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Twenty-One Day Administration of Dienogest Reversibly Suppresses Gonadotropins and Testosterone in Normal Men

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Androgen-progestin combinations are promising male contraceptive regimens. Optimization of these regimens includes the development of new progestins with more favorable biological properties. In this context we tested the effects of the progestin dienogest (DNG) on reproductive hormones and metabolic parameters in men.

After a 3-wk control period, 25 men were randomly assigned to receive daily doses of 2, 5, or 10 mg DNG or placebo and 10 mg cyproterone acetate for 21 d (n = 5 subjects/group). Subjects were followed for 2 wk after cessation of hormone administration. Weekly blood samples, physical examinations, hormone and chemistry measurements, semen analysis, and sexual/behavioral assessments were performed. These parameters were compared among study groups and within each group at different time points throughout the study periods.

DNG and cyproterone acetate administration resulted in profound suppression of gonadotropins and T with no change in SHBG levels. No adverse effects were detected in any subject. Hormone levels returned to baseline after stopping hormone intake.

DNG is a potent suppressor of gonadotropins and T in men and does not induce adverse effects over a 21-d administration. DNG is a promising progestin to be used in clinical trials for male contraception. (*J Clin Endocrinol Metab* 87: 2107–2113, 2002)

THE ULTIMATE GOAL of hormonal contraceptive regimens is the induction of sperm suppression sufficient to provide effective contraception (1–3). To that end, recent studies have shown that androgen-progestin combinations are promising regimens to be used for male contraception (4). Progestins tested over the last decade in male contraceptive trials include levonorgestrel (LNG), medroxyprogesterone acetate, cyproterone acetate (CPA), desogestrel (DSG), and norethisterone enanthate (NETE). When LNG, CPA, DSG, or NETE was given in combination with T enanthate (100 mg/wk), T undecanoate, or T pellets, profound sperm suppression was achieved in all men, with azoospermia induced in about 70–80% of the men (5–11). However, LNG, NETE, and DSG caused side effects, such as decreased high density lipoprotein cholesterol, weight gain, and increased hematological parameters. These effects are probably due to the residual androgenic activity of these progestins, which adds to that of the androgen. On the other hand, CPA induced changes in hematological parameters and a slight decrease in body weight (12). These effects seemed to be related to the dose of CPA and were probably due to its strong antiandrogenic activity (13). Both androgenic and antiandrogenic effects were less pronounced or disappeared when lower doses were used, but the level of sperm suppression was also significantly decreased. These studies demonstrated that achieving the required sperm suppression necessitates administering progestins at high doses. At such high dosages, some biological properties of these compounds can induce adverse effects that potentially reduce the safety and acceptability of these regimens. Therefore, the development of new progestins more potent in terms of sperm suppression and with favorable biological properties (*i.e.* less androgenic and less antiandrogenic) could be an important factor in the development of optimal contraceptive regimens in terms of both effectiveness and safety.

A new segment between the pharmacodynamic profiles of modern 19-norprogestins and the more conventional derivatives of natural progesterone has been created by norprogestins with 17-substituents other than the common 17-ethinyl group pioneered by dienogest (17-cyanomethyl-17β-hydroxy-4,9′-estradien-3-one;codeSTS557; DNG) and followed by drospirenone. DNG combines the benefits of the norprogestins (strong progestational activity on the endometrium, receptor selectivity, short half-life, high oral bioavailability, low liver impact, and normal toxicological or genotoxicological patterns) with typical properties of the progesterone derivatives, such as excellent tolerability, moderate antiandrogenic action, and definite antiproliferative activities. Other characteristics of DNG include no binding to serum transport proteins with high levels of unbound, free drug in the blood serum, no accumulation after daily intake, and easy microbial degradation in the environment. Fur-

Abbreviations: CPA, Cyproterone acetate; DNG, dienogest; DSG, desogestrel; LNG, levonorgestrel; NETE, norethisterone enanthate; PSA, prostate-specific antigen.

thermore, DNG showed male antifertility action in mice, rats, rabbits, and monkeys (14). The antiandrogenic activity of DNG is lower than that of CPA. Therefore, if used in combination with T to suppress spermatogenesis, it could offer an advantage over CPA of not causing side effects due to strong androgen deprivation. However, there are no clinical reports on the effects of DNG on male reproductive function.

In view of the possible use of this progestin in male contraceptive regimens, the purpose of this study was to evaluate the ability of DNG to suppress gonadotropins and T and to assess the potential systemic adverse effects compared with CPA, the effectiveness of which in contraceptive combined regimens is already known.

Subjects and Methods

Study design and volunteers

The study was performed in a single-blind, randomized, placebo-controlled fashion. The human subject committee of University of Bologna approved the protocol. Written informed consent was obtained from all volunteers before the study. Enrollment criteria included an uneventful medical history; normal physical examination; normal routine blood chemistry; basal serum LH, FSH, and T levels within normal range; and normal seminal analysis according to the criteria established by the WHO (15). Twenty-five men, aged 21–45 yr, were enrolled. Demographic, clinical characteristics, gonadotropin levels, and sperm counts at baseline are reported in Table 1.

The study lasted 8 wk. This included a 3-wk control phase (d −14, −7, and 0), a 3-wk treatment phase (d 7, 14, and 21), and a 2-wk recovery phase (d 28 and 35). After the control phase, subjects were randomized into five groups to receive 10 mg/d DNG (DNG-10), 5 mg/d DNG (DNG-5), 2 mg/d DNG (DNG-2), 10 mg/d CPA (CPA-10), or placebo (DNG-0), orally, for 21 d (3 wk). During the control phase (d −14, −7, and 0), the treatment phase (d 7, 14, and 21) and the recovery phase (d 28 and 25) subjects came to the clinic for a visit that included physical examinations, weight and blood pressure measurements, drawing of fasting blood samples (for hormone, chemistry, and hematology measurements), sperm analyses, and completion of a sexual and behavioral questionnaire each week during the control phase.

Measurements

We compared sperm concentrations and gonadotropin, T, SHBG, estrogen, and prostate-specific antigen (PSA) levels, and reported sexual and behavioral patterns among all groups throughout the study.

Serum LH and FSH levels were analyzed using the Delfia fluoroimmunoassay (Wallac, Inc., Turku, Finland), (7). The minimum sensitivity was 0.0188 IU/liter for the LH assay and 0.0156 IU/liter for the FSH assay. The interassay coefficients of variation in the high, medium, and low parts of the standard curve were 9.5%, 12.5%, and 11.2% for the LH assay and 6.2%, 6.1%, and 17.9% for the FSH assay. The intraassay coefficients of variation in the high, medium, and low parts of the curve for LH and FSH assays were 2.6%, 3.2%, and 7.6% and 2.7%, 2.9%, and 6.8%, respectively. Serum T levels were measured by Delfia fluoroimmunoassay (Wallac, Inc.). The minimum sensitivity of the assay was 0.5 nmol/liter. The interassay coefficients of variation in the high, medium, and low parts of the standard curve were 16.0%, 11.5%, and 13.4% for the T assay. The intraassay coefficients of variation in the high, medium, and low parts of the curve were 5.3%, 8.7%, and 9.3%. Serum estrogen levels were measured by RIAs (ICN Biomedicals, Inc., Costa Mesa, CA). The minimum sensitivity for this assay was 110 pmol/liter. The interassay coefficients of variation in the high, medium, and low parts of the standard curve were 12.5%, 14.6%, and 16.9%. The intraassay coefficients of variation in the high, medium, and low parts of the curve were 6.4%, 6.7%, and 9.1%. SHBG levels were measured by fluoroimmunoassay (AUTODELFIA, Wallac, Inc.). The minimum sensitivity of this assay was 6.25 nmol/liter. The interassay coefficients of variation in the high and low parts of the standard curve were 5.0% and 9.5%. The intraassay coefficients of variation in the high and low parts of the curve were 3.2% and 4.8%. PSA levels were measured by immunofluorescent assays (KRYPTOR; CIS-Bio International, Oris Group, Gif-sur-Yvette, France). The minimum sensitivity of this assay was 0.04 ng/ml. The interassay coefficients of variation in the high and low parts of the standard curve were 2.1% and 2.1%. The intraassay coefficients of variation in the high and low parts of the curve were 1.5% and 0.6%.

DNG levels in serum and seminal plasma were also measured in all samples from DNG- and placebo-treated subjects. DNG RIAs were performed at Jenapharm (16) according to previously validated procedures using a rabbit antidienogest 3-carboxymethyloxime-BSA antiserum, AS14 (17), and [14α,15α-^{3}H]dienogest as tracer. DNG levels in serum and seminal plasma were determined after extraction with diethyl ether and reconstitution of the extracts in buffer. The values were corrected for the extraction yields (88.6% for serum and 88.1% for seminal plasma). Suitable parts of the reconstituted extracts were taken for RIA to obtain measurement ranges of 1–56.5 ng DNG/ml serum and 0.25–14.25 ng/ml seminal plasma. For serum, intra- and interassay coefficients of variation were between 2.0–9.4% (at the limit of quantitation), and between 2.8–12.2%, respectively. For seminal plasma, the coefficients of variation were between 2.8–8.9% (intraassay) and between 1.1–15.8% (interassay). Clinical chemistry and hematology were performed by routine assays according to previously described procedures (12).

Semen analysis was performed according to the WHO manual (15). Volunteers were asked to maintain abstinence from last ejaculation (spontaneous or sexual intercourse) of 2–5 d. Days of abstinence were recorded at each semen analysis. Semen volume and sperm concentration, motility, and morphology were recorded. Sexual and behavioral parameters were monitored using a previously used questionnaire (18).

The same operator performed all testicular measurements by the Prader orchidometer.

Prostatic ultrasound examinations were performed using a 7.5-MHz transrectal transducer (2 cm in diameter) and an ESAOTE ultrasound machine (ESAOTE S.p.A., Genova, Italy). After applying ultrasound transmisssion gel, a transducer was covered with a sterile lubricated disposable rubber sheath. The three maximal diameters of the total and central areas of the prostate were recorded. Volumes of the central and total areas were calculated using the standard ellipsoidal formula. The central zone of the prostate was defined according to criteria established by Bosch *et al.* (19). The same urologist performed all prostatic ultrasound examinations.

Statistical analysis

Data are reported as the mean ± SEM. The normal distribution of the data was tested by means of the Kolmogorov-Smirnov test (20). One-way and repeated measurement ANOVA were applied (21). Statistical eval-

TABLE 1. Demographic data, baseline hormone levels, and sperm concentration (mean + SEM) in the 25 men, according to their treatment group

	DNG-0	DNG-2	DNG-5	DNG-10	CPA-10
Age (yr)	23.0 ± 0.3	24.0 ± 1.1	28.2 ± 1.9	24.6 ± 1.4	22.4 ± 0.7
Body weight (kg)	75.2 ± 3.3	77.2 ± 3.1	71.7 ± 8.5	74.3 ± 4.8	71.9 ± 1.5
Body mass index (kg/m^2)	23.9 ± 1.0	24.1 ± 0.6	22.1 ± 1.1	23.5 ± 1.4	22.1 ± 0.8
LH (IU/liter)	3.38 ± 0.76	4.76 ± 0.54	4.02 ± 0.90	3.46 ± 0.84	3.02 ± 0.60
FSH (IU/liter)	2.85 ± 0.97	2.50 ± 0.34	2.58 ± 0.51	2.22 ± 0.37	1.80 ± 0.23
T (nmol/ml)	26.9 ± 6.2	34.9 ± 7.6	26.7 ± 6.2	23.9 ± 6.2	20.8 ± 5.3
Sperm concentration (million/ml)	48.6 ± 7.2	46.6 ± 5.7	56.0 ± 2.4	52.0 ± 2.5	52.0 ± 12.7

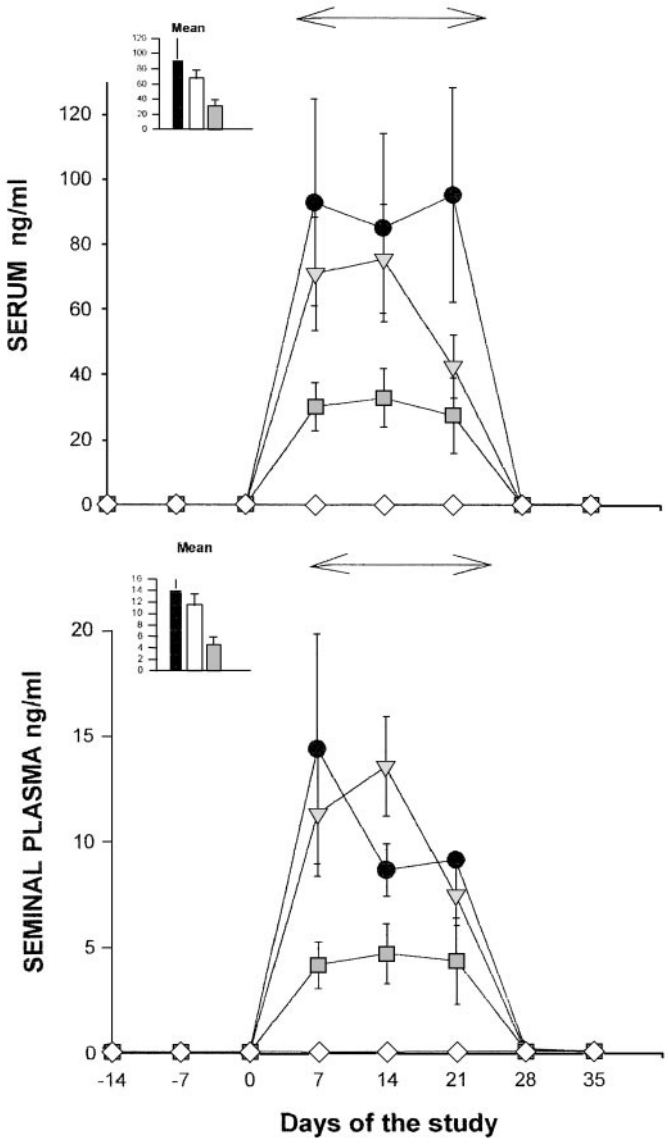

FIG. 1. DNG levels in serum (*upper panel*) and seminal plasma (*lower panel*) throughout the study. The *inset* shows mean DNG levels during the 21 d of treatment in the three DNG-treated groups (●, DNG-10; ▽, DNG-5; □, DNG-2) and in the placebo group (♦, DNG-0).

uations were performed by running the SPSS/PC$^+$ package (SPSS, Inc., Chicago, IL) on a personal computer (22). Two-tailed *P* value less than 0.05 was considered statistically significant.

Results

Hormones

DNG levels in serum and seminal plasma were undetectable at baseline, increased in all subjects who received DNG in a dose-dependent manner, and remained elevated until the end of hormone administration (d 21), then returned to undetectable levels. DNG levels in seminal plasma were detectable in only one subject 7 d after the end of DNG intake (d 28 of the study) and became undetectable in the last seminal sample of the study (d 35). DNG serum and seminal plasma levels remained undetectable in the placebo group throughout the entire study (Fig. 1). The DNG concentrations of seminal plasma correlated well to those of serum. DNG levels in seminal plasma were about 16% of those in serum ($r^2 = 0.96$; Fig. 2).

No significant difference was found in mean baseline FSH, LH, T, and SHBG levels among groups or within each group among the three baseline samples (Fig. 3). No significant changes in FSH, LH, and T were detected in the placebo group (DNG-0) at any time throughout the study period.

During hormone administration, FSH levels significantly decreased compared with baseline values and levels in the DNG-0 group in all treatment groups. The FSH decrease was dependent on the dose of DNG ($P = 0.003$). The FSH decrease was already significant at wk 7 of DNG or CPA administration and remained low until the end of hormone administration in all subjects. In the DNG groups, the decrease in FSH was significantly greater in group DNG-10 than in group DNG-2 and in group DNG-5 than in group DNG-2, but not in group DNG-10 compared with group DNG-5. No significant difference in the FSH decrease was detected between the CPA and DNG groups. On d 21 of hormone administration, the mean (±SEM) percent FSH decrease was 73 ± 3 in the DNG-10 group, 57 ± 10 in the DNG-5 group, 30 ± 6 in the DNG-2 group, and 59 ± 5 in the CPA-10 group. After stopping DNG or CPA administration, FSH levels returned to baseline levels in all groups except group CPA-10 on d 28 (lower than basal) and DNG-5 on d 35 (higher than basal).

During hormone administration, LH levels significantly decreased compared with baseline values and levels in the DNG-0 group in all DNG treatment groups. In the CPA group, LH levels decreased, but the decrease achieved sig-

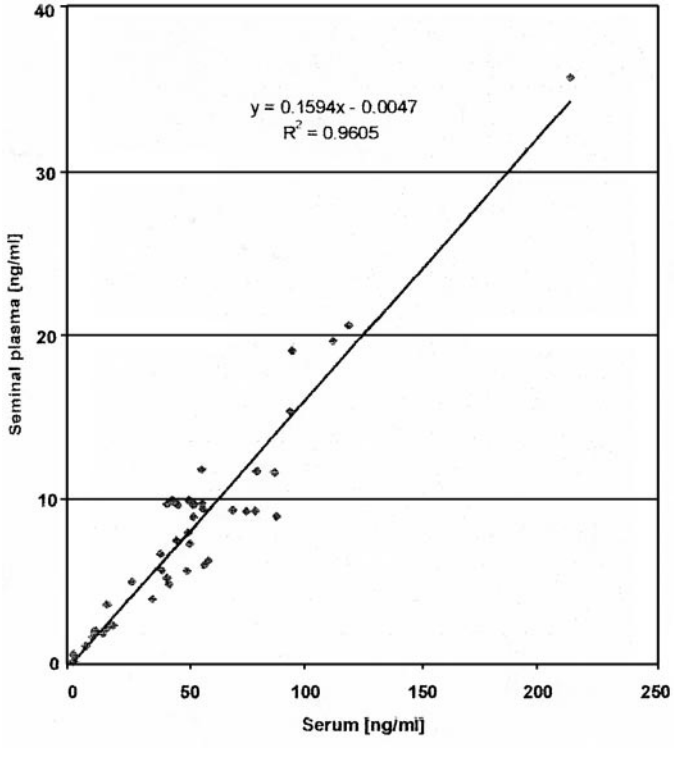

FIG. 2. Correlation of DNG levels in seminal plasma and serum (subjects 1–20).

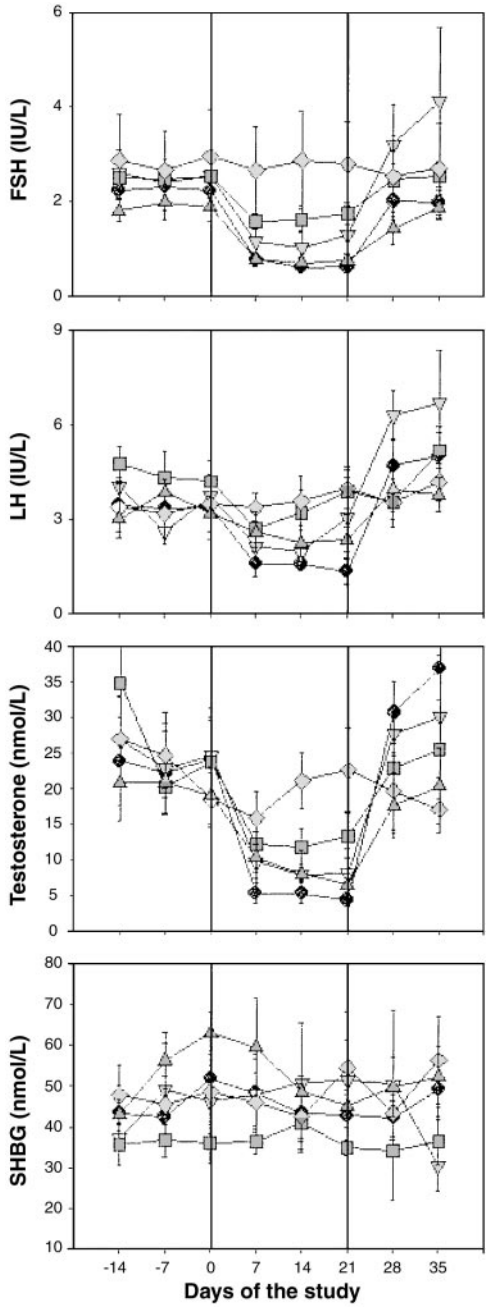

FIG. 3. Serum FSH, LH (*upper panel*), T, and SHBG (*lower panel*) levels (mean ± SEM) in the five groups of men throughout the study.

nificance only on d 14 and 21 of hormone administration. On d 21 of DNG administration, the LH decrease was significantly greater in group DNG-10 compared with groups DNG-5 and DNG-2. No significant difference in the LH decrease was detected between the CPA-10 and DNG-10 groups. On d 21 of hormone administration, the mean (±SEM) percent LH decreases were 59 ± 6%, 16 ± 36%, 4 ± 15%, and 21 ± 15% in the DNG-10, DNG-5, DNG-2, and CPA-10 groups, respectively. After stopping hormone administration, LH levels increased and were significantly higher than baseline in group DNG-5 on d 28 and 35 and in group DNG-10 on d 28.

During hormone administration, T levels significantly decreased compared with baseline values and levels in the DNG-0 group in all DNG and CPA treatment groups. The decrease in T was already significant on d 7 of hormone administration and remained significantly decreased until d 21 in all groups. On d 21 of the study the mean (±SEM) percent T decreases were 81 ± 4%, 70 ± 7%, 43 ± 5%, and 66 ± 4% in the DNG-10, DNG-5, DNG-2, and CPA-10 groups, respectively. In all groups, T levels increased after stopping hormone administration and were significantly higher than baseline at the end of the recovery phase (d 35) in group DNG-10.

No significant change in SHBG levels was detected in any group at any time (Fig. 3).

Spermatogenesis

Sperm concentration, motility, and morphology did not differ among the five groups at baseline and within each group among the three baseline samples. During the treatment phase, the sperm concentration did not show significant difference among any groups at any time (Fig. 4). On d 35 the sperm concentration was significantly decreased compared with baseline in groups DNG-10, DNG-5, and DNG-2. Sperm motility did not show any significant change at any time or in any group throughout the study period. Normal morphology was also significantly decreased in group DNG-10 on d 21, 28, and 35 of the study and in the CPA-10 on d 35.

Clinical

Mean (±SEM) demographic parameters and basal reproductive hormones are shown in Table 1. No significant differences in these parameters were found among the five groups at baseline and within each group among the three baseline samples. No significant changes in clinical parameters (blood pressure, body weight, or testis volume) were found in any group at any time throughout the study. Total volume and volume of the central zone of the prostate did not show any significant change at the end of hormone administration compared with baseline (Table 2). No adverse effects or changes in biochemical parameters were observed, and DNG and CPA were well tolerated by all subjects (Table 3). Volunteers did not report major mood or behavioral changes in any phase of the study. However, one volunteer from group DNG-5 withdrew from the study because of mood changes and decreased libido. After the intake of 10 mg DNG, T decreased to less than 5 nmol/liter, and there was a significant decrease in morning erections, spontaneous erections, and frequency of intercourse and masturbation ($P < 0.05$). These parameters were not significantly decreased in any other group.

Discussion

The findings of the present study demonstrate that the administration of DNG to normal men results in a profound suppression of gonadotropins and T. A rapid return to baseline levels occurred after stopping hormone intake in all subjects, and no side effects were reported with any dose at

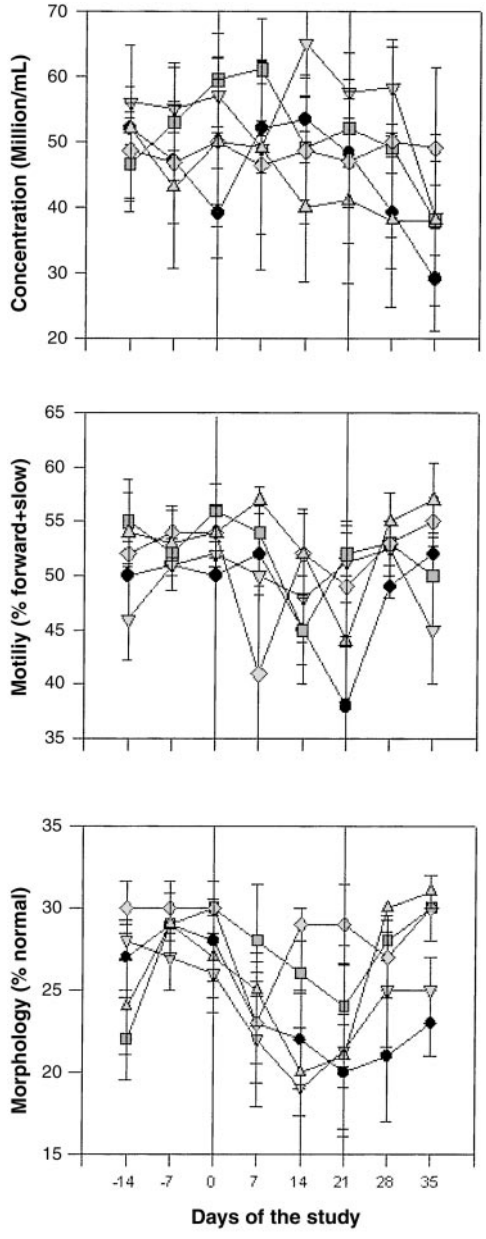

FIG. 4. Seminal parameters (concentration, *upper panel*; motility, *middle panel*; morphology, *lower panel*) in the five groups of men throughout the study (mean ± SEM).

any time. These results suggest that DNG is a promising progestin to be tested in androgen-progestin combined regimens for safe and effective male contraception.

DNG is called a hybrid progestin because of its unique ability to combine properties of 19-norderived progestins with those of progesterone-derived progestins (23). Similar to other 19-norderived progestins, DNG has a high oral bioavailability (16), but unlike the other progestins of this class, it retains a very low androgenic activity. DNG has an antiandrogenic activity approximately one third that of CPA. These characteristics, the results of preclinical studies, and its long-standing use in women have suggested that DNG might have favorable properties for a compound to be used in androgen-progestin male contraceptive regimens (24, 25).

TABLE 2. Mean (+SEM) testis volume, prostate ultrasound measurements, and PSA serum levels in the five groups of men at baseline (first control sample), d 21 of hormone intake (treatment), and 14 d after stopping hormone intake (recovery)

	Testis volume (cc)		Prostate volume (cc)		PSA (ng/ml)
	Right	Left	Central prostate	Total prostate	
DNG-0					
Control	19 ± 1	18 ± 1	4.9 ± 1.0	21.5 ± 2.5	0.6 ± 0.1
Treatment	19 ± 1	19 ± 1	5.1 ± 0.8	18.5 ± 1.7	0.6 ± 0.1
Recovery	19 ± 1	19 ± 1	6.4 ± 0.7	18.0 ± 2.0	0.7 ± 0.1
DNG-2					
Control	19 ± 0	19 ± 0	4.4 ± 0.3	20.8 ± 2.1	0.8 ± 0.2
Treatment	20 ± 0	20 ± 0	4.5 ± 0.5	20.0 ± 1.6	0.6 ± 0.2
Recovery	19 ± 0	19 ± 0	6.4 ± 0.8	21.3 ± 2.8	0.9 ± 0.2
DNG-5					
Control	20 ± 1	19 ± 1	3.7 ± 0.6	16.0 ± 3.0	0.6 ± 0.1
Treatment	18 ± 1	18 ± 0	3.6 ± 0.4	16.2 ± 2.6	0.3 ± 0.1
Recovery	19 ± 1	19 ± 1	4.3 ± 1.0	15.6 ± 1.8	0.3 ± 0.1
DNG-10					
Control	20 ± 1	20 ± 1	3.2 ± 0.9	19.6 ± 1.4	1.1 ± 0.2
Treatment	20 ± 2	20 ± 2	3.8 ± 0.8	15.7 ± 2.1	1.4 ± 0.7
Recovery	19 ± 2	19 ± 2	5.6 ± 0.5	19.1 ± 1.8	1.4 ± 0.4
CPA-10					
Control	18 ± 0	18 ± 1	5.4 ± 1.1	20.0 ± 2.0	0.6 ± 0.0
Treatment	20 ± 1	19 ± 1	3.9 ± 0.5	18.7 ± 1.8	0.3 ± 0.0
Recovery	20 ± 1	19 ± 1	6.0 ± 0.7	23.3 ± 1.9	0.6 ± 0.1

In a previous pharmacokinetic study carried out in a randomized, open cross-over design in 22 male volunteers receiving oral or iv single administration of 2 mg DNG, pharmacokinetic parameters were calculated, and the absolute bioavailability was determined (26). This study showed that after oral administration of 2 mg DNG, the time of maximum concentration was 1.6 h, and terminal elimination half-life was 8.5 h, but DNG was detectable in the circulation for almost 24 h. However, blood sampling lasted only 48 h, and no hormone measurements were performed. Therefore, in this study we evaluated the effectiveness of DNG in suppressing gonadotropins and T and its safety in normal men over a 21-d administration.

The results of the present trial show that DNG can induce a profound suppression of FSH, LH, and T with no effect on SHBG levels in men. The decrease in both gonadotropins tended to depend on the dose of DNG. Suppression of FSH, LH, and T was profound with the highest dose of DNG and was similar to or even more profound than that achieved with CPA. This rapid and profound suppression of gonadotropins and T suggests that in a combined regimen with an androgen DNG can induce a rapid and profound suppression of sperm production. As shown in previous studies in women, 21-d administration of DNG results in stable serum levels, indicating that no accumulation of DNG occurs (24). This aspect of DNG will be advantageous in a male contraceptive setting for a rapid return to fertility after stopping intake.

The concentrations of DNG in seminal plasma were much lower than those in serum (~16% of the serum levels). This fact may explain why only high doses have direct influence on sperm (see below). The levels of most endogenous steroids were much lower in seminal plasma compared with serum levels (27) even if the sulfoconjugates were included (28), which are the main forms in seminal plasma (29). On the other hand, conjugates of DNG itself are at least of secondary

TABLE 3. Mean (+SEM) body weight and chemistry tests in the five groups of men at baseline (first control sample), d 21 of hormone intake (treatment), and 14 d after stopping hormone intake (recovery)

	Body weight (kg)	Total cholesterol (nmol/ml)	HDL cholesterol (nmol/ml)	LDL cholesterol (nmol/ml)	Tryglicerides (nmol/ml)	GOT (U/liter)	GPT (U/liter)
DNG-0							
Control	75.2 ± 3.3	5.11 ± 0.31	1.40 ± 0.08	3.55 ± 0.31	0.80 ± 0.07	23 ± 2	24 ± 3
Treatment	75.1 ± 3.2	4.90 ± 0.32	1.46 ± 0.12	3.28 ± 0.39	0.78 ± 0.12	23 ± 4	19 ± 4
Recovery	75.3 ± 3.0	5.15 ± 0.34	1.49 ± 0.15	3.46 ± 0.38	1.01 ± 0.13	21 ± 2	15 ± 2
DNG-2							
Control	77.2 ± 3.1	5.17 ± 0.41	1.37 ± 0.18	3.61 ± 0.42	0.98 ± 0.20	21 ± 1	19 ± 2
Treatment	76.0 ± 3.2	4.73 ± 0.33	1.39 ± 0.19	3.19 ± 0.25	0.75 ± 0.09	21 ± 2	14 ± 1
Recovery	77.2 ± 3.6	4.55 ± 0.35	1.45 ± 0.13	2.94 ± 0.32	0.81 ± 0.12	18 ± 2	14 ± 1
DNG-5							
Control	71.7 ± 8.5	4.83 ± 0.32	1.40 ± 0.18	3.19 ± 0.27	1.18 ± 0.18	23 ± 4	19 ± 3
Treatment	71.8 ± 8.2	4.65 ± 0.21	1.41 ± 0.21	3.08 ± 0.27	0.81 ± 0.08	20 ± 3	18 ± 2
Recovery	71.8 ± 7.6	4.90 ± 0.41	1.55 ± 0.18	3.14 ± 0.35	1.06 ± 0.15	20 ± 3	16 ± 2
DNG-10							
Control	74.3 ± 4.8	4.11 ± 0.30	1.32 ± 0.12	2.63 ± 0.21	0.79 ± 0.16	31 ± 4	38 ± 7
Treatment	74.5 ± 4.6	3.80 ± 0.29	1.40 ± 0.12	2.29 ± 0.19	0.55 ± 0.03	23 ± 2	18 ± 2
Recovery	75.3 ± 4.3	4.23 ± 0.33	1.58 ± 0.14	2.51 ± 0.21	0.70 ± 0.07	22 ± 3	21 ± 3
CPA-10							
Control	71.9 ± 1.5	4.73 ± 0.53	1.76 ± 0.22	2.82 ± 0.53	0.77 ± 0.15	23 ± 4	19 ± 4
Treatment	69.7 ± 1.4	4.21 ± 0.29	1.56 ± 0.11	2.52 ± 0.29	0.65 ± 0.13	22 ± 2	21 ± 4
Recovery	69.6 ± 1.0	4.11 ± 0.22	1.41 ± 0.11	2.59 ± 0.25	0.54 ± 0.09	20 ± 3	20 ± 6

GOT, Glutamic-ossalacetic transaminase; GPT, glutamic-pyruvic transaminase.

TABLE 4. Mean (+SEM) sexual function and behavior parameters in the five groups of men at baseline (first control sample), d 21 of hormone intake (treatment), and 14 d after stopping hormone intake (recovery)

	Morning erections[a]	Sexual activity[b] (intercourse or masturbation)	Frequency of spontaneous erections[b]	Mood[c]
DNG-0				
Control	1.67 ± 0.16	2.30 ± 0.52	7.87 ± 0.09	2.53 ± 0.34
Treatment	2.00 ± 0.00	2.40 ± 0.92	7.80 ± 0.20	2.00 ± 0.55
Recovery	1.80 ± 0.20	2.00 ± 0.79	7.80 ± 0.20	2.20 ± 0.58
DNG-2				
Control	1.27 ± 0.21	0.93 ± 0.20	5.87 ± 0.63	2.73 ± 0.28
Treatment	1.20 ± 0.49	1.60 ± 0.65	6.40 ± 0.87	2.60 ± 0.24
Recovery	1.20 ± 0.37	0.70 ± 0.33	5.40 ± 1.21	2.00 ± 0.32
DNG-5				
Control	0.77 ± 0.20	2.68 ± 0.43	7.00 ± 0.47	2.21 ± 0.24
Treatment	0.50 ± 0.29	1.88 ± 0.30	5.50 ± 1.44	2.00 ± 0.58
Recovery	1.00 ± 0.41	2.25 ± 0.31	7.50 ± 0.29	1.75 ± 0.48
DNG-10				
Control	1.27 ± 0.21	2.33 ± 0.39	6.93 ± 0.43	2.27 ± 0.27
Treatment	0.20 ± 0.20[d]	1.70 ± 0.63[d]	5.00 ± 1.64[d]	2.40 ± 0.40
Recovery	1.60 ± 0.24	2.50 ± 0.89	5.60 ± 1.29	1.80 ± 0.37
CPA-10				
Control	0.73 ± 0.18	1.13 ± 0.21	6.13 ± 0.52	3.27 ± 0.27
Treatment	0.80 ± 0.37	1.80 ± 0.76	4.20 ± 1.39[d]	2.40 ± 0.51
Recovery	1.40 ± 0.40	1.50 ± 0.34	5.20 ± 1.36	1.60 ± 0.40

[a] Scores ranged from 0 (no morning erection) to 1 (partial erection) and 2 (complete erection).
[b] Scores ranged from 0 (not at all) to 8 (more than once a day).
[c] Scores ranged from 1 (not at all) to 6 (feel angry daily or more than once a day).
[d] $P < 0.05$.

importance in the metabolization of DNG (24). When DNG was measured in another body fluid, saliva, its concentrations were 8% of the serum levels (16).

In previous studies we have shown that CPA plus T induces a profound and consistent suppression of spermatogenesis (12, 13). In these studies the effects of CPA were dose dependent, and azoospermia was achieved only with high doses of CPA (at least 50–100 mg/d). Gonadotropin suppression was similar to that found with other progestins despite the more profound sperm suppression (4). Therefore, it was hypothesized that at these high dosages CPA might act not only by suppressing gonadotropins, but also by blocking the intratesticular androgen stimulatory effects on sperm development. However, at these doses CPA also induced a 8–10% decrease in hematological parameters and a slight decrease in body weight. Although it is not known whether these decreases could be progressive, they can certainly affect the safety of long-term use of this regimen and thus blunt its acceptability. Therefore, the lower antiandrogenic potency together with its greater ability to suppress LH/T

could make DNG a more effective and safer alternative to CPA for combined androgen-progestin regimens.

No changes in sperm concentrations were found in any group at any time in the study. However, because of the short duration of the hormone administration, this study was not aimed at evaluating the effects of this progestin on seminal parameters. Previous studies performed *in vitro* and in male mice and rabbits have suggested that DNG given at high doses impairs sperm motility and normal morphology due to a direct testicular or a posttesticular action (30–35). When DNG was administered to monkeys at lower doses, these effects were not confirmed (36, 37). In the present study a significant decrease in sperm normal morphology was detected only after administration of 10 mg DNG and CPA. These changes suggest a direct effect of DNG and CPA on the epididymis and may result in a decrease in the fertilizing ability of the spermatozoa. These possible direct testicular effects of DNG should be further explored in men.

In conclusion, recent studies in which prototype androgen-progestin regimens have been tested indicate that these regimens are promising candidates for male contraception. The development of new progestins with more favorable biological properties could improve the safety, effectiveness, and acceptability of the current regimens. Our study demonstrates that DNG is a potent suppressor of gonadotropins and does not induce adverse effects on health over a 21-d administration. Thus, DNG may be an optimal candidate to be tested in male contraceptive clinical trials.

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