Norethisterone Enanthate Plus Testosterone Undecanoate for Male Contraception: Effects of Various Injection Intervals on Spermatogenesis, Reproductive Hormones, Testis, and Prostate

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The goal of this study was to find the most favorable injection interval of norethisterone enanthate (NETE) plus testosterone undecanoate (TU) in terms of gonadotropin, sperm suppression, and prostatic effects. Fifty normal men were randomly assigned to receive NETE 200 mg plus TU 1000 mg every 8 wk (n = 10), every 12 wk (n = 10), every 6 wk for 12 wk and then every 12 wk (n = 10), and every 6 wk for 12 wk and thereafter TU 1000 mg plus placebo every 12 wk (n = 10), and placebo plus placebo every 6 wk for 12 wk and then every 12 wk (n = 10) for 48 wk. Semen analyses, blood drawings, physical examinations, and prostate ultrasounds were performed throughout the study. Of the men in the 8-wk injection group, 90% (nine of 10) achieved azoospermia, compared with 37.5% (three of eight) in the 12-wk injection group (P = 0.019). TU plus placebo injected every 12 wk did not maintain sperm suppression. Prostate volumes did not change significantly in either group. In conclusion, these data suggest that the combined administration of NETE and TU at 8-wk intervals represents an effective hormonal contraceptive regimen. (J Clin Endocrinol Metab 90: 2005–2014, 2005)

N ORETHISTERONE ENANTHATE (NETE) is a nor-derived progestin with strong progestational and androgenic activity. It has been shown to induce gonadotropin and testosterone (T) suppression when administered to men (1). Because of these characteristics, NETE was tested for use in hormonal contraceptive regimens for men (2). Injected in combination with testosterone undecanoate (TU) every 6 wk, it induces profound spermatogenic suppression (3). Although these injections seem to be very well tolerated, men might find this injection scheme too frequent for long-term use, which could potentially hinder the acceptability of such a contraceptive. A longer injection interval would increase acceptability and reduce total steroid dose, thus improving safety and reducing the cost.

In hypogonadal men, TU injections can be spaced apart up to 12 wk and still maintain serum T levels within the normal range (4). However, NETE remains in the bloodstream for about 18 d in men (1). Therefore, it is not clear whether spacing the NETE plus TU injection interval beyond 6 wk would still allow for maintenance of profound gonadotropin and thus sperm suppression.

Previous studies on hormonal male contraception have demonstrated that once sperm suppression has been induced with a higher hormonal load, lower doses can maintain sperm suppression (5, 6). In our previous study, injections of TU at 8-wk intervals maintained suppression of spermatogenesis for 32 wk in a small group of eight volunteers in which sperm suppression was induced with cyproterone acetate (CPA) plus TU. Whether even longer TU injection intervals can maintain gonadotropin and sperm suppression has never been tested.

In light of these considerations and with the ultimate goal of developing a hormonal contraceptive based on the lowest and thus safest possible hormonal dose, in this study we administered NETE plus TU at longer injection intervals, compared with previous studies, either from the beginning or after a 12-wk suppression phase. The purpose of this study was to determine which interval would be the most favorable in terms of gonadotropins and sperm suppression. In this paper we report the effects of these regimens on spermatogenesis, reproductive hormones, prostate, and testis, in comparison with a control group in which only placebo injections were administered.

Subjects and Methods

Population

Fifty Caucasian healthy male subjects, aged 18–50 yr, were enrolled in the study. All men had clinical examinations and routine clinical chemistries within the normal range. They had normal reproductive function as assessed by reproductive hormones and semen analysis. All volunteers signed a consent form. The Ethics Committee of the S. Orsola Hospital and University of Bologna approved the study.

Study design

A prospective, monocentric, randomized, controlled, five-arm and single-blind design was used.

The study consisted of a baseline phase lasting at least 4 wk, a treatment phase lasting 48 wk, and a recovery phase that lasted until each subject had at least two sperm counts within his own baseline range.

Baseline phase. During this period volunteers provided three seminal fluid samples and three fasting blood samples. They underwent a complete physical and andrological examination.

Treatment phase. At completion of the baseline period, the 50 subjects were randomly assigned to receive im one of the following regimens (10 subjects each group):

1. NETE 200 mg plus TU 1000 mg every 8 wk (NETE-8 group).
2. NETE 200 mg plus TU 1000 mg every 12 wk (NETE-12 group).
3. NETE 200 mg plus TU 1000 mg every 6 wk for 12 wk (suppression phase) and then NETE 200 mg plus TU 1000 mg every 12 wk (maintenance phase) (NETE-6/12 group).
4. NETE 200 mg plus TU 1000 mg every 6 wk for 12 wk (suppression phase) and then placebo plus TU 1000 mg every 12 wk (maintenance phase) (NETE-6/12/0 group).
5. Placebo plus placebo every 6 wk for 12 wk (suppression phase) and then placebo plus placebo every 12 wk (maintenance phase) (NETE-0/0 group).

During the 48 wk of the treatment period, subjects came to the clinic to receive their injections according to the schedule of the group to which they were assigned. On these occasions, they underwent complete physical and andrological examinations and fasting (10 h) blood drawings. All blood samples were drawn right before the next NETE and TU injections. Subjects provided biweekly seminal fluid samples throughout the entire treatment phase.

Recovery phase. During the recovery phase, subjects came to the clinic every 6 wk for 18 wk to undergo physical and andrological examinations and collection of fasting blood samples. They provided biweekly seminal fluid samples until fulfillment of recovery criteria (at least two sperm counts within individual baseline range).

Metabolic, hematological, coagulation, and anthropological parameters were also monitored throughout the study and will be reported later in a subsequent paper.

Drugs

TU (4 ml, im; Jenapharm, Jena, Germany) as well as NETE (1 ml, im; Schering, Berlin, Germany) were administered in an oily solution of castor oil and benzyl benzoate. Both drugs were administered at the same time but separately, one in each gluteus.

Effect variables

Primary effect variables reported in this study were sperm concentration and sperm suppression at wk 48 to the two thresholds of azoospermia or severe oligozoospermia. Secondary effect variables were gonadotropin levels, T, free T, estradiol (E2), SHBG, prostate volume, prostate-specific antigen (PSA) and testis volumes.

Measurements

Semen. Semen analysis was performed according to the World Health Organization criteria 1999 (7). Semen volume, sperm concentration, motility, and morphology were recorded. Azoospermia was defined as zero sperm count after centrifugation at greater than $3000 \times g$ for 15 min and analysis of the pellet. Severe oligozoospermia was considered as sperm count 1 million/ml or more. Recovery of sperm count was defined as at least two sperm counts within the individual's baseline range.

Serum hormones. Blood samples for hormone measurements were stored at -20 C and assayed at the end of the study. Serum samples from subjects of different groups were run in the same assay. Serum levels of LH, FSH, and SHBG were measured by highly specific time-resolved fluoroimmunoassays (DELFIA; Wallac, Inc., Turku, Finland). T was measured by RIA (TKTT5; Diagnostic Products Corp., Los Angeles, CA).

Free T was calculated using the formula of Vermeulen *et al.* (8). PSA levels were measured by immunofluorescent assays (KRYPTOR; CIS-Bio International, Oris Group, Gif-sur-Yvette, France). The lower limits of quantitation were 0.019 IU/liter, 0.016 IU/liter, 0.35 nmol/liter, 1.56 nmol/liter, and 0.04 ng/ml for LH, FSH, T, SHBG, and PSA, respectively. Mean intraassay coefficients of variation were 10.5 and 5.0% for LH, 8.3 and 2.3% for FSH, 10.0 and 6.6% for T, 3.8 and 2.2% for SHBG, and 0.6 and 1.5% for PSA in the low and high parts of the standard curve, respectively. Mean interassay coefficients of variation were 20.5 and 9.6% for LH, 16.8 and 4.3% for FSH, 13.6 and 6.8% for T, 3.1 and 6.8% for SHBG, and 2.1 and 2.1% for PSA in the low and high parts of the standard curve, respectively. E2 was measured by RIA (DSL-39100; Diagnostic System Laboratories, Inc., Webster, TX). The lower limit of quantitation was 5.5 pmol/liter. Mean intraassay coefficients of variation in the low and high parts of the standard curve were 5.6 and 5.3%. Mean interassay coefficients of variation were 7.0 and 8.9%. Prolactin was measured by immunochemiluminescent assay (ADVIA CENTAUR; Chiron Diagnostics, Los Angeles, CA). The lower limit of quantitation limit of the assay was 6.4 ng/ml. Mean intraassay coefficient of variation was 5.0%.

Clinical: instrumental

Clinical examination consisted of general and genital inspection, measurement of body weight, blood pressure and pulse rate, and an interview to evaluate possible occurrence of adverse events and compliance with drug intake. Prostatic ultrasound examinations were performed according to standardized procedures (9). A 7.5-MHz transrectal transducer (2 cm diameter) and an ESAOTE ultrasound machine (Esaote, Genova, Italy) were used. After applying ultrasound transmission gel, a transducer was covered with a sterile lubricated disposable rubber sheath. The three maximal diameters of the total area of the prostate were recorded. Volumes of the total area were calculated using the standard ellipsoidal formula. The same urologist performed all prostatic ultrasound examinations and was blinded to the treatment group.

The same operator performed measurements of testis volumes using the Prader orchiometer (ASSI, Westbury, NY).

Statistics

Unless otherwise stated, results are reported as mean $\pm$ sd and were analyzed by means of the repeated-measure ANOVA. Where appropriate, parameters were log transformed before analysis. The simple contrast was used to compare pairs of groups (10). The Kaplan-Meier method and log-rank tests were used to evaluate the achievement of azoospermia and the recovery of sperm counts; median times, together with their 95% confidence intervals (95% CIs) were also evaluated. Statistical evaluations were performed by running the SPSS/PC plus (version 8.0; SPSS Inc., Chicago, IL) (11) package on a personal computer. Two-tailed $P < 0.05$ was considered statistically significant.

Baseline values were calculated as the mean of the three samples. Time to azoospermia was calculated during the treatment phase starting from the beginning of the treatment (wk 0). Time to recovery was considered the period from the end of the maintenance phase (wk 48) to the first of two consecutive samples within baseline range. The percentage of azoospermia or oligozoospermia in each group at each time point was calculated excluding dropouts.

Results

Forty-three of the 50 enrolled subjects completed the study. Six of the seven men dropped out of the study before completing the treatment phase: one complained of loss of libido and two because they did not like the injections. Four men discontinued the study for reasons unrelated to the study protocol. Of these men, one subject was in the NETE-0/0 group; two subjects in the NETE-12 group, two subjects in the NETE-6/12/0 group and one subject in the NETE-6/12 group. One subject in the NETE-6/12/0 group dropped out at wk 14 of the recovery phase.

Injections were well tolerated by all the other men who completed the study.

Semen parameters

No significant difference was found among the five groups at baseline (Fig. 1 and Table 1) ($P = 0.436$).

In the NETE-0/0 group, no significant changes of sperm concentration were recorded at any time throughout the study periods.

In the NETE-8 group, mean sperm count was profoundly suppressed throughout the entire treatment period. At wk 48, nine subjects were azoospermic and one had a sperm count of 1 million/ml. Median time to achieve azoospermia was 16 ± 3 wk (95% CI 10–22) and median time to severe oligozoospermia was 8 ± 2 wk (95% CI 5–11) (Fig. 2).

In group NETE-12, mean sperm count was significantly suppressed, compared with baseline, throughout the entire treatment period. However, individual sperm suppression was quite variable among the subjects during the treatment phase. At wk 48, three men were azoospermic (azoospermia achieved at wk 22, 30, and 34), one man had a sperm count below 1 million/ml, and the other four men had sperm counts of 1.5, 10, 25, and 40 million/ml, respectively (Fig. 2).

In the NETE-6/12 and NETE-6/12/0 groups, mean sperm count was profoundly suppressed in all men by wk 12 of hormone administration (suppression phase). At wk 12, six of 18 men were azoospermic (four subjects were in the NETE-6/12 group and two subjects in the NETE-6/12/0 group), and eight subjects were severely oligozoospermic (three subjects were in the NETE-6/12 group and five subjects in the NETE-6/12/0 group). Two subjects of each group had a sperm count above 1 million/ml (3.0, 1.8 and 1.5, 2.0

million/ml in group NETE-6/12 and NETE-6/12/0, respectively).

In the NETE-6/12 group at wk 48, seven subjects were azoospermic, and the remaining two subjects had a sperm count of 1.3 and 10 million/ml, respectively. Four of the seven azoospermic men at wk 48 were already azoospermic at wk 12 and maintained azoospermia or severe oligozoospermia throughout the treatment phase. The other three azoospermic men at wk 48 were severely oligozoospermic at wk 12. In this group, median time to achieve azoospermia was 16 ± 3 wk (95% CI 10–22). Median time to severe oligozoospermia was 12 ± 3 wk (95% CI 6–18) (Fig. 2).

In the NETE-6/12/0 group at wk 48, two subjects were azoospermic (one was already azoospermic at wk 12, whereas the other one achieved azoospermia at wk 14), one had sperm count less than 1 million/ml, and the other five subjects had sperm count of 1.5, 2, 6, 7, and 20 million/ml, respectively.

At wk 48, the percentage of azoospermic subjects was significantly higher in the NETE-8 group, compared with the NETE-12 group (90 *vs.* 37.5%, $P = 0.019$), NETE-8 *vs.* NETE-6/12/0 group (90 *vs.* 25%, $P = 0.005$), and NETE-6/12 *vs.* NETE-6/12/0 group (78 *vs.* 25%, $P = 0.030$). At wk 48, percentage of severe oligozoospermic subjects (≤ 1 million/ml) was significantly higher in the NETE-8 group, compared with the NETE-12 group (100 *vs.* 50% $P = 0.011$), NETE-8 *vs.* NETE-6/12/0 (100 *vs.* 37.5, $P = 0.003$).

Sperm count recovered in all subjects of all groups. Median time to recovery was 16.0 ± 6.8 wk (95% CI 2.6–29.4), 27.0 ± 1.1 (95% CI 24.9–29.1), 16.0 ± 3.0 (95% CI 10.2–21.8), and 14.0 ± 4.7 (95% CI 4.8–23.2) in the NETE-12, NETE-8, NETE-6/12, and NETE-6/12/0 groups, respectively (significantly
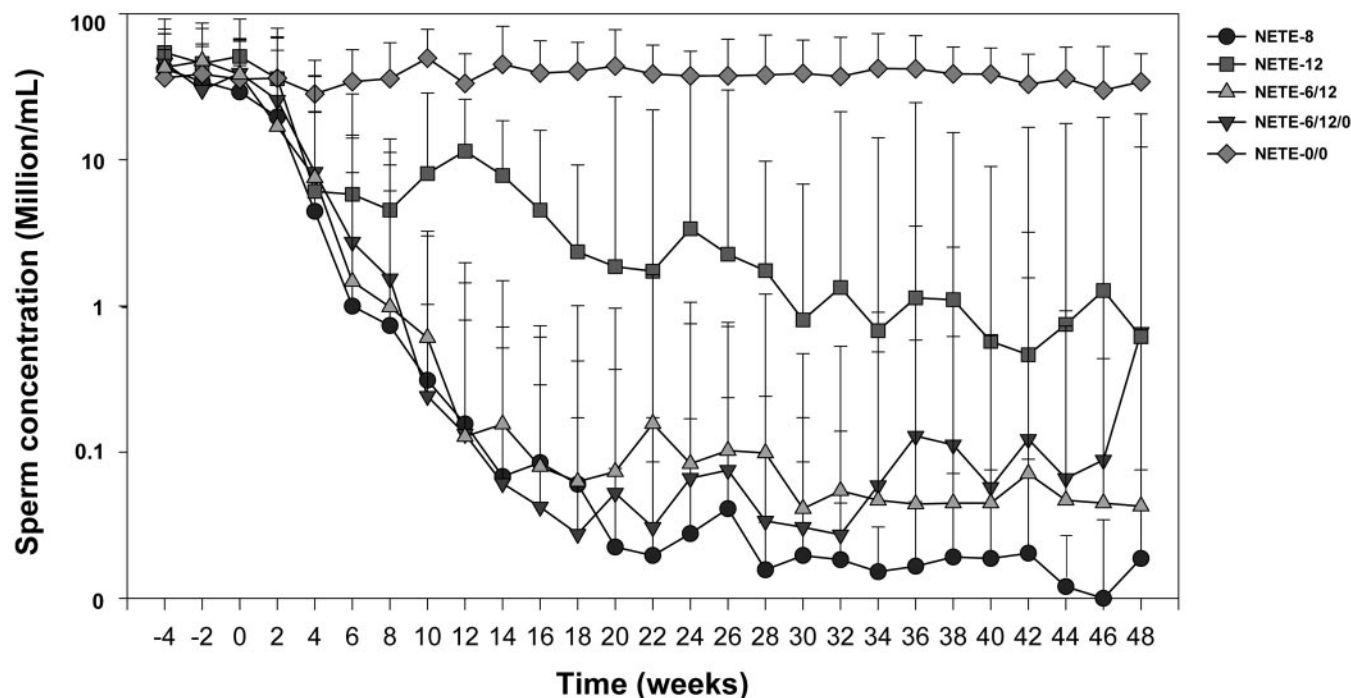

FIG. 1. Mean ($\pm$ SD) sperm concentrations in the five groups during baseline and treatment phases.

TABLE 1. Demographic parameter (mean ± SD) in the five groups of men at baseline

|	NETE-8	NETE-6/12/0	NETE-6/12	NETE-0/0	NETE-12
Age (yr)	30.1 ± 5.0	29.4 ± 7.1	31.1 ± 7.1	28.2 ± 9.22	26.3 ± 4.8
BMI (kg/m^2)	24.56 ± 1.82	23.65 ± 2.29	25.31 ± 2.14	23.66 ± 3.14	24.73 ± 3.14
Testis size (right + left)/2 (ml)	20.10 ± 2.51	21.30 ± 1.82	21.10 ± 2.76	20.40 ± 2.67	20.20 ± 2.04
Sperm concentration (million/ml)	40.13 ± 21.77	43.36 ± 19.60	49.13 ± 22.82	41.56 ± 17.68	57.90 ± 27.90
LH (IU/liter)	4.07 ± 0.91	4.10 ± 0.86	3.31 ± 1.15	4.31 ± 1.19	4.78 ± 1.00
FSH (IU/liter)	3.26 ± 2.20	2.59 ± 0.83	2.68 ± 1.24	2.60 ± 1.03	2.40 ± 0.77
T (nmol/liter)	17.60 ± 6.34	17.26 ± 3.89	15.40 ± 2.23	19.20 ± 3.71	20.20 ± 4.86
SHBG (nmol/liter)	30.56 ± 8.38	34.53 ± 9.98	26.33 ± 8.56	30.70 ± 7.25	29.40 ± 10.08

BMI, Body mass index.
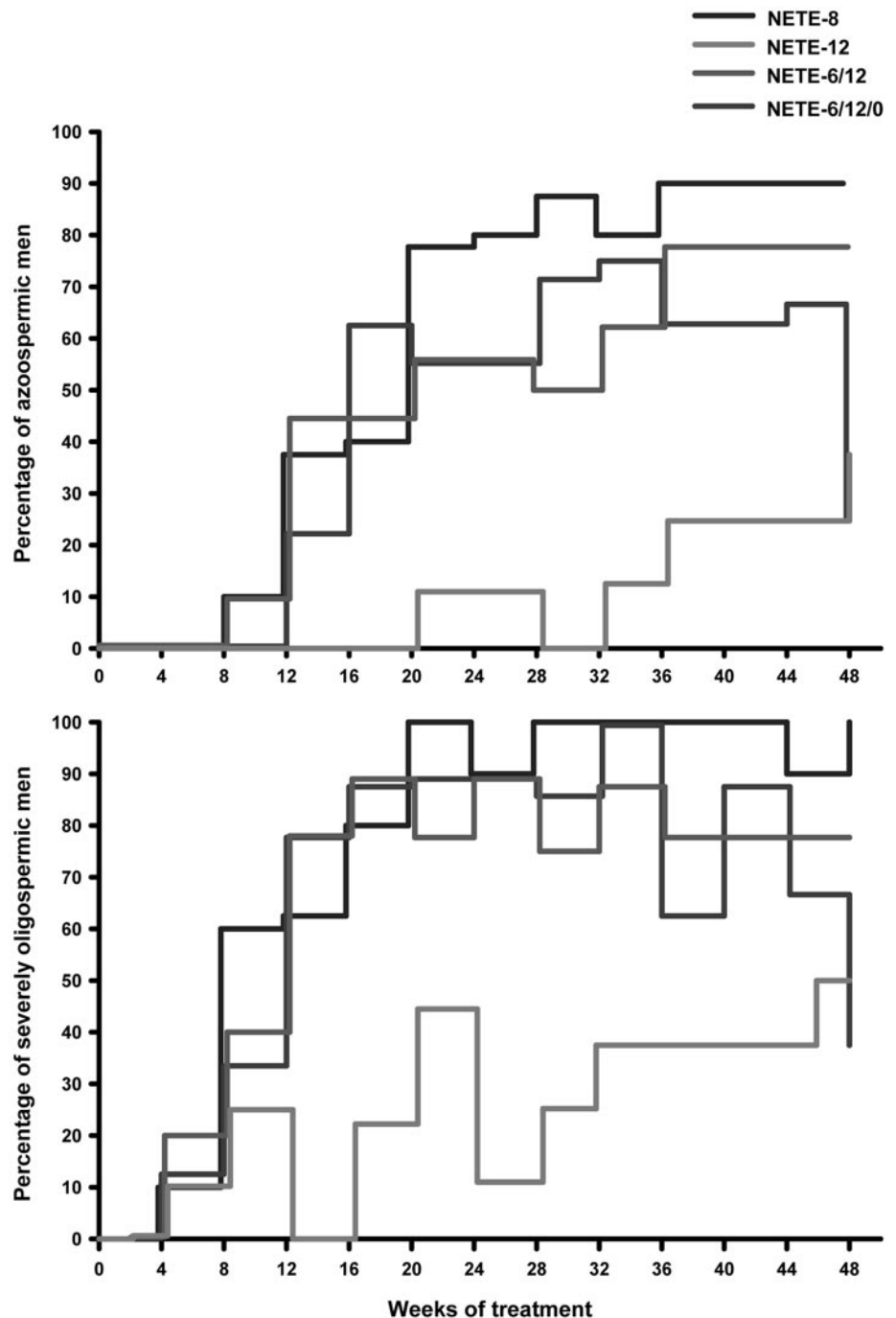
FIG. 2. Percentage of subjects who achieved azoospermia (*upper panel*) and severe oligozoospermia (≤1 million/ml) (*lower panel*) in the four groups throughout the treatment phase (placebo group was excluded). Refer to *Results* for statistical differences among groups.

slower in NETE-8 *vs.* NETE-6/12/0 group, $P < 0.001$, and *vs.* NETE-12 group, $P = 0.002$) (Fig. 3).

Reproductive hormone levels

No significant differences in mean serum LH, FSH, T, free T, E2, and SHBG levels were found at baseline among the five groups (Figs. 4 and 5 and Table 1).

Treatment phase. No significant changes of LH, FSH, T, free T, E2, and SHBG occurred in the NETE-0/0 group at any time throughout the treatment phase.

In the NETE-8 group, LH and FSH levels were already significantly decreased, compared with baseline, at wk 8 (baseline: LH 4.07 ± 0.91 IU/liter; wk 8: LH 0.52 ± 0.78 IU/liter; $P < 0.001$ and baseline: FSH 3.20 ± 2.21 IU/liter; wk 8: FSH 0.93 ± 0.93 IU/liter; $P = 0.006$) and remained profoundly suppressed until the end of the treatment phase (wk 48: 0.02 ± 0.01 IU/liter and 0.33 ± 0.49 IU/liter, $P < 0.001$ and $P < 0.001$ *vs.* baseline for LH and FSH, respectively). Nadir serum T levels were significantly decreased, compared with baseline, at wk 8 ($P = 0.013$). Afterward they started to increase and at wk 40 and 48 were significantly increased over baseline ($P = 0.033$ and 0.001) but were still within normal range. Nadir serum free T levels were significantly increased, compared with baseline at wk 16 ($P = 0.046$) and remained significantly increased until the end of the treatment phase ($P < 0.001$). Serum E2 levels were significantly decreased, compared with baseline, at wk 8 ($P = 0.004$). Afterward they started to increase and at wk 32, 40, and 48 were significantly increased over baseline ($P = 0.039, 0.030,$ and 0.020) but still within normal range. Serum SHBG levels were significantly decreased, compared with baseline, from wk 8 to wk 48 of treatment ($P < 0.001$).

In group NETE-12, LH and FSH levels decreased significantly from baseline but remained significantly higher than group NETE-8 at all times throughout the entire treatment phase ($P < 0.05$). At wk 48, LH and FSH levels were significantly higher in subjects with sperm count greater than 1 million/ml (n = 4), compared with subjects with sperm count 1 million/ml or less (n = 4) (LH: 4.85 ± 2.76 IU/liter *vs.* 0.53 ± 0.98 IU/liter, $P = 0.026$; FSH: 2.33 ± 0.05 IU/liter *vs.* 0.77 ± 1.09 IU/liter, $P = 0.030$). Nadir serum T levels were significantly decreased, compared with baseline, at wk 12 ($P = 0.007$) and wk 36 of hormone administration ($P = 0.048$). No significant changes of nadir free T and E2 were found at any time throughout the treatment phase. SHBG levels were significantly decreased at wk 36 of hormone administration ($P = 0.003$).

In the NETE-6/12 and NETE-6/12/0 groups at wk 12 (end of suppression phase), both LH and FSH were profoundly suppressed to the assay detection limit in all subjects ($P < 0.001$ *vs.* baseline). In the NETE-6/12 group, LH suppression was maintained until the end of treatment in all subjects ($P = $ n.s. wk 12 *vs.* wk 48), whereas FSH levels showed a modest but significant increase (FSH wk 12: 0.09 ± 0.04 IU/liter, wk 48: 0.53 ± 0.43; $P = 0.010$). In this group at wk 48, serum levels of both gonadotropins were not significantly different from the NETE-8 group. In the NETE-6/12/0 group during the maintenance phase (wk 12–48), both gonadotropins increased and at wk 48 became significantly higher than wk 12 (LH wk 12: 0.09 ± 0.20 IU/liter, wk 48: 1.32 ± 1.28 IU/liter,
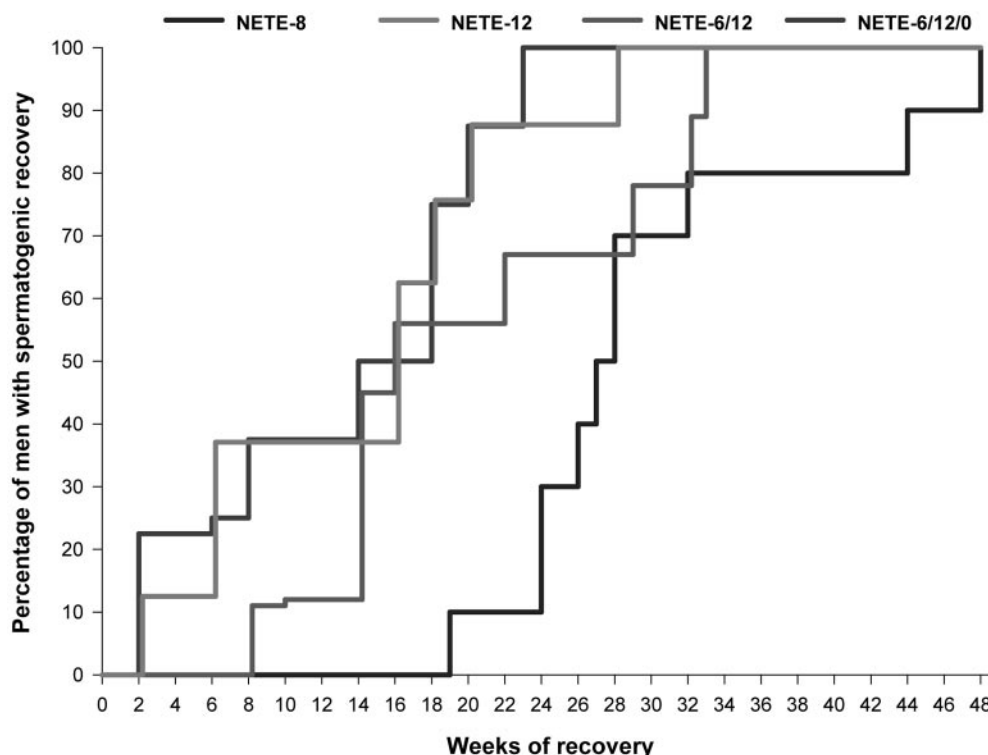

FIG. 3. Percentage of men who recovered to baseline values (first of two consecutive sperm counts within baseline values) after stopping hormone administration in the four treatment groups ($P < 0.001$ among the four groups; placebo group was excluded).
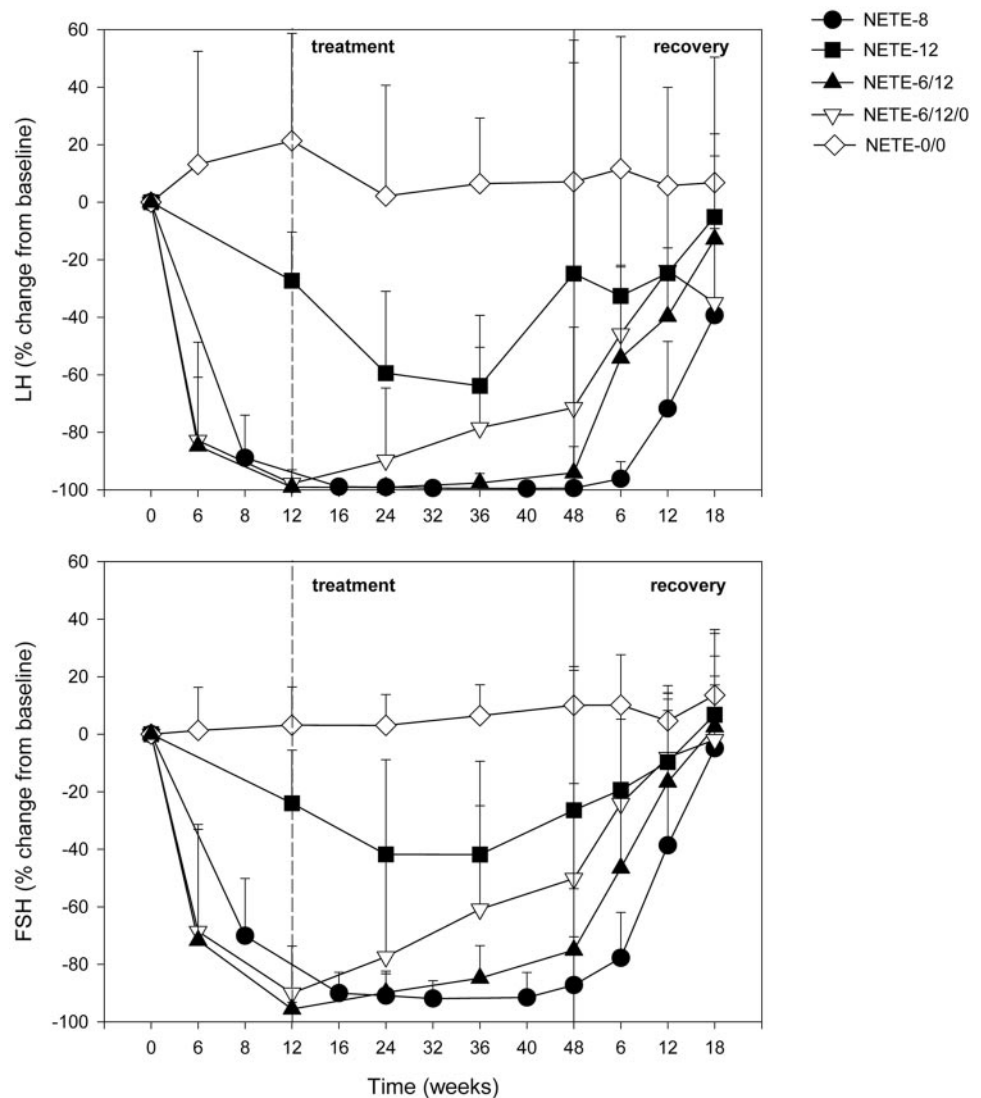
FIG. 4. Mean ± SD percentage changes of LH and FSH levels from baseline in the five groups of subjects throughout the study periods.

$P = 0.023$; FSH wk 12: 0.22 ± 0.29 IU/liter, wk 48: 1.28 ± 0.94 IU/liter, $P = 0.011$). In particular, a rebound of gonadotropins was present in the five subjects who at wk 48 had sperm count 1 million/ml or more, compared with the other three subjects with a sperm count less than 1 million/ml (wk 48 LH: 2.10 ± 0.92 IU/liter *vs.* 0.02 ± 0.01 IU/liter, $P < 0.001$; FSH 1.77 ± 0.79 IU/liter *vs.* 0.45 ± 0.48 IU/liter, $P = 0.041$). At wk 48, mean LH and FSH levels were significantly higher in this group, compared with the NETE-8 group ($P = 0.001$ and 0.012 for LH and FSH, respectively).

There were no significant changes of nadir serum T levels throughout the treatment phases in either group. In both groups, nadir serum-free T levels were significantly increased, compared with baseline, at wk 12 ($P = 0.016$ and 0.001 in the NETE-6/12/0 and NETE-6/12 groups, respectively). Afterward in the NETE-6/12/0 group, free T levels decreased and were not significantly different from baseline at any times throughout the maintenance phase. In the NETE-6/12 group, free T levels were still significantly higher than baseline at wk 24 and 36 ($P = 0.003$ and 0.054, respectively) but were not significantly different from baseline at

wk 48. There were no significant changes of serum E2 levels throughout the treatment phases in the NETE-6/12/0 group. In the NETE-6/12 group, E2 levels were increased, compared with baseline, during the maintenance phase (significant at wk 12 and 48: $P = 0.049$ and 0.021). SHBG levels significantly decreased at wk 6 and 12 in both groups ($P = 0.001$ and $P < 0.001$ in the NETE-6/12/0 and NETE-6/12 groups, respectively). They returned to baseline values in the NETE-6/12/0 group by wk 24 but were significantly lower than baseline in the NETE-6/12 group at wk 24 and 48 ($P = 0.021$ and 0.017).

Recovery phase. After stopping hormone administration, gonadotropin, total T, free T, E2, and SHBG levels started to increase in all subjects. At wk 18 of the recovery phase when the last blood sample was drawn, LH, FSH, T, free T, E2, and SHBG were not different from baseline in all subjects of most groups except the NETE-8 and NETE-6/12/0 groups, in which LH levels were still lower than baseline ($P < 0.001$ and 0.008, respectively), and the NETE-6/12 group, in which E2 levels were still higher than baseline ($P = 0.034$) (Figs. 4 and 5).
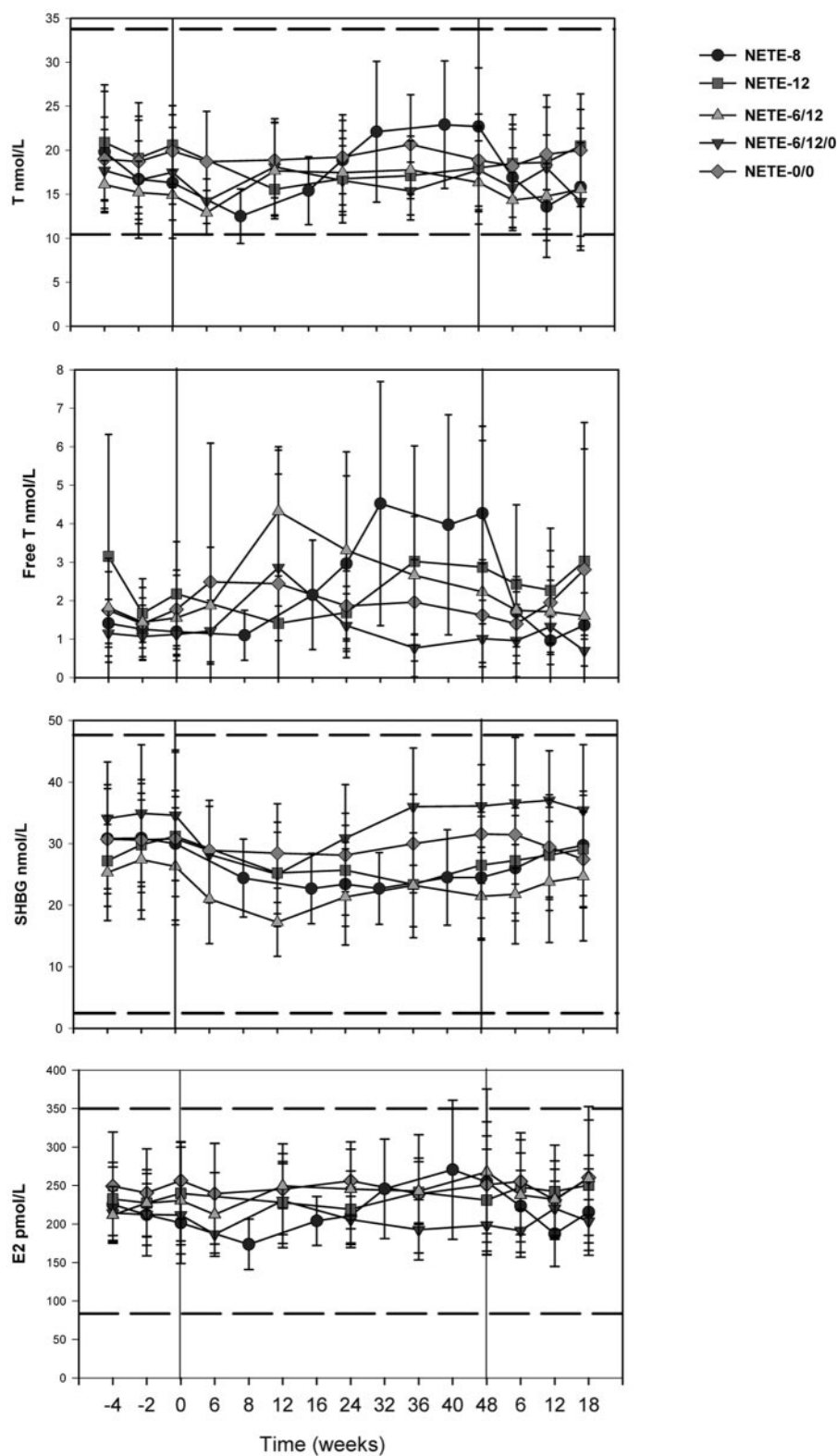
FIG. 5. Mean (± SD) serum total T, free T, SHBG, and E2 levels in the five groups of subjects throughout the study periods.

Clinical examinations

Baseline testis size was within the normal range for all subjects and did not significantly change in any subject of the NETE-0/0 group (Table 2). At wk 48, testis sizes were significantly decreased, compared with baseline, in subjects of the NETE-8, NETE-6/12, NETE-6/12/0, and NETE-12 groups ($P < 0.001$, 0.001, 0.001, and 0.041, respectively). Testis volumes were returned to baseline values by wk 18 of the recovery phase (last measurement) in all groups but NETE-8 and NETE-6/12/0 groups in which they were still signifi-

TABLE 2. Mean ± SD total testis volumes, total prostate volumes, and PSA serum levels in the five groups of men throughout the study periods

	Testis volume (ml) (mean right and left)	Prostate total volume (ml)	PSA (ng/ml)
NETE-8			
Baseline	20.10 ± 2.51	19.7 ± 6.1	0.72 ± 0.58
wk 24	15.00 ± 0.80[a]		0.98 ± 0.80
wk 48	14.70 ± 1.49[a]	19.5 ± 5.8	0.83 ± 0.69
Recovery	17.80 ± 1.93[a,b]	19.9 ± 6.1	0.84 ± 0.60
NETE-6/12/0			
Baseline	21.30 ± 1.82	21.3 ± 5.3	0.81 ± 0.52
wk 24	16.22 ± 1.78[a]		0.74 ± 0.33
wk 48	17.37 ± 1.76[a]	20.1 ± 5.3	0.61 ± 0.39
Recovery	19.42 ± 1.90[a,b]	22.3 ± 4.3	0.57 ± 0.38
NETE-6/12			
Baseline	21.10 ± 2.76	22.7 ± 5.2	0.61 ± 0.22
wk 24	14.70 ± 2.20[a]		0.70 ± 0.23
wk 48	15.22 ± 1.39[a]	25.3 ± 6.4	0.69 ± 0.29
Recovery	20.11 ± 2.93[b]	23.4 ± 5.4	0.60 ± 0.26
NETE-0/0			
Baseline	20.40 ± 2.67	20.5 ± 3.4	0.67 ± 0.24
wk 24	20.44 ± 2.65		0.83 ± 0.32
wk 48	20.44 ± 2.65	20.4 ± 5.4	0.87 ± 0.50
Recovery	20.44 ± 2.65	20.7 ± 3.6	0.69 ± 0.20
NETE-12			
Baseline	20.20 ± 2.04	22.9 ± 6.5	0.90 ± 0.66
wk 24	16.00 ± 2.29[a]		0.95 ± 0.59
wk 48	18.12 ± 2.35[a]	23.1 ± 4.9	0.95 ± 0.73
Recovery	19.25 ± 1.58	24.4 ± 5.0	0.93 ± 0.63

[a] $P < 0.05$ *vs.* baseline.
[b] $P < 0.05$ *vs.* wk 48.

cantly reduced ($P = 0.001$ and 0.011, respectively). A later (1 yr) andrological examination of subjects of these groups showed that all testis volumes had returned to normal.

Prostate volumes (total volume) at baseline and wk 48 and 18 of the recovery phase are reported in Table 2. No significant difference in baseline total volumes was found among the five groups. No significant changes of prostate volumes were detected in any groups at any times.

Discussion

In this study we administered a prototype hormonal male contraceptive regimen based on NETE plus TU at different injection intervals to four groups of 10 healthy normal men. The effects of these regimens were compared with those seen in a group receiving placebo plus placebo injections for the same length of time. We found that the 8-wk injection regimen of TU plus NETE induces a profound suppression of sperm production. The increase of the injection interval from 8 to 12 wk did not allow for induction of profound sperm suppression, and TU alone injected at 12-wk intervals did not maintain sperm suppression. Testis volume decreased significantly in all hormonally treated groups but remained unchanged in the placebo group. Total prostate volumes and PSA did not change significantly in any of the groups at any time throughout the study periods.

Previous studies demonstrated that the NETE plus TU regimen injected every 6 wk can induce profound spermatogenic suppression in men (2, 3). In this study, injections of NETE plus TU at 8-wk intervals induced profound sperm suppression in all subjects. At the end of 48 wk of admin-

istration, sperm count was decreased less than 1 million/ml in all subjects, and nine of 10 subjects had achieved azoospermia. In previous large-scale trials supported by the World Health Organization, it has been demonstrated that spermatogenic suppression less than 1 million/ml provides a pregnancy rate of 0.7/100 couple-years that is similar to that provided by the best female contraceptives and clearly much better than that achieved with condoms (12–14). Therefore, this NETE plus TU regimen, by suppressing sperm count less than 1 million/ml, would be a good candidate for use in large-scale efficacy clinical trials. When the injection interval was increased to 12 wk, sperm suppression was very poor, and only three of eight subjects achieved azoospermia. NETE has been reported to remain in the bloodstream as long as 18 d in men (1). Therefore, when NETE is injected in combination with TU at longer intervals, the androgen has to maintain gonadotropin suppression for the remaining weeks. Based on the pharmacokinetic data of TU in hypogonadal men, the very low levels of TU present 8 wk after injections can explain the escape from suppression in the 12-wk injection regimen (4). For the same reason, TU alone when injected every 12 wk was not able to maintain sperm suppression induced by the 6-wk injection of NETE plus TU. This is in contrast with the 8-wk TU injection regimen that in our previous study was able to maintain sperm suppression for 32 wk after it was induced by TU 1000 mg per 6 wk plus CPA 20 mg/d (6). On the other hand, NETE plus TU injected at 12-wk intervals was able to maintain suppression in those subjects that had already achieved azoospermia or severe oligospermia, whereas the subjects that were not profoundly suppressed at wk 12 never suppressed any further during the maintenance phase. These results seem to confirm previous data suggesting that a lower hormonal dose is needed to maintain rather than to suppress spermatogenesis (15).

By using a borderline suppressive dose, like TU plus NETE injected every 12 wk, the intersubject difference in the sensitivity to the steroid's suppressive effects becomes evident. In fact, with this regimen, whereas four subjects achieved very profound gonadotropin and spermatogenic suppression (azoospermia or severe oligozoospermia), in the other three subjects, sperm counts remained above 1 million/ml in most of the samples. This phenomenon of a different sensitivity to the gonadotropin suppressive effects of steroid hormones, described in most of the previously reported studies that used suboptimal hormonal dose regimens, remains unexplained (16–21). On the other hand, the use of a higher steroid dose may overcome this difference, as described in the literature and demonstrated in this study in the 8-wk injection group in which spermatogenesis was eventually suppressed in all men.

Compared with previously described regimens such as depot medroxyprogesterone acetate plus T pellets, desogestrel plus T pellets, or CPA plus TU, 8-wk injection of NETE plus TU seems to suppress sperm production more slowly (6, 14, 22). In the 8-wk interval group, three of 10 subjects (30%) were azoospermic and four of 10 (40%) had sperm count 1 million/ml or less at wk 12. In our previous study, CPA plus TU suppressed sperm count less than 1 million/ml in all 24 subjects by wk 12 and 14 of 24 (58%) were azoospermic. In this study, the injection interval of 6 wk did

not seem to also improve the speed of sperm suppression significantly. At wk 12, six of 18 subjects of the NETE-6/12/0 and NETE-6/12 groups (33%) were azoospermic, whereas 14 of 18 had a sperm count of 1 million/ml or less (78%). The effects of a shorter injection interval of NETE plus TU, like four weekly injections, have never been evaluated, but in view of previously published data, it is unlikely that an increase of the androgenic dose could result in better sperm suppression (23). The increase of the NETE dose to 400 mg was reported to be nonbeneficial (3). Potentially, a third agent such as a GnRH antagonist or another progestin could be added to speed up gonadotropin suppression. This could lead to a block of spermatozoa maturation and an acceleration of spermiation that would deplete the testis of spermatozoa and result in an early and more predictable block of spermatogenesis.

In this study, in agreement with what was previously reported in trials testing hormonal male contraceptives, recovery of spermatogenesis has been universal after stopping hormone administration (18). However, full recovery of spermatogenesis, defined as sperm count within the baseline range of each subject, took quite a long time (27 wk) in the NETE-8 group, in which spermatogenesis was profoundly suppressed. In this group, the slow recovery was probably due to the slow recovery of LH. In fact, the mean LH after wk 18 in this group was below the normal range. Slow recovery of spermatogenesis has been previously reported with other hormonal contraceptive regimens (13, 21). One may argue that the slow recovery of spermatogenesis after stopping hormone intake may hinder the acceptability of a male hormonal contraceptive regimen. However, we should consider that sperms are present in seminal fluid much earlier than the time at which sperm count finally reaches baseline values. Based on the knowledge that we have of the relationship between sperm count and fertility potential during sperm suppression induced by hormonal contraceptive regimens (12), we may speculate that fertility recovers much sooner than sperm count reaches the normal and/or baseline range. This issue of spermatogenesis recovery is clearly very important. Further trials are required to clarify several points such as the mechanisms of sperm recovery after hormone administration, the relationship between sperm count and fertility potential in the recovery phase, and possible treatments for accelerating recovery of fertility in these subjects.

In the 8-wk injection group, there was a tendency toward an increase in serum total and free T levels from wk 8 to wk 40. Serum total and free T levels remained stable from wk 32 to wk 48 within the normal range. It should also be mentioned that in this study blood samples for T measurements were drawn before the next injections. Therefore, our data show trough T levels. Peak levels according to the previous studies occur about 9 d after injections in hypogonadal men (4). Therefore, it is not known whether long-term TU administration could lead to supraphysiological T levels after injection. A detailed pharmacokinetic study of TU alone or in combination with NETE in normal men has never been performed.

The lack of any effect on prostate volumes and PSA levels during 48 wk of hormone administration is reassuring when considering the long-term safety of this regimen. As in our previous study, the addition of a progestin to TU (in this case NETE) seems to reduce the stimulatory effect of the androgen on the prostate (6). The issue of the possible effects of these contraceptive hormonal regimen on the prostate is, however, very important and will need further and more detailed exploration in long-term studies.

In conclusion, our study shows that the hormonal contraceptive regimen based on 8-wk injections of NETE plus TU very effectively suppresses spermatogenesis in normal men. Longer injection intervals of NETE plus TU could not induce but could maintain sperm suppression, whereas TU alone was not able to do so. These data should encourage the planning of large-scale contraceptive efficacy trials in men with the administration of this combination.

Acknowledgments

References

1. **Kamischke A, Diebacker J, Nieschlag E** 2000 Potential of norethisterone enanthate for male contraception: pharmacokinetics and suppression of pituitary and gonadal function. Clin Endocrinol (Oxf) 53:351–358
2. **Kamischke A, Venherm S, Ploger D, von Eckardstein S, Nieschlag E** 2001 Intramuscular testosterone undecanoate and norethisterone enanthate in a clinical trial for male contraception. J Clin Endocrinol Metab 86:303–309
3. **Kamischke A, Heuermann T, Kruger K, von Eckardstein S, Schellschmidt I, Rubig A, Nieschlag E** 2002 An effective hormonal male contraceptive using testosterone undecanoate with oral or injectable norethisterone preparations. J Clin Endocrinol Metab 87:530–539
4. **Von Eckardstein S, Nieschlag E** 2002 Treatment of male hypogonadism with testosterone undecanoate injected at extended intervals of 12 weeks: a phase II study. J Androl 23:419–425
5. **Swerdloff RS, Bagatell CJ, Wang C, Anawalt BD, Berman N, Steiner B, Bremner WJ** 1998 Suppression of spermatogenesis in man induced by Nal-Glu gonadotropin releasing hormone antagonist and testosterone enanthate (TE) is maintained by TE alone. J Clin Endocrinol Metab 83:3527–3533
6. **Meriggiola MC, Costantino A, Cerpolini S, Bremner WJ, Huebler D, Morselli-Labate AM, Kirsch B, Bertaccini A, Pelusi C, Pelusi G** 2003 Testosterone undecanoate maintains spermatogenic suppression induced by cyproterone acetate plus testosterone undecanoate in normal men. J Clin Endocrinol Metab 88:5818–5826
7. **WHO Task Force on Methods for the Regulation of Male Fertility** 1999 WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge, MA: Cambridge University Press
8. **Vermeulen A, Verdonck L, Kaufman JM** 1999 A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84:3666–3672
9. **Bosch JL, Hop WC, Niemer AQ, Bangma CH, Kirkels WJ, Schroder FH** 1994 Parameters of prostate volume and shape in a community based population of men 55 to 74 years old. J Urol 152:1501–1505
10. **Snedecor GW, Cochran WG** 1989 Statistical methods. 8th ed. Ames, IA: Iowa State University Press
11. **Norusis M, SPSS Inc.** 1998 SPSS base 8.0 user's and applications guides. Chicago: SPSS Inc.
12. **WHO Task Force on Methods for the Regulation of Male Fertility** 1996 Efficacy of testosterone-induced azoospermia and oligozoospermia in normal men. Fertil Steril 65:821–829
13. **Gu YQ, Wang XH, Xu D, Peng L, Cheng LF, Huang MK, Huang ZJ, Zhang GY** 2003 A multicenter contraceptive efficacy study of injectable testoster-

one undecanoate in healthy Chinese men. J Clin Endocrinol Metab 88:559–561

14. **Turner L, Conway AJ, Jimenez M, Liu PY, Forbes E, McLachlan R, Handelsman DJ** 2003 Contraceptive efficacy of a depot progestin and androgen combination in men. J Clin Endocrinol Metab 88:4659–4667

15. **Weinbauer GF, Limberger A, Behre HM, Nieschlag E** 1994 Can testosterone alone maintain the gonadotropin-releasing hormone antagonist-induced suppression of spermatogenesis in the non-human primate? J Endocrinol 142:485–495

16. **Amory JK, Bremner WJ** 2003 Regulation of testicular function in men: implications for male hormonal contraceptive development. J Steroid Biochem Mol Biol 85:357–361

17. **Anderson RA, Baird DT** 2002 Male contraception. Endocr Rev 23:735–762

18. **Wang C, Swerdloff RS** 2002 Male contraception. Best Pract Res Clin Obstet Gynecol 16:193–203

19. **Meriggiola MC, Farley TMM, Mbizvo MT** 2003 Review of androgen-progestin regimens for male contraception. J Androl 24:466–483

20. **Kamischke A, Nieschlag E** 2004 Progress towards hormonal male contraception. Trends Pharmacol Sci 25:49–57

21. **McLachlan RI, Robertson DM, Pruysers E, Ugoni A, Matsumoto AM, Anawalt BD, Bremner WJ, Meriggiola MC** 2004 Relationship between serum gonadotropins and spermatogenic suppression in men undergoing steroidal contraceptive treatment. J Clin Endocrinol Metab 89:142–149

22. **Kinniburgh D, Zhu H, Cheng L, Kicman AT, Baird DT, Anderson RA** 2002 Oral desogestrel with testosterone pellets induces consistent suppression of spermatogenesis to azoospermia in both Caucasian and Chinese men. Hum Reprod 17:1490–1501

23. **Meriggiola MC, Costantino A, Bremner WJ, Morselli-Labate AM** 2002 Higher testosterone dose impairs sperm suppression induced by a combined androgen-progestin regimen. J Androl 23:684–690