was earlier (week 8 *vs.* week 12) in the NETE group compared with that in the group given TU alone (Fig. 1). In the TU alone group 7 of 14 and in the NETE group 13 of 14 men reached azoospermia (Fig. 2). The mean time till achievement of azoospermia was not significantly different between the groups. The highest azoospermia rate in the TU/NETE group was achieved 8 weeks after the end of the treatment period (14 weeks after the last injection). One volunteer with very high initial sperm counts (mean, 190 million/mL at baseline) could be suppressed only to oligozoospermia (10.2 million/mL) with a progressive motility of 41% and 13% normal morphology in study week 28. In the TU alone group 5 of 14 achieved severe oligozoospermia (<3 million/mL), and additionally 2 had lowest sperm concentrations of 4.4 million/mL and 7.2 million/mL, respectively.

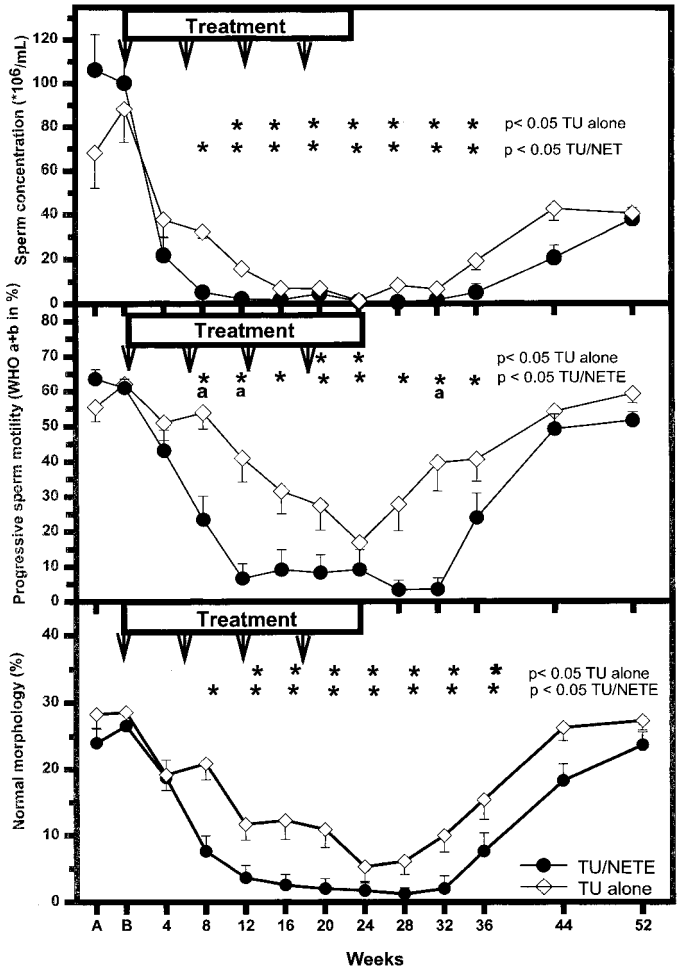

FIG. 1. Sperm concentration, sperm motility (WHO a+b), and sperm morphology in the TU/NETE group (●) and the TU alone group (◇). Results are the mean ± SEM of all volunteers; if volunteers achieved azoospermia, values for motility and morphology are counted as zero. Significant differences from baseline are indicated as *stars*. Significant differences between the groups are indicated as *lowercase letters*. Injection time points are indicated as *arrows*.

Forward sperm motility (grades a and b) was significantly reduced in the NETE group (weeks 8, 12, 16, 20, 24, 28, 32, and 36) and in the TU alone group (weeks 20 and 24). Suppression of sperm motility was significantly more pronounced in the NETE group compared with the TU alone group in weeks 8, 12, and 32 (Fig. 1).

The percentage of normal sperm morphology (Fig. 1) was significantly reduced between weeks 8 and 36 in the NETE and between weeks 12 and 36 in the TU alone group. No significant differences could be detected between the groups at any investigated time point.

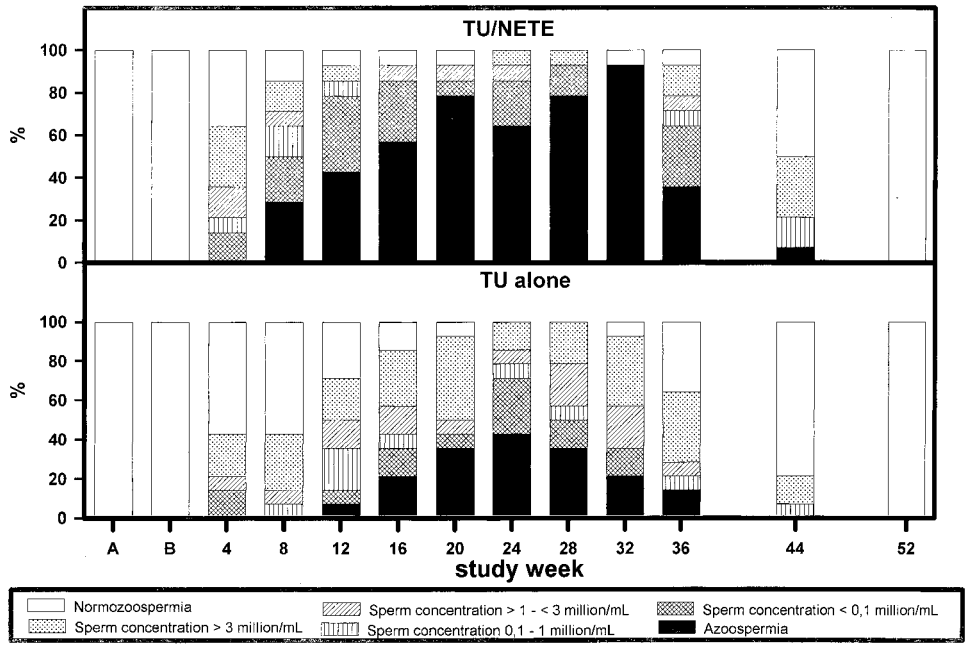

FIG. 2. Distribution of volunteers achieving azoospermia, sperm concentration less than 0.1 million/mL, sperm concentration between 0.1–1 million/mL, sperm concentration less than 3 million/mL, sperm concentration less than 20 million/mL, and sperm concentration less than 20 million/mL over time. In volunteers who recovered after week 52, the normal ejaculate achieved was counted. Normozoospermia was defined as more than 20 million/mL.

Hormones

In both groups FSH and LH concentrations were significantly suppressed from week 4 until week 32 (except for FSH in week 6 in the TU alone group; Fig. 3). FSH and LH suppression in the NETE group was significantly more pronounced compared with that in the group given TU alone ($P < 0.0001$). A significant difference in FSH levels could be detected between the groups at week 6. There was a significant increase in PRL in weeks 4, 8, 16, 18, 20, and 24 in the NETE group and in week 20 in the TU alone group (Table 1). Between the groups PRL levels were significantly different in study weeks 8, 16, and 18 (Table 1). The increase in PRL appeared to be of no clinical relevance.

Apart from significantly elevated testosterone and estradiol serum levels in weeks 8 and 20 in both groups and a significant reduction of estradiol levels in week 6 in the NETE group, testosterone and estradiol levels remained unchanged. Between the groups testosterone levels were significantly different in week 20 (Fig. 3). In the NETE group SHBG (Fig. 3) was significantly decreased from week 4 to week 24, whereas SHBG remained unchanged (apart from a reduction at week 8) in the TU alone group.

Clinical chemistry and hematology

Values from routine clinical chemistry showed no significant changes during the study period, except for alkaline phosphatase (Table 2), which was significantly decreased from week 8 to week 44 in the TU alone group and from week 8 to week 24 in the NETE group. In the NETE group a significant increase in erythrocytes (week 12 to week 28), hemoglobin (week 12 to week 32), and hematocrit (week 4 to week 32) could be detected, whereas hematological parameters in the TU alone group showed no significant differences.

Lipid parameters

No significant differences from baseline or between the groups could be observed for apoB and triglyceride at any investigated time point (Table 2). In the NETE group low density lipoprotein (LDL) cholesterol (weeks 20, 24, 32, and 36) and cholesterol (week 36) were significantly increased, and Lp(a) (weeks 8, 16, and 20), HDL cholesterol (weeks 4, 8, 16, 20, and 24), and apoA-I (weeks 8, 16, and 20) were significantly decreased. In the TU alone group cholesterol (week 24), HDL cholesterol (week 16 to week 24), and apoA-I (week 4 to week 24) were significantly decreased compared with baseline values. No differences were observed for Lp(a) and LDL within the TU alone group (Table 2). Despite significant overall variations between the groups in the ANOVA ($P < 0.0001$) for cholesterol and LDL, no significant differences were seen at any investigated time point. No differences between the groups were detected for HDL, Lp(a), and apoA-I. Basal and augmented glucose levels remained unchanged during the entire study within and between the groups.

Testes and prostates

In both treatment groups total testes volumes (right plus left sides) were significantly reduced from week 12 to week 36 and returned to baseline values at the end of the study (Table 1).

Prostate volumes did not show any significant differences within and between the study groups during the entire study (Table 1). Except for a significant elevation of PSA in week 20 in the NETE group, no differences in PSA could be detected in either group.

Discussion

The combination of NETE and TU resulted in a profound suppression of gonadotropins and spermatogenesis that was

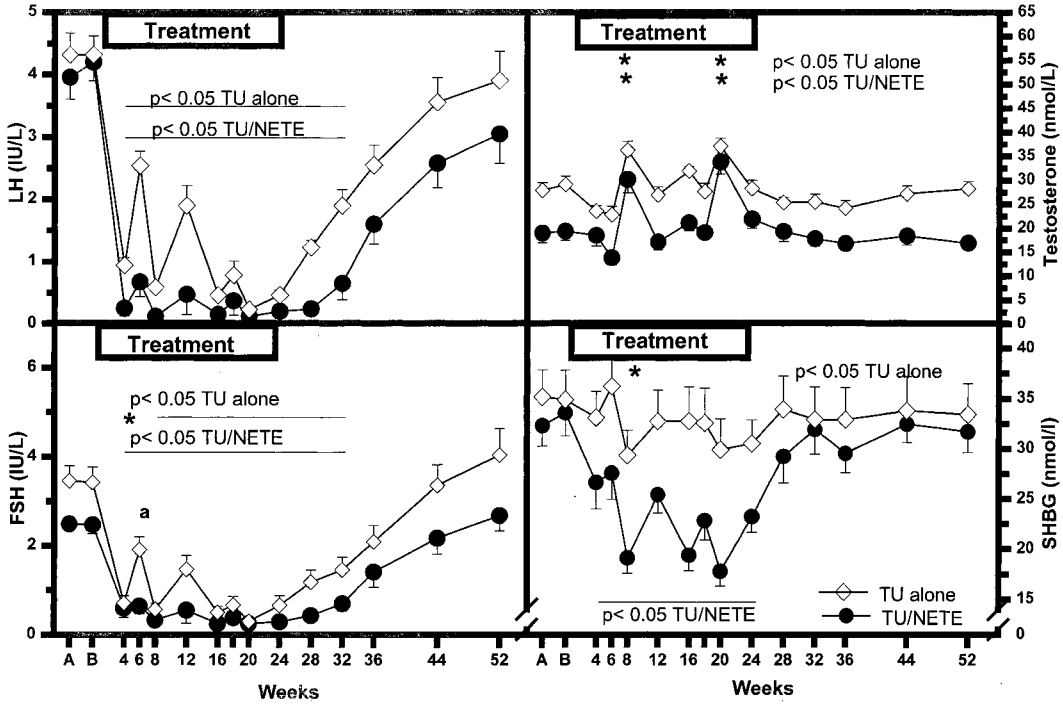

FIG. 3. FSH, LH, T, and SHBG serum levels before and during treatment and in the recovery period (mean ± SEM). Significant differences from baseline are indicated as *stars* and *lines*. Significant differences between the groups are indicated as *lowercase letters*.

greater than those achieved in nearly all other previous studies for hormonal male contraception. In view of the excellent results and only minor side-effects, the combination of TU and NETE offers great potential for the development of a hormonal male contraceptive.

In hormonal male contraception long intervals between testosterone injections appear mandatory for general acceptance and a monthly to 3-monthly injection interval appears acceptable to approximately 40% of men (27) and women (28). Although acceptability can only be truly tested in the marketplace, these studies suggest that the 6-weekly injection interval in our study is in the range of acceptability for quite a number of men. In addition, TU provides mostly normal serum testosterone levels; elevated levels were only seen 2 weeks after the last TU injections. At this time point pharmacokinetic studies also showed maximum concentrations (t_{max}) (13, 14). In another study with TU alone azoospermia was achieved in 11 of 12 and 12 of 12 Chinese volunteers receiving 500 or 1000 mg TU at 4-week intervals (15). In our study with Caucasian men azoospermia could be achieved only in 7 of 14 volunteers, whereas another 5 of 14 volunteers became severely oligozoospermic. Compared with weekly injections of TE, im injections of TU provide similar suppression of gonadotropins and spermatogenesis in Chinese and Caucasian volunteers, with the great advantage of longer injection intervals.

Studies in Caucasians using a combination of TE and depot medroxyprogesterone have shown azoospermia rates of 60% (29). More recent trials have focused on the combination of TE and desogestrel (30) or levonorgestrel (LNG) (4, 5). Desogestrel has shown promising initial results, with azoospermia obtained in all 8 volunteers given oral 300 μg desogestrel daily and 50 mg TE weekly. However, in the other groups with a higher androgen dose (100 mg/week) or a lower desogestrel dose (daily 150 μg desogestrel, orally) the effect was much less pronounced (66% azoospermia). Furthermore, results of studies with a combination of a desogestrel and a long-acting testosterone ester are not available. Among the studies using TE (100 mg/week) in combination with LNG, the best results were obtained with a daily LNG dose of 250 μg, which resulted in azoospermia in 14 of 18 and severe oligozoospermia in 2 additional volunteers (5). However, when combined in different regimens with long-acting im injections of 250 mg TU every 4 weeks (31) or 1000 mg TU every 6 weeks (32), the combination with LNG implants (31) or 250 μg orally (17) achieved azoospermia only in 6 of 16 Chinese and 7 of 14 Caucasian volunteers, respectively. Better suppression of spermatogenesis was achieved when TE (100 mg/week) was combined with cyproterone acetate (CPA; 25–100 mg), resulting in azoospermia in all volunteers (7, 8). However, 16 weeks after initiation of the treatment a significant reduction of hemoglobin was seen under these CPA doses, whereas a lower CPA dose (12.5 mg/day) showed low efficacy (8). This is in agreement with older studies using 5–10 mg CPA (32). Whether CPA at higher doses in combination with TU injections will lead to similar results as those obtained with TE, but without hemoglobin decrease, remains to be investigated.

In view of the results achieved for hormonal male contraception with LNG and CPA, a search for more appropriate gestagens is warranted. Among the candidates, we chose NETE because of its long-lasting gonadotropin suppressive effect and high efficacy in women (33). In addition, as NET binds to the androgen receptor, resulting in androgenic activity equal to approximately 10% that of testosterone (34), it exhibits androgenic properties undesirable in women but

TABLE 2. Clinical chemistry, lipids, and hematology parameters at baseline and in weeks 12, 24, 36, and 52

Parameters	Group	Mean baseline	Week 12	Week 24	Week 36	Week 52
Erythrocytes ($10^6/\mu$L)	TU/NETE	5.0 ± 0.1	5.3 ± 0.1[a]	5.4 ± 0.1[a]	5.1 ± 0.1	5.1 ± 0.1
	TU alone	5.1 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.1 ± 0.1	5.1 ± 0.1
Hemoglobin (g/dL)	TU/NETE	14.6 ± 0.2	15.4 ± 0.2[a]	15.6 ± 0.3[a]	14.8 ± 0.2	14.9 ± 0.2
	TU alone	15.1 ± 0.2	15.5 ± 0.3	15.4 ± 0.2	14.9 ± 0.2	15.2 ± 0.2
Hematocrit (%)	TU/NETE	43.8 ± 0.5	46.7 ± 0.7[a]	47.4 ± 0.6[a]	44.7 ± 0.6	44.0 ± 0.6
	TU alone	45.91 ± 0.6	47.7 ± 0.9	46.7 ± 0.6	44.5 ± 0.4	45.9 ± 0.5
Platelets ($10^3/\mu$L)	TU/NETE	228 ± 12	238 ± 12	243 ± 13	238 ± 11	231 ± 10
	TU alone	233 ± 9	245 ± 11	240 ± 10	234 ± 9	233 ± 10
Alkaline phosphatase (U/L)	TU/NETE	104 ± 6	92 ± 5[a]	87 ± 5[a]	104 ± 8	106 ± 6
	TU alone	116 ± 9	102 ± 8[a]	100 ± 7[a]	103 ± 8[a]	109 ± 8[a]
Cholesterol (mg/dL)	TU/NETE	173 ± 6	178 ± 8	187 ± 8	194 ± 7[a]	182 ± 6
	TU alone	209 ± 9	199 ± 11	183 ± 8[a]	191 ± 7	223 ± 10
HDL-C (mg/dL)	TU/NETE	54 ± 3	49 ± 3	45 ± 2[a]	53 ± 4	51 ± 3
	TU alone	51 ± 2	49 ± 2	46 ± 1[a]	47 ± 2	50 ± 3
Triglycerides (mg/dL)	TU/NETE	90 ± 14	97 ± 25	101 ± 21	94 ± 17	92 ± 16
	TU alone	90 ± 11	103 ± 12	84 ± 9	117 ± 15	108 ± 12
LDL-C (mg/dL)	TU/NETE	102 ± 7	110 ± 7	122 ± 8[a]	122 ± 9[a]	113 ± 6
	TU alone	140 ± 8	130 ± 10	120 ± 8	123 ± 7	151 ± 10
Lp(a) (mg/dL)	TU/NETE	16 ± 4	13 ± 3	13 ± 3	15 ± 3	17 ± 5
	TU alone	25 ± 11	23 ± 10	22 ± 9	22 ± 9	22 ± 0
ApoA-I (mg/dL)	TU/NETE	131 ± 5	117 ± 5	115 ± 3	131 ± 5	141 ± 5
	TU alone	140 ± 5	121 ± 4[a]	115 ± 3[a]	114 ± 4	121 ± 4

Data are given as the mean and SEM.
[a] $P < 0.05$ compared with baseline.

that might be of advantage in male contraception. In a pharmacokinetic study we could confirm that the NETE profile in men is similar to that in women with comparable maximal concentrations, area under the curve, time points of maximal concentrations, and terminal elimination half-life (19, 35). In preclinical studies in bonnet monkeys monthly im injections of NETE resulted in azoospermia in all monkeys within 60–150 days (36). In humans NET acetate (10 mg/day, orally) was capable of suppressing spermatogenesis to azoospermia in all 5 volunteers within 2 months when combined with 250 mg percutaneous testosterone gel daily (37). The present study confirms the good results of these initial studies, with 13 of 14 volunteers achieving azoospermia. Why 1 volunteer in the NETE group did not achieve azoospermia remains unclear. However, he also showed a marked suppression of spermatogenesis, as his sperm concentration declined from 189.5 million/mL at baseline to 10.2 million/mL in week 28. Gonadotropin suppression in this volunteer was not as pronounced as that in volunteers becoming azoospermic. One might speculate that adjustments of the doses or frequency of injections could also cause azoospermia in this man. However, in general, suppression of gonadotropins was significantly better than that in the TU alone group during the entire treatment period. As the suppression of gonadotropins was similar with LNG (17) or NETE, we attribute the better suppression of spermatogenesis to an additional direct testicular effect of NET. It was shown in rats that unilateral NET implants in the epididymal fat pad lead to drastic reduction of testicular size and weight as well as sperm production at the ipsilateral site, whereas the contralateral testis was not affected (38).

The rapidity and the degree of gonadotropin suppression observed with TU/NETE are comparable to or better than those seen with other regimens using GnRH antagonists (39–43), CPA (7, 8), or desogestrel in combination with TE (50 mg/week) (30). Altogether 45 volunteers were treated with daily GnRH antagonist injections in male contraception trials. In these studies azoospermia and severe oligozoospermia were obtained in 82% and 13% of volunteers, respectively (39–43). Thus, results using GnRH antagonists or NETE are comparable. However, the necessity of daily sc injections of the GnRH antagonist make this approach impractical, whereas NETE has to be injected only every 6 weeks.

In general, treatment with TU and NETE was well tolerated by all volunteers, and none of our volunteers discontinued treatment. However, as in other studies with im testosterone injections, local pain and induration at the injection site as well as mild acne and weight gain can occur occasionally. In addition, increased nocturnal sweating seems to be associated with the gestagen treatment. Other unfavorable effects were TU-associated significant decreases in the antiatherogenic parameters HDL cholesterol and apoA-I and an increased atherogenic LDL cholesterol/HDL cholesterol index. These results are consistent with the results of other studies, which have shown that exogenous testosterone application influences atherogenic risk factors (for review, see Ref. 44). In the NETE group this effect is more pronounced, as gestagens are known to decrease HDL cholesterol (45, 46). In addition, a significant increase in LDL cholesterol, erythrocytes, hematocrit, and hemoglobin was seen in the TU plus NETE group. However, these anabolic effects of TU were only moderate, and values did not exceed normal values, casting doubt on the clinical relevance of these findings in long-term studies. However, extending the injection interval after the third or fourth injection might overcome the possible risk of polycythemia in long-term users. The decrease in total alkaline phosphatase might reflect decreased bone formation. However, as no markers for bone resorption have been measured, and androgens are known to increase bone formation in hypogonadal patients (47), the clinical relevance of this finding remains to be elucidated.

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