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Testosterone Undecanoate Maintains Spermatogenic Suppression Induced by Cyproterone Acetate Plus Testosterone Undecanoate in Normal Men

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In this study we evaluated whether testosterone undecanoate (TU), alone or combined with low dose cyproterone acetate (CPA), can maintain spermatogenic suppression induced by higher doses of CPA plus TU. Twenty-four men received for 12 wk 20 mg/d CPA plus 1000 mg/6 wk TU and then 1000 mg/8 wk TU plus 20 mg/d CPA (n = 8), 2 mg/d CPA (n = 8), or plus placebo (n = 8) for 32 wk. Blood samples, physical examinations, hormones, chemistry, hematology, semen analysis, and sexual/behavioral assessments were performed throughout the study. Sperm counts decreased to less than 1 million/ml in all subjects by wk 12, and 54% of them achieved azoospermia. Suppression of sperm counts was maintained until wk 44. Serum LH and FSH levels were suppressed by wk 12 of hormone administration and remained suppressed until wk 44. No significant changes in any biochemical parameters were detected at wk 44 in any group. There was a slight increase in total prostate volume to within the normal range at wk 44 that returned to baseline 1 yr after stopping hormone administration. In conclusion, TU alone or combined with lower doses of CPA maintains sperm suppression induced by higher dose CPA plus TU for 32 wk. This prototype regimen represents a promising male contraceptive regimen. (*J Clin Endocrinol Metab* 88: 5818–5826, 2003)

THERE IS A general concern about long-term steroid intake, and in both in females and males, the aim of hormonal contraceptive research is to find the lowest hormonal dose that would allow reliable contraception and at the same time maximally reduce the incidence of complications and the likelihood of developing long-term side effects (1–4).

Most recently developed androgen-progestin combinations have been shown to be highly effective in terms of sperm suppression and fully reversible contraceptive regimens in men (5–15). In the short-term, these hormonal combinations do not induce any major changes in clinical and laboratory parameters, thus promising to also be safe for long-term use (1). Therefore, androgen-progestin regimens hold great promise to be further developed and become a real option for contraception in the male. Establishing the minimum effective combined dose of the two steroids of this contraceptive regimen would further improve its long-term safety.

Among recently tested prototype androgen-progestin regimens, the combined administration of CPA plus low dose testosterone enanthate (TE) proved to induce rapid and profound sperm suppression (5, 16, 17). However, the need for weekly injections of TE would not be acceptable for contraception. Moreover, although no major changes in laboratory parameters were detected with this prototype regimen, a decrease in hemoglobin and hematocrit was found that seemed to be dependent on the antiandrogenic activity of CPA and that would certainly decrease the acceptability of such a regimen.

The recent development of the long-acting injectable testosterone undecanoate (TU) formulation represents a major breakthrough in the andrology field and in particular will greatly improve hormonal contraceptive regimens. With this preparation, testosterone (T) levels can be maintained within the physiological range for 12 wk in hypogonadal men (18). The reduction of supraphysiological testosterone peaks that were present with previously used im T preparations will contribute to improving sperm suppression and reducing androgen-related side effects. TU has been used in male contraceptive trials alone or in combination with the progestins levonorgestrel or norethisterone enanthate (19–21). In these studies it has proved to be at least as effective as previously tested androgens, such as TE, but is more acceptable because of the longer injection intervals at which it can be administered.

Preliminary studies in nonhuman primates and in humans have suggested that sperm suppression induced with a higher hormonal load can be maintained with a lower hormonal dose (22, 23). This maneuver would allow for the use of higher hormonal doses for a short period of time needed to induce a rapid and profound sperm suppression that can be maintained by lower, and probably safer for long-term use, hormonal doses.

Therefore, in the present study we tested whether the long-acting T preparation TU, alone or in combination with

Abbreviations: CI, Confidence interval; CPA, cyproterone acetate; E2, estradiol; HDL, high-density lipoprotein; PRL, prolactin; PSA, prostate-specific antigen; T, testosterone; TE, testosterone enanthate; TU, testosterone undecanoate.

lower CPA doses, could maintain sperm suppression induced with higher dose CPA and TU for 12 wk.

Subjects and Methods

Population

Twenty-four Caucasian male subjects, aged 18–45 yr, were enrolled in the study (Table 1). All men were healthy by medical history, clinical examination, and routine clinical chemistry. They had normal reproductive function as assessed by reproductive hormones and semen analysis. All volunteers signed the consent form to participate in the trial. The ethics committee of the S. Orsola Hospital and University of Bologna approved the study.

Study design

A prospective, monocentric, randomized, controlled, and single-blind design was used. The study consisted of a baseline phase lasting at least 4 wk, a treatment phase lasting 44 wk, and a recovery phase that lasted until each subject had at least two sperm counts within his own baseline range. The treatment phase was divided into a suppression phase that lasted 12 wk and a maintenance phase that lasted 32 wk.

Baseline phase. During this period volunteers provided three seminal fluid samples and three fasting blood samples. They filled out a sexual and behavior questionnaire three times (wk −4, −2, and 0) and underwent a transrectal prostatic ultrasound.

Suppression phase. During this period all 24 subjects received 20 mg/d CPA, orally, and 1000 mg TU injected every 6 wk until wk 12.

Maintenance phase. At wk 12, all volunteers were randomly divided into three groups to receive 1000 mg/8 wk TU plus 20 mg/d CPA (CPA-20; n = 8 subjects), plus 2 mg/d CPA (CPA-2; n = 8 subjects), or plus placebo (CPA-0; n = 8 subjects). During the suppression and maintenance phases, subjects came to the clinic every 6 wk for the first 12 wk and then every 8 wk until wk 44, respectively, for clinical examination. During these visits to the clinic, volunteers provided fasting (10 h) blood samples and completed sexual and behavioral questionnaires. All blood samples were drawn immediately before next TU injection. Subjects provided biweekly seminal fluid samples throughout the entire treatment phase (suppression and maintenance phases). At the end of the maintenance phase, at wk 44, all subjects underwent prostatic transrectal ultrasounds.

Recovery phase. During the recovery phase, subjects were asked to come to the clinic every 6 wk for the collection of a fasting blood specimen and the completion of sexual and behavioral questionnaires. Volunteers provided biweekly seminal fluid samples until fulfillment of recovery criteria (at least two sperm counts within individual baseline range). Subjects underwent transrectal prostatic ultrasound at wk 18 of the recovery phase. One year after the end of hormone administration, subjects underwent prostatic transrectal ultrasound and measurements of testis volumes.

Effect variables recorded in this study were sperm concentration, sperm suppression to the two thresholds of azoospermia or severe oligozoospermia (≤1 million/ml), gonadotropins, T, estradiol (E2), SHBG, and prolactin (PRL). Safety measurements included hematology and clinical chemistry, prostate-specific antigen (PSA), prostate sonography, clinical examination, and interview and questioning about local tolerability of TU injection, sexual function, and behavior.

Measurements

Semen analysis was performed according to the WHO criteria (24). Semen volume, sperm concentration, motility, and morphology were recorded. Azoospermia was defined as at least two consecutive azoospermic specimens and a zero sperm count at the end of treatment (wk 44) after centrifugation at more than 3000 rpm for 15 min and analysis of the pellet. Recovery of sperm count was defined as at least two sperm counts within individual baseline range.

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, T, and SHBG were measured by highly specific time resolved fluoroimmunoassays (DELFIA, Wallac, Inc., Turku, Finland). PSA levels were measured by immunofluorescent assays (Kryptor, CIS-Bio International, Oris Group, Gif-sur-Yvette, France). The lower detection limits were 0.0156 IU/liter, 0.0188 IU/liter, 1.5 nmol/liter, 6.25 nmol/liter, and 0.04 ng/ml for FSH, LH, T, SHBG, and PSA, respectively. Mean intraassay coefficients of variation were 12.0% and 2.9% for FSH, 6.5% and 5.4% for LH, 5.6% and 7.9% for T, 4.1% and 4.1% 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 22.3% and 8.3% for FSH, 17.7% and 14.1% for LH, 8.2% and 8.0% for T, 10.7% and 3.5% for SHBG, and 2.1% and 2.1% for PSA in the low and high parts of the standard curve, respectively. Estradiol was measured by RIA (ICN Biomedicals, Inc., Costa Mesa, CA). The lower detection limit was 37 pmol/liter. Mean intraassay coefficients of variation in the high and low parts of the standard curve were 9.5% and 5.4%, respectively. Mean interassay coefficients of variation in the high and low parts of the standard curve were 8.0% and 10.5%, respectively. PRL was measured by immunochemiluminescent assay (ADVIA Centaur, Chiron Diagnostics, Emeryville, CA). The lower detection limit of the assay was 6.4 mU/liter. The mean intraassay coefficient of variation was 5%. Other measurements included hematology (red cell count, hemoglobin, hematocrit, white cell count, and platelet count) and clinical chemistry (total cholesterol, triglycerides, high (HDL) and low density lipoproteins, glucose, alkaline phosphatase, urea, creatinine, aspartate aminotransferase, alanine aminotransferase, total bilirubin, sodium, potassium, calcium, and phosphate) and were performed according to previously described methodologies (16).

Clinical examination consisted of general and genital inspections; measurement of body weight, blood pressure, and pulse rate; and an interview to evaluate possible occurrence of adverse events and compliance with drug intake. The same operator always performed testis volumes measured with Prader orchidometer.

Sexual and behavioral parameters were monitored using a previously reported questionnaire (25).

Prostatic ultrasound examinations were performed using a 7.5-MHz transrectal transducer (2 cm in diameter) and an Esaote ultrasound machine (Esaote, Genova, Italy). 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. Total area volumes were calculated using the standard ellipsoidal formula. The same urologist performed all prostatic ultrasound examinations and was blinded to the treatment group. His interassay coefficient of variation is 2.3% calculated on prostate total volume measurements made in placebo-treated subjects.

TABLE 1. Demographic parameters in the three groups of men at baseline

	CPA-20	CPA-2	CPA-0	*P* value
Age (yr)	35.25 ± 2.36	32.38 ± 2.76	27.88 ± 1.99	0.114
BMI (kg/m^2)	25.13 ± 1.12	25.50 ± 0.83	24.19 ± 0.60	0.562
Testis size (right + left)/2 (ml)	20.88 ± 0.77	20.86 ± 0.83	20.71 ± 1.70	0.994
Sperm density (million/ml)	46.46 ± 8.16	36.88 ± 6.58	58.25 ± 8.83	0.185
LH (IU/liter)	3.46 ± 0.31	3.02 ± 0.27	2.95 ± 0.31	0.437
FSH (IU/liter)	3.87 ± 0.90	3.19 ± 0.51	2.93 ± 0.43	0.580
T (nmol/liter)	21.56 ± 1.96	21.42 ± 1.19	21.00 ± 1.82	0.971

BMI, Body mass index.

Drugs

TU was administered in a castor oil suspension of 4 ml, im. CPA (Jenapharm GmbH & Co. KG, Jena, Germany) was taken orally.

Statistics

Data are reported as the mean ± SE and were analyzed by repeated measure ANOVA (26). To avoid multiple comparisons, the simple contrast was used to compare pairs of groups. The Kaplan-Meier method and the log-rank test (27) were used to evaluate the achievement of azoospermia and the recovery of sperm counts; median times together with their 95% confidence intervals (CI) were reported. Statistical evaluations were performed by running the SPSS/PC$^+$ version 8.0 (28) package (SPSS, Inc., Chicago, IL) 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 suppression and maintenance phases starting from the beginning of the suppression treatment (wk 0); the calculation was performed considering the first of two consecutive azoospermic samples. Time to recovery was considered the period from the end of the maintenance phase (wk 44) to the first of two consecutive samples within baseline range.

Results

Semen parameters

No significant difference was found among the three groups at baseline and within each group among the three baseline samples (Fig. 1).

Suppression phase. At wk 12 of hormone administration (end of suppression phase), 13 men were azoospermic [13 of 24; 54%; time to azoospermia was 11.79 ± 0.84 (mean ± SE); range, 8–12 wk], and sperm count was suppressed to less than 1 million/ml in all men (Figs. 1 and 2).

Maintenance phase. Two subjects dropped out of the study at wk 14 (group CPA-2) and wk 18 (group CPA-0), respectively, for reasons possibly unrelated to hormone administration. The 2 men who dropped out of the study were severely oligozoospermic (<1 million/ml) and azoospermic, respectively. At the end of the maintenance phase (wk 44), 18 of 22 men had achieved azoospermia (82%), and all of the other men had sperm counts below 1 million/ml (Figs. 1 and 2).

All 13 subjects who were azoospermic at wk 12 were

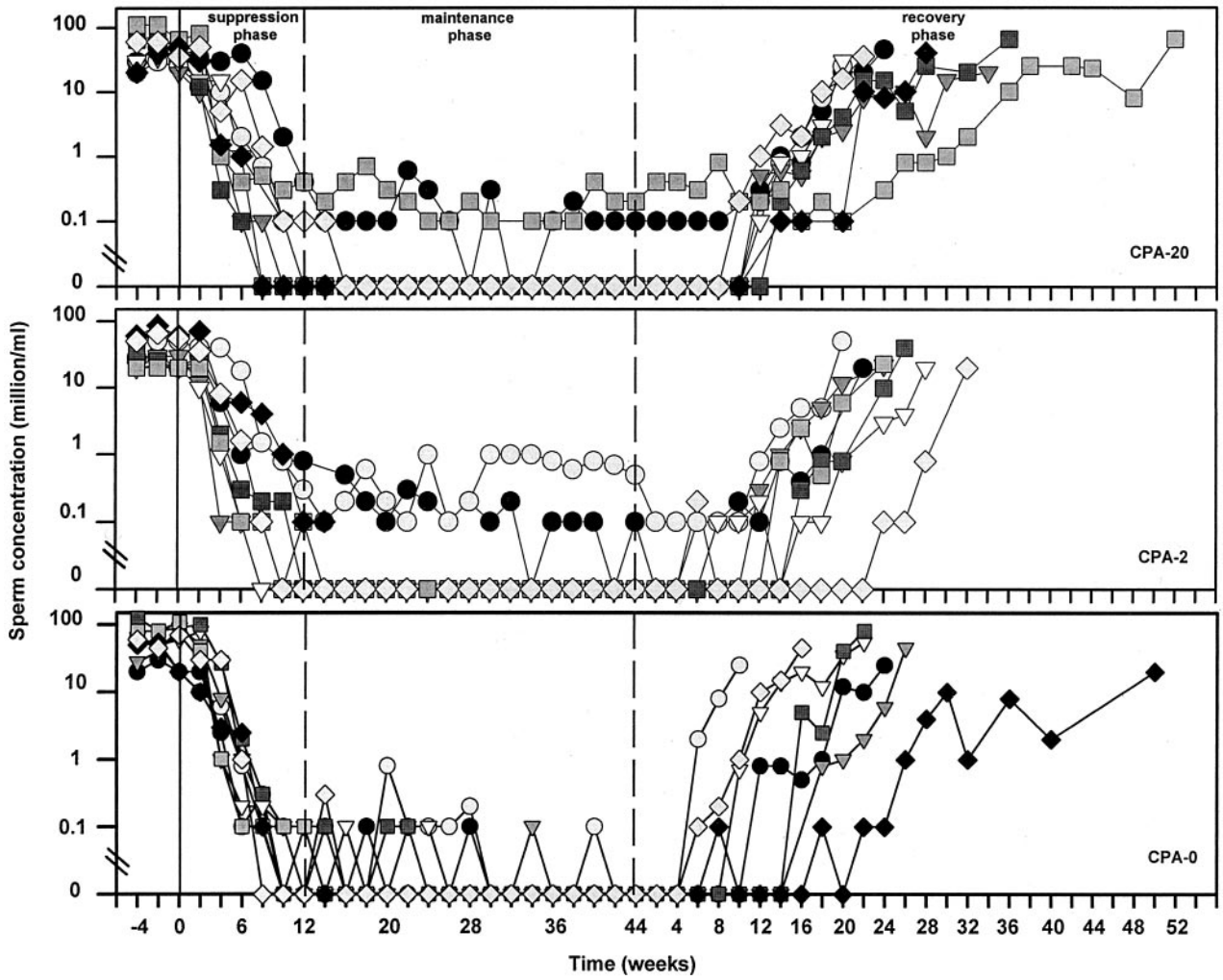

FIG. 1. Individual sperm concentrations in subjects of the three groups (CPA-20, CPA-2, and CPA-0) during baseline, suppression, maintenance, and recovery phases.

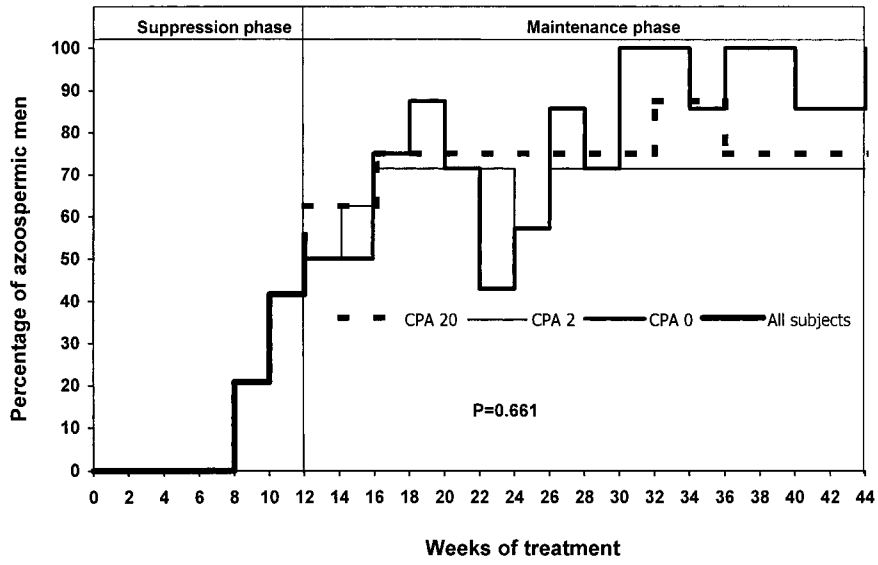

FIG. 2. Percentage of all azoospermic men combined over the first 12 wk of treatment (suppression phase) and separated into three groups throughout the maintenance phase ($P = 0.661$ among the three groups). Azoospermia was defined as the first of two azoospermic samples.

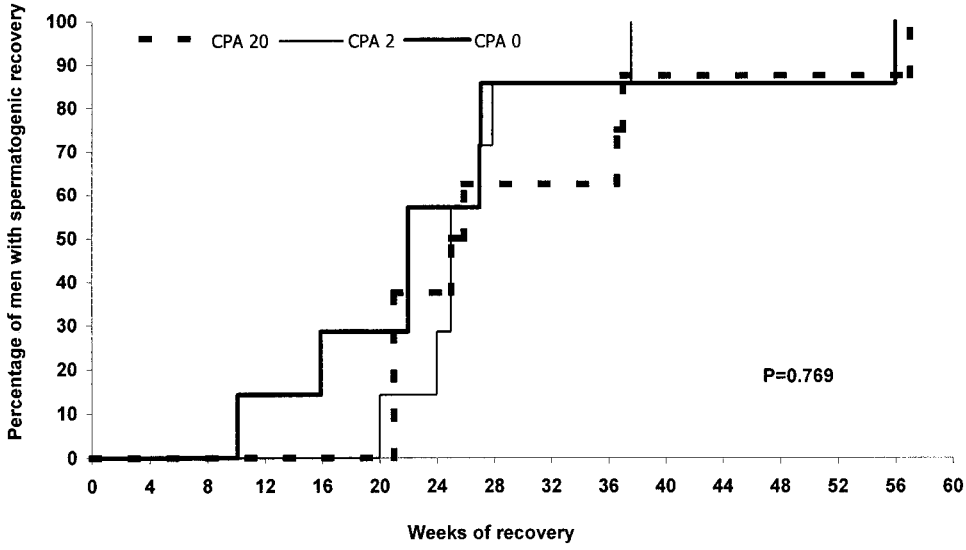

FIG. 3. Recovery of spermatogenesis to baseline values (first sperm count within baseline values) after stopping hormone administration in the three groups of men ($P = 0.769$ among the three groups).

azoospermic at wk 44 regardless of the treatment they received in the maintenance phase. Five subjects who were severely oligozoospermic (<1 million/ml) at wk 12 became azoospermic at wk 44. Four subjects remained oligozoospermic. No significant difference in the rate of azoospermia or in mean sperm count was found at any time point during the maintenance phase among the three groups.

In the CPA-20 group, five of eight men were azoospermic at wk 12 and six of eight men were azoospermic at wk 44. No rebound of sperm count occurred at any time in any azoospermic subject in this group between wk 12 and wk 44.

In the CPA-2 group, four of eight men were azoospermic at wk 12, and five of seven men were azoospermic at wk 44. No rebound of sperm count occurred at any time in any azoospermic subject in this group between wk 12 and wk 44.

In the CPA-0 group, four of eight men were azoospermic at wk 12 and seven of seven men were azoospermic at wk 44. Three of the four men in the CPA-0 group who were azoospermic at wk 12 and 44 occasionally had rebound of spermatogenesis (to <1 million/ml) during the maintenance phase. These fluctuations are displayed in Fig. 1.

Recovery phase. Spermatogenesis returned to normal levels in all men (Figs. 1 and 3). The recovery rate is reported in Fig. 3. Median time to recovery was 25.5 (95% CI, 18.2–31.8), 25.0 (95% CI, 23.7–26.3), 22.0 (95% CI, 14.2–29.8) in the CPA-20, CPA-2, and CPA-0 groups, respectively ($P = 0.769$ among different groups).

Reproductive hormone levels

No significant differences in mean serum LH, FSH, T, E2, SHBG, and PRL levels were found at baseline among the three groups (Figs. 4–6).

Suppression phase. After the administration of CPA plus TU or TU alone, FSH and LH significantly decreased in all subjects from baseline to wk 12: FSH: baseline, 3.33 ± 0.37 IU/liter; wk 12, 0.10 ± 0.03 IU/liter; LH: baseline, 3.14 ± 0.17

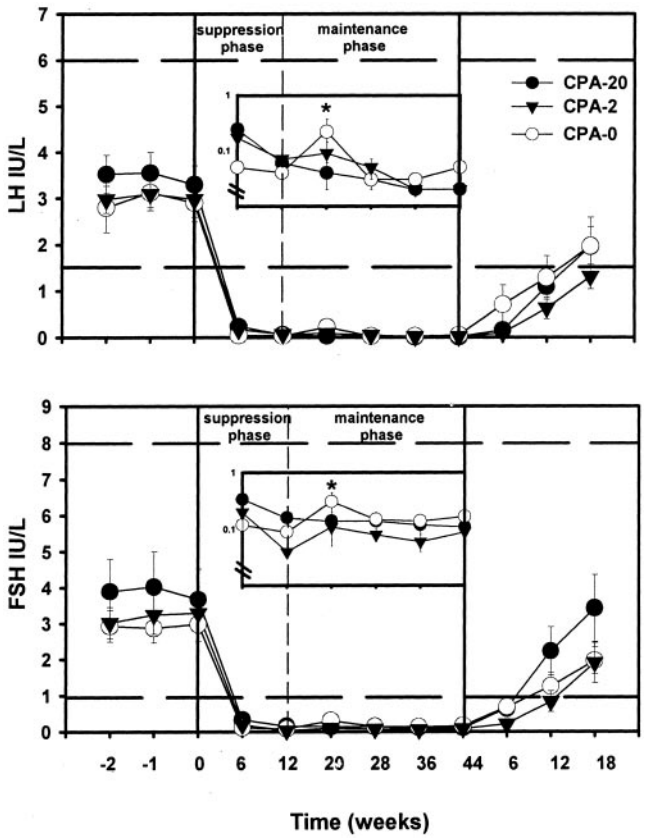

FIG. 4. Mean (±SE) serum FSH and LH levels in the three groups of subjects throughout the study periods. *, $P < 0.05$ at wk 20 *vs.* wk 12 in group CPA-0. *Inset*, Changes in LH and FSH on a log scale from wk 6 to wk 44 of treatment.

IU/liter; wk 12, 0.05 ± 0.01 IU/liter ($P < 0.001$ *vs.* baseline both for LH and FSH; mean ± SE of all 24 subjects; Fig. 4). Low levels are clearly shown in the log scale graph of the *inset*. Mean serum T levels did not significantly change from baseline to wk 12: baseline, 21.33 ± 0.93 nmol/liter; wk 12, 20.71 ± 1.43 nmol/liter ($P = 0.680$; mean ± SE of all 24 subjects; Fig. 5). Mean serum SHBG levels were significantly decreased from baseline to wk 12: baseline, 36.32 ± 3.07 nmol/liter; wk 12, 30.13 ± 2.53 nmol/liter ($P = 0.001$; mean ± SE of all 24 subjects). Mean E2 levels were significantly decreased from baseline to wk 12: baseline, 95.20 ± 6.53 pmol/liter; wk 12, 75.63 ± 5.60 pmol/liter ($P = 0.013$; mean ± SE of all 24 subjects). PRL levels were significantly increased in all subjects from baseline to wk 12: baseline, 195.62 ± 16.79 mIU/liter; wk 12, 383.53 ± 32.83 mIU/liter ($P < 0.001$; mean ± SE of all 24 subjects; Fig. 6).

Maintenance phase. At wk 20, serum FSH and LH were significantly increased in group CPA-0 compared with wk 12 ($P = 0.016$ and 0.039 for FSH and LH, respectively). These changes were due in particular to an increase in serum FSH and LH levels in two subjects. Both gonadotropins were suppressed again below the normal range by wk 28 in these two subjects and remained suppressed until wk 44 in all subjects of group CPA-0. From wk 12 to wk 44 both serum FSH and LH levels remained suppressed below the normal range in all subjects in groups CPA-20 and CPA-2. From wk

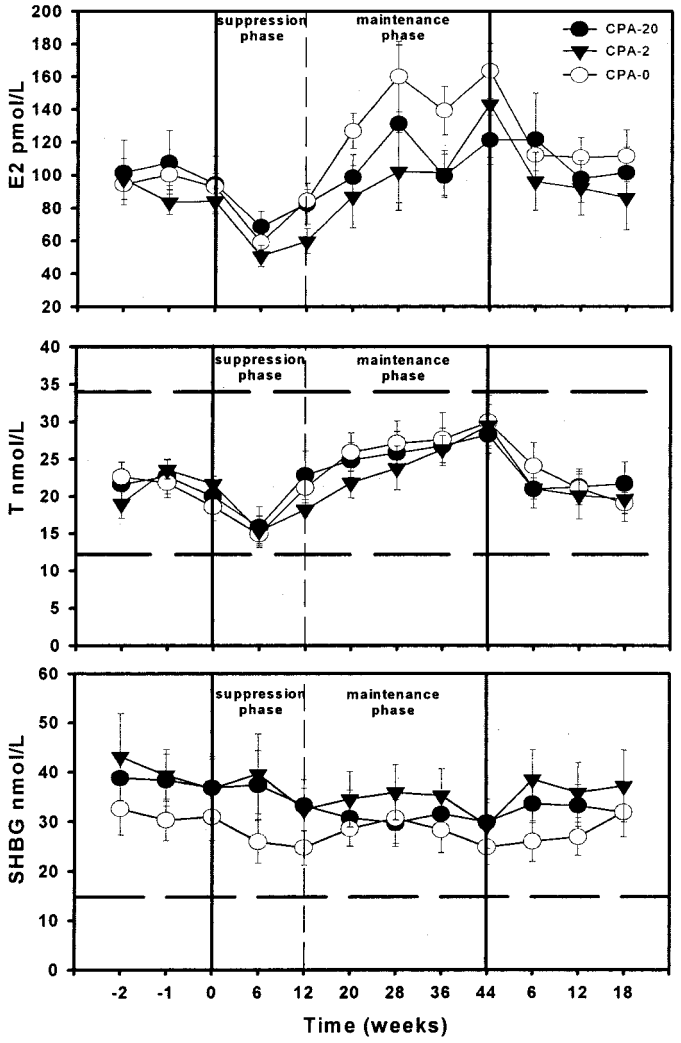

FIG. 5. Mean (±SE) serum T, E2, and SHBG levels in the three groups of subjects throughout the study periods.

12 to wk 44 T levels tended to gradually increase in all three groups. At wk 44 serum T levels were significantly increased compared with baseline levels in all three groups (CPA-20, $P = 0.008$; CPA-2, $P = 0.002$; CPA-0, $P = 0.002$) and compared with wk 12 in groups CPA-2 and CPA-0 ($P = 0.003$ and $P = 0.024$). At wk 44 serum SHBG levels were significantly lower than baseline in all three groups ($P = 0.030$, 0.037, and 0.051 in groups CPA-20, CPA-2, and CPA-0, respectively), but were not different from wk 12 levels. At this time, SHBG levels did not differ among the three treatment groups. PRL had returned to baseline levels by wk 28 and 44 in the CPA-0 and CPA-2 groups, respectively. In group CPA-20, PRL levels were maintained significantly higher than baseline until wk 44 (Fig. 6; $P = 0.005$).

Recovery phase. After stopping hormone administration, gonadotropin levels started to increase in all subjects. At wk 18 of the recovery phase, when the last blood sample was drawn, mean serum FSH levels were not significantly different from baseline in the three groups, whereas LH levels were still significantly lower than baseline in all three groups ($P = 0.001$, 0.001, and 0.031 in CPA-20, CPA-2, and CPA-0,

respectively). Serum T levels had returned to baseline by wk 6 of the recovery phase. By wk 18 of the recovery phase, serum SHBG and E2 levels had returned to baseline in all groups. At wk 18 of the recovery phase, PRL levels were not significantly different from baseline in all three groups.

Hematology and clinical chemistry

At wk 12, hematocrit and hemoglobin were significantly lower compared with baseline in all 24 subjects: hematocrit: baseline, 45.28 ± 0.49%; wk 12, 43.52 ± 0.60% ($P = 0.004$); hemoglobin: baseline, 15.11 ± 0.15 g/dl; wk 12, 14.76 ± 0.20 g/dl ($P = 0.019$; mean ± SE of all 24 subjects). By wk 44, both hematocrit and hemoglobin were found to be increased compared with wk 12 values (Table 2).

Chemistry parameters throughout the study periods are displayed in Table 3. Plasma HDL cholesterol levels were significantly decreased at wk 12 of hormone administration compared with baseline; baseline 1.26 ± 0.04 mmol/liter; wk 12 1.10 ± 0.04 mmol/liter; $P < 0.001$ (mean ± SE of all 24 subjects). At wk 44, HDL cholesterol levels were not significantly different from baseline in all three groups. Plasma total cholesterol levels were significantly decreased at wk 12 of hormone administration compared with baseline: baseline, 4.91 ± 0.18 mmol/liter; wk 12, 4.56 ± 0.17 mmol/liter ($P = 0.001$; mean ± SE of all 24 subjects). At wk 44, total cholesterol levels were not significantly different from baseline in all three groups.

No significant changes in liver tests were found at any time in any group throughout the study periods (Table 3).

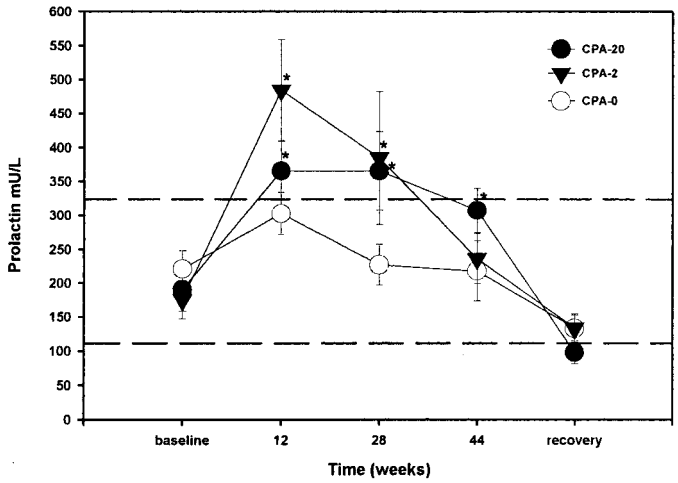

FIG. 6. Mean (±SE) serum PRL levels in the three groups of subjects throughout the study periods. *, $P < 0.05$ *vs.* baseline.

Clinical examinations

The hormonal treatment was well accepted by all subjects. Two subjects dropped out of the study for reasons not definitely related to the hormonal treatment. Injections were well tolerated, and six subjects experienced six adverse events related to the TU injection (five complained of pain at the injection site, and one reported a reaction at the injection site). No significant changes in any clinical signs, blood pressure, pulse rate, and body weight were detected in any subject at any time.

At wk 12 and 44, testis size was significantly decreased compared with baseline in all subjects, with no difference among the three groups (Table 4). Testis volumes were still significantly lower than baseline at wk 18 of the recovery phase, but had returned to baseline in the measurements performed 1 yr later. Prostate volumes (total volume) at baseline, wk 44, wk 18 of the recovery phase, and 1 yr after stopping hormone administration are reported in Table 4. No difference in baseline total volume was found among the three groups. At wk 44, all three groups showed a slight increase in total volumes of the prostate that attained statistical significance only in the CPA-0 group. Measurements performed 1 yr later showed that prostate volumes had returned to baseline in all subjects of all groups. PSA levels tended to decrease at wk 12 in all three groups and to return to baseline at wk 44 in all three groups.

No significant changes in any sexual parameter, well-being, or mood were reported at any time throughout the study periods.

Discussion

In this study we tested whether TU injected every 8 wk alone or in combination with low dose CPA was able to

TABLE 2. Hematological parameters (mean ± SE) in the groups of men at baseline (mean of three samples), at the end of the suppression phase (wk 12), at the end of the treatment phase (wk 44), and after 18 months of the recovery phase

	All subjects	CPA-20	CPA-2	CPA-0
Hemoglobin (g/dl)				
Baseline	15.11 ± 0.15	15.30 ± 0.28	14.97 ± 0.26	15.07 ± 0.23
Wk 12	14.76 ± 0.20[a]	14.96 ± 0.37	14.70 ± 0.39	14.60 ± 0.32
Wk 44		15.16 ± 0.26	15.51 ± 0.28[b]	14.99 ± 0.50
Recovery		15.56 ± 0.42	15.40 ± 0.34	15.36 ± 0.38
Hematocrit (%)				
Baseline	45.28 ± 0.49	45.99 ± 0.87	44.85 ± 1.02	45.00 ± 0.64
Wk 12	43.52 ± 0.60[a]	43.83 ± 1.16[a]	43.19 ± 1.09	43.50 ± 0.99
Wk 44		45.84 ± 0.90[b]	47.26 ± 0.88[a,b]	45.43 ± 1.43[b]
Recovery		46.35 ± 1.18	45.71 ± 1.17	45.03 ± 1.11
Red blood cells ($10^6 \times mm^3$)				
Baseline	5.16 ± 0.06	5.20 ± 0.08	5.18 ± 0.14	5.11 ± 0.08
Wk 12	4.98 ± 0.06[a]	5.05 ± 0.09	4.95 ± 0.11	4.93 ± 0.11
Wk 44		5.23 ± 0.11	5.32 ± 0.14[b]	5.19 ± 0.18[b]
Recovery		5.36 ± 0.08	5.19 ± 0.10	5.13 ± 0.05

[a] $P < 0.05$ *vs.* baseline.
[b] $P < 0.05$ *vs.* wk 12.

TABLE 3. Chemical parameters (mean ± SE) in the groups of men at baseline (mean of three samples), at the end of the suppression phase (wk 12), at the end of the treatment phase (wk 44), and at the end of the recovery phase (wk 18)

	All subjects	CPA-20	CPA-2	CPA-0
Body weight (kg)				
Baseline	79.27 ± 2.24	78.41 ± 4.96	79.58 ± 3.89	79.83 ± 3.10
Wk 12	78.38 ± 2.04	77.81 ± 4.14	78.56 ± 3.87	78.75 ± 2.98
Wk 44		78.94 ± 3.94	80.43 ± 4.89	80.14 ± 3.68[a]
Recovery		79.75 ± 4.21	80.07 ± 5.64	79.36 ± 3.99
Total cholesterol (mmol/liter)				
Baseline	4.91 ± 0.18	5.20 ± 0.41	4.58 ± 0.18	4.96 ± 0.29
Wk 12	4.56 ± 0.17[b]	4.85 ± 0.38[b]	4.38 ± 0.23	4.48 ± 0.28[b]
Wk 44		5.33 ± 0.39[a]	4.30 ± 0.19	4.80 ± 0.34
Recovery		5.88 ± 0.52	4.71 ± 0.25	4.89 ± 0.48
HDL cholesterol (mmol/liter)				
Baseline	1.26 ± 0.04	1.24 ± 0.07	1.22 ± 0.04	1.31 ± 0.07
Wk 12	1.10 ± 0.04	1.09 ± 0.08[b]	1.10 ± 0.05[b]	1.12 ± 0.06[b]
Wk 44		1.11 ± 0.10	1.17 ± 0.03	1.34 ± 0.11[a]
Recovery		1.29 ± 0.09	1.20 ± 0.07	1.28 ± 0.09
LDL cholesterol (mmol/liter)				
Baseline	3.21 ± 0.15	3.45 ± 0.34	2.95 ± 0.18	3.22 ± 0.28
Wk 12	3.04 ± 0.13	3.25 ± 0.25	2.83 ± 0.18	3.03 ± 0.25
Wk 44		3.69 ± 0.30	2.69 ± 0.18	3.04 ± 0.30
Recovery		3.88 ± 0.49	3.00 ± 0.22	3.11 ± 0.43
Triglycerides (mmol/liter)				
Baseline	0.98 ± 0.09	1.13 ± 0.22	0.88 ± 0.05	0.93 ± 0.13
Wk 12	0.93 ± 0.11	1.12 ± 0.24	0.93 ± 0.22	0.73 ± 0.07
Wk 44		1.13 ± 0.22	0.95 ± 0.17[a]	0.91 ± 0.07
Recovery		1.11 ± 0.28	1.11 ± 0.22	1.08 ± 0.18
ASAT (U/liter)				
Baseline	24.25 ± 1.51	22.58 ± 2.37	25.75 ± 3.63	25.33 ± 1.68
Wk 12	23.58 ± 2.05	21.13 ± 2.40	27.75 ± 5.32	21.88 ± 1.93
Wk 44		18.50 ± 1.71	24.00 ± 4.04	29.86 ± 5.85
Recovery		23.25 ± 1.10	28.57 ± 4.54	27.57 ± 3.52
ALAT (U/liter)				
Baseline	26.90 ± 2.11	26.04 ± 3.47	24.46 ± 3.99	30.21 ± 3.66
Wk 12	23.17 ± 1.79	29.13 ± 3.87	19.88 ± 1.51	20.50 ± 2.61[b]
Wk 44		24.25 ± 3.67	24.00 ± 2.77	32.29 ± 9.23[a]
Recovery		28.50 ± 2.06	25.71 ± 3.78	31.00 ± 4.32

LDL, Low-density lipoproteins; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase.
[a] $P < 0.05$ *vs.* wk 12.
[b] $P < 0.05$ *vs.* baseline.

TABLE 4. Total prostate volumes, testis volumes, and PSA serum levels in the groups of men throughout the study periods

	All subjects	CPA-20	CPA-2	CPA-0
Testis volume [ml; (right + left)/2]				
Baseline	20.82 ± 0.63	20.88 ± 0.77	20.86 ± 0.83	20.71 ± 1.70
Wk 12	13.73 ± 0.41[a]	14.00 ± 0.91	14.00 ± 0.72	13.14 ± 0.40
Wk 44		12.13 ± 0.81[a,b]	12.29 ± 0.36[a,b]	12.71 ± 0.47[a]
Recovery		19.13 ± 0.83[a]	18.00 ± 0.87[a]	18.88 ± 0.96[a]
1 yr later		21.30 ± 1.86	21.20 ± 0.97	21.00 ± 2.05
Prostate volume (ml)				
Baseline	20.17 ± 0.92	21.88 ± 1.73	20.01 ± 1.38	19.22 ± 1.76
Wk 44		22.54 ± 1.65	23.36 ± 2.44	22.42 ± 2.52[a]
Recovery		23.88 ± 2.18	22.70 ± 2.41	21.18 ± 2.20
1 yr later		21.88 ± 1.18	20.25 ± 1.90[c]	20.68 ± 2.34[c]
PSA (ng/ml)				
Baseline	0.69 ± 0.08	0.63 ± 0.08	0.83 ± 0.20	0.61 ± 0.09
Wk 12	0.55 ± 0.05[a]	0.56 ± 0.07	0.54 ± 0.10[a]	0.55 ± 0.08
Wk 44		0.75 ± 0.10[b]	0.84 ± 0.16[b]	0.76 ± 0.14[b]
Recovery		0.85 ± 0.14	0.81 ± 0.15	0.94 ± 0.35

Values are the mean ± SE.
[a] $P < 0.05$ *vs.* baseline.
[b] $P < 0.05$ *vs.* wk 12.
[c] $P < 0.05$ *vs.* wk 44.

maintain sperm suppression induced by the combined administration of higher dose CPA plus TU. CPA (20 mg/d) plus TU (1000 mg/6 wk) administered for 12 wk induced profound sperm suppression in all men. TU injected at a dose of 1000 mg every 8 wk with placebo or with 2 mg/d CPA was as effective as TU given in combination with 20 mg/d CPA

in the maintenance of sperm suppression for the following 32 wk. None of the regimens induced any major change in metabolic parameters.

Studies performed a few decades ago suggested that once spermatogenesis is profoundly decreased with a higher hormonal load, the suppression can be maintained by lower dose hormonal regimens (29–34). However, none of those studies proved the validity of the concept, probably because the regimens used to maintain the spermatogenic suppression were not adequate. This paradigm was repeated in monkeys in which sperm suppression was induced with the combined administration of GnRH antagonist plus T (22). All animals became azoospermic, and T alone maintained the suppression for 12 wk. In humans this regimen held conflicting results. One study reported that weekly injections of TE maintained sperm suppression induced with TE plus a GnRH antagonist for 20 wk (23). However, in another study 19-nortestosterone was not able to maintain gonadotropin, and thus sperm suppression, induced with the combination of the same androgen plus a GnRH antagonist (35). Differences in the nature and doses of the two androgens may be accounted for by the different abilities to maintain gonadotropin, and thus sperm suppression, with the two regimens.

In this study we used a prototype androgen progestin regimen consisting of daily oral intake of cyproterone acetate and injections of TU. This regimen was selected because in previous studies the combined administration of CPA plus T induced a rapid and profound decrease in sperm count in all tested men (5, 16, 17). In this study the combination of 20 mg CPA and injections of 1000 mg TU every 6 wk induced rapid and profound gonadotropin and sperm suppression in all subjects. At the end of 48 wk of administration, 82% of the subjects were azoospermic, and all of the other subjects had achieved severe oligozoospermia (<1 million/ml). These results confirm previous preliminary data suggesting the high effectiveness of this hormonal combination. A previous study showed that injections of 1000 mg TU every 6 wk induced azoospermia in only about 50% of the subjects (19). Therefore, the rapid and profound sperm suppression that we found in this study also confirms other preliminary studies suggesting that the addition of a progestin, in this case CPA, greatly enhances the speed and degree of spermatogenic suppression compared with T alone (20, 21). The degree of sperm suppression achieved with this androgen-progestin combination provided an optimal contraceptive protection in the WHO multicenter trial (36, 37). Therefore, these results confirm that androgen-progestin combinations represent a promising new contraceptive option for men.

On the other hand, these results also suggest that progestin is not absolutely needed to maintain sperm suppression once it has been induced. In fact, in this study every 8 wk injections of TU were able to maintain sperm suppression for 32 wk when injected with placebo as well as when given in combination with 2 or 20 mg CPA. However, although sperm counts remained suppressed below 1 million/ml, the occasional presence of sperm was detected in a few samples. It is not clear whether this sperm rebound may have resulted directly from an escape from gonadotropin suppression, because it also occurred in subjects that did not show gonadotropin escape at sampling points. Alternatively, higher T levels, intratesticularly occurring in the absence of the antiandrogenic effect of CPA or resulting from escape from gonadotropin suppression, may have allowed for completion of spermiation in a few subjects (38). Also, the relevance of this escape from sperm suppression from the point of view of the contraceptive efficacy remains to be clarified. In a previous study TE maintained sperm suppression induced by TE plus GnRH antagonist for 20 wk (23). In this study we extended the maintenance period to 32 wk and demonstrated for the first time that injections of the newly developed androgen formulation TU every 8 wk can maintain sperm suppression. Whether TU injected alone without the progestin would maintain sperm suppression for even longer times is not known.

T levels did not differ among the three groups at any time point throughout the study period. Serum T levels remained within the physiological range even if a trend toward an increase in T levels during the maintenance phase was observed. This increase can result from an accumulation of TU in the tissue and may confirm data in hypogonadal men showing that even longer injection intervals could maintain T levels within the normal range (18).

The results of this study showed that CPA had a mild stimulatory effect on PRL levels. This effect was clearly related to progestin, as shown by the fact that it was promptly reversed when CPA intake was suspended in the CPA-0 group. The PRL increase was completely reversible at the end of the study and was not accompanied by any other clinical sign. Previous studies have also reported a stimulatory effect of CPA on PRL in men (39). In our study the increase in PRL was modest and was not accompanied by any sign of gynecomastia or galactorrhea. The clinical relevance of this increase remains uncertain.

In this study an androgen-progestin contraceptive regimen was administered to healthy Caucasian men for almost 1 yr. In previous studies a high dose T regimen was given to healthy men for over 1 yr (36, 37). Changes, such as decreases in HDL cholesterol or increases in body weight, were registered in that study (25, 40, 41). The addition of a nor-derived progestin to lower dose TE also induced a dose-dependent decrease in HDL cholesterol and an increase in body weight (7). Although a slight, but significant, decrease in HDL cholesterol and total cholesterol was detected at wk 12, this decrease was not present in any group at the end of the study. Neither a change in body weight nor any other changes in metabolic parameters were detected at any time in these groups. These results suggest that the addition of a nonandrogenic progestin to T may be advantageous for avoiding metabolic adverse effects.

In this study a slight, but significant, increase in total prostate volume was detected only in the group that did not receive CPA, whereas it was negligible in the other two groups. PSA levels did not show any significant increase in any group. This increase was completely reversed 1 yr after stopping hormone administration. An increase in prostate volumes was also reported after 1 yr of TE injections (42), but was never detected with other androgen-progestin combinations (20). These results again underline the importance of the addition of a progestin to an androgen to minimize long-term risks of a hormonal contraceptive regimen. The effects of male hormonal contraceptives on the prostate are clearly very important and will certainly need further attention in the future.

In conclusion, our study confirms and further extends

previous preliminary data suggesting that a lower hormonal dose is needed to maintain than to induce sperm suppression. Although tested in a small number of subjects, the high effectiveness in terms of sperm suppression and maintenance and the lack of major adverse effects over 1-yr administration suggest that combined androgen-progestin regimens may represent promising contraceptives for men.

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