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Oral Progestogen Combined with Testosterone as a Potential Male Contraceptive: Additive Effects between Desogestrel and Testosterone Enanthate in Suppression of Spermatogenesis, Pituitary-Testicular Axis, and Lipid Metabolism*

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ABSTRACT

The effects of a synthetic oral progestogen, desogestrel (DSG), administered with low dose testosterone (T) were investigated to determine the optimal combination for suppression of gonadotropins and spermatogenesis to targets compatible with effective male contraception. Twenty-four healthy male volunteers (33.2 ± 0.9 yr) were randomly assigned to 3 groups (n = 8) to receive: 1) 300 μg DSG orally daily and 100 mg T enanthate, im, weekly; 2) 300 μg DSG and 50 mg T enanthate; or 3) 150 μg DSG and 100 mg T enanthate for 24 weeks. To investigate the individual contribution to the combined action, DSG was administered alone for the first 3 weeks, and T enanthate was added on day 22. After 24-week treatment, sperm density in 78% (18 of 23) of the subjects became azoospermic, whereas 91.7% (22 of 24) and 95.8% (23 of 24) suppressed to less than 1 million/mL and less than 3 million/mL, respectively. The 300 μg DSG with 50 mg T enanthate combination induced azoospermia in 8 of 8 subjects, and the suppression of sperm density was significantly greater than that in the 300 μg DSG/100 mg T enanthate group, but was not different from that in the 150 μg DSG/100 mg T enanthate group. DSG (300 or 150 μg daily) alone in the first 3 weeks suppressed LH, FSH, and T to 60.6%, 48.0%, and 35.4%, respectively, of the baseline. Addition of T enanthate (50 and 100 mg weekly) raised plasma T to the physiological range and induced a further fall in LH and FSH to the limits of assay detection. There was no consistent difference in mean LH and FSH levels among the three groups during treatment or recovery, except that FSH remained detectable in a higher proportion of samples from the group receiving 300 μg DSG with 50 mg T enanthate. Total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol decreased by 9.3 ± 1.7%, 10.3 ± 2.6%, and 7.7 ± 2.8%, respectively, during treatment with DSG alone with no difference between 300 and 150 μg. Addition of T enanthate (both 50 and 100 mg weekly) induced a further fall only in high density lipoprotein cholesterol to 22.6 ± 3.7% from the baseline. In summary, the combined actions of oral DSG with low doses of T enanthate were highly effective in suppressing pituitary-testicular functions in adult men. The optimal regimen for inducing azoospermia was 300 μg DSG daily with 50 mg T enanthate weekly. Oral DSG exerted discernible effects on lipid metabolism. We conclude that the combination of oral progestogens with low dose T is a promising approach to achieve effective reversible male contraception. (*J Clin Endocrinol Metab* **84:** 112–122, 1999)

HORMONAL male contraception is achieved through the suppression of LH and FSH. The hypogonadotropic state and depletion of intratesticular testosterone (T), in turn, lead to an arrest of spermatogenesis to an extent that vouchsafes reliable, but reversible, infertility (1). A number of sex steroids, administered alone or in combination, are potentially capable of accomplishing this goal through their inhibitory action on pituitary gonadotropin secretion. Early exploratory studies showed that medroxyprogesterone acetate (MPA) (2–5), danazol, (6), cyproterone acetate (7), norethisterone acetate (8), or levonorgestrel (9, 10) combined with subreplacement doses of T were effective in inducing azoospermia in 40–80% of Caucasian men. The principle that sex steroid-induced azoospermia and oligozoospermia can confer effective contraceptive protection was established in two multicenter efficacy trials (11, 12) using an androgen-only regimen: 200 mg T enanthate, im, weekly. The unsatisfactory pharmacokinetics of T enanthate required that a relatively high dose (200 mg) be administered at weekly intervals to ensure adequate suppression of spermatogenesis (13, 14). This produced supraphysiological and markedly fluctuating levels of T with repeated peaks (15) that induced significant androgen-related effects on lipid metabolism [lowering high density lipoprotein cholesterol (HDL-C)], skin, muscle, liver, and hemopoiesis (16). Improved long acting T formulations with stable dose-sparing pharmacokinetics or combining reduced doses of T with a second nonandrogenic suppressive agent, such as progestogen or GnRH antagonist, are clearly required. T

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buciclate promised improved pharmacokinetics with prolonged action (17), which can potentially be exploited to advantage in male contraception (18) but is currently unavailable. Three recent studies reexplored the use of the progestogens, levonorgestrel, cyproterone acetate, and MPA, combined with lower doses of T (19–21). All three combinations conferred highly effective suppression of spermatogenesis with reduced nonreproductive androgenic effects. However, significant suppression of HDL-C was still observed with oral levonorgestrel combined with T enanthate (19). The relative contribution of progestogen *vs.* T to the antigonadotropic and metabolic effects is unclear, as all previous studies using the combination have administered both agents simultaneously from the start.

3-Ketodesogestrel or etonogestrel (ENG; 13-ethyl-17-hydroxy-11-methylene-18,19-dinor-17α-pregn-4-en-20-en-3-one) is a potent and highly selective synthetic progestogen (22) that is considered to have less androgenic properties than other 19-nortestosterone-derived gonane progestogens, such as levonorgestrel and norethisterone acetate (23). ENG does not lower circulating HDL-C or apolipoprotein A1 when coadministered with estrogens in women (24). Based on the results of steady state studies and on excretion data, ENG (unlike MPA) does not accumulate in the body (25). Desogestrel (DSG), which is converted by the liver to ENG as the active metabolite, has been used widely in the combined oral female contraceptive (Marvelon, N.V. Organon, Oss, The Netherlands) since 1981. The effects of DSG or ENG in men have not previously been investigated. We hypothesized that oral DSG combined with T can reversibly suppress spermatogenesis in healthy men with minimal metabolic effects.

Based on the knowledge that 150 μg DSG are used in the female oral contraceptive pill for ovulation suppression and that between 125–500 μg levonorgestrel daily with T enanthate can suppress spermatogenesis (19, 26), we selected DSG doses of 300 and 150 μg daily for the present study. In a pilot study we have shown that increasing the dose of DSG from 300 to 450 μg daily did not produce greater gonadotropin suppression in men (27). In the same study it was demonstrated that LH, FSH, and T were all suppressed to the nadir by DSG within 3 weeks. Thus, administering DSG alone for the initial 3 weeks and deferring the addition of T by 3 weeks should provide a unique opportunity to determine the individual contributions to the synergism between the two steroids.

We have conducted a downward dose-ranging study to investigate the impact of combining DSG with reducing physiological replacement doses of T on suppression of spermatogenesis and androgen-related nonreproductive effects. The aim of the study was to address the following specific questions. 1) How much DSG is required to be combined with low doses of T to suppress spermatogenesis? 2) What is the lowest dose of T (enanthate) compatible with maintaining secondary sexual and physiological functions as well as contributing to the contraceptive action of the combination? 3) To what extent do the two components contribute to the overall actions of the combination?

Subjects and Methods

Subjects

Twenty-four healthy male Caucasian volunteers (mean age, 33.2 $\pm$ 0.9 yr) were recruited from the community. Of 289 respondents to our advertisements, 40 were suitable for screening. Twelve did not wish to proceed further after the initial interview and screening tests, three were excluded because of low sperm counts, and one was excluded because of a blood coagulation disorder.

Study design

Subjects who met the admission criteria after screening were randomized into three treatment groups (n = 8) to receive: 1) 300 μg DSG, orally, daily and 100 mg T enanthate, im, weekly; 2) 300 μg DSG, orally, daily and 50 mg T enanthate, im, weekly; 3) 150 μg DSG, orally, daily and 100 mg T enanthate, im, weekly for 24 weeks in a single blind, parallel group design. Each subject was studied in three phases: 1) control phase: a screening medical examination, two baseline semen analyses, and hormonal and biochemical assessments were carried out over 4 weeks; 2) treatment phase: each subject was randomly allocated to one of the three treatment groups [for all subjects, desogestrel was administered during the total treatment period (weeks 1–24), and T enanthate was given during weeks 4–24; subjects received the first dose of T enanthate on day 22 after starting DSG; a medical review, including physical examination, semen analyses, and blood sampling, was carried out every 4 weeks except during the first 6 weeks, when blood samples were obtained on days 3, 5, 7, 14, 21, 28, and 56]; and 3) recovery phase: all subjects were monitored every 4 weeks by medical review, semen analyses, and blood sampling until they attained the recovery criteria, *i.e.* when the geometric mean pretreatment sperm density was reached or two consecutive specimens showed sperm density greater than 20 million/mL. All subjects provided informed written consent and were advised to continue with alternative forms of contraception during the study. The study was approved by the Central Manchester ethical committee for medical research.

Medications

DSG (150 μg) and matching placebo tablets were supplied by NV Organon. Each subject received one active and one placebo (150 μg group) or two active tablets (300 μg groups). T was administered weekly by the study personnel as deep im injections of 0.2 or 0.4 mL Testoviron Depot (250 mg T enanthate in 1 mL castor oil; Schering AG, Berlin, Germany), giving 50 or 100 mg T enanthate, respectively.

Clinical monitoring

Subjects were interviewed monthly and were examined at 3-month intervals throughout the study, with particular emphasis on eliciting any side-effects and monitoring sexual function, endocrine system, body weight, blood pressure, and testicular size (by orchidometer). A digital prostate examination was carried out pretreatment, at the end of treatment, and on recovery. Sexual function and moods were recorded by weekly diaries.

Semen analysis

Semen collection and analysis of semen volume, sperm density, motility, and morphology were carried out according to the *WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction* (28). Azoospermia was verified by centrifugation of the whole semen sample. Semen analyses were carried out twice during the control phase and at monthly intervals until recovery, as defined above, was achieved.

Blood tests

Blood samples were obtained twice pretreatment; on days 3, 5, 7, 14, 21, 28, and 56 in the treatment phase; and thereafter at 4-week intervals for hormone measurements [T, LH, FSH, and sex hormone-binding globulin (SHBG)]. Blood samples were also obtained immediately before the daily dose of DSG on days 7 and 28 and then at weeks 4, 8, 12, 16,

20, and 24 for measurement of ENG concentrations. Additional fasting blood samples were taken for hematological (hemoglobin, hematocrit, and white cell count), biochemical (urea, creatinine, electrolytes, albumin, liver enzymes, glucose, and hemoglobin A_{1c}), and lipid profiles [total cholesterol, low density lipoprotein cholesterol (LDL-C), HDL-C, triglyceride, and apolipoprotein A_1) at baseline and at weeks 3, 6, 12, 20, 24, 32, 40, and 48.

Hormone assays

All plasma samples were stored at −20 C until assay. Plasma gonadotropins were assayed by previously reported highly sensitive immunofluorometric assays (Delfia, Pharmacia-Wallac, Turku, Finland) (29) with an assay sensitivity of 0.05 IU/L for both LH and FSH, T was determined by a previously described RIA (30) with an assay sensitivity of 0.3 nmol/L, SHBG was determined by an immunoradiometric assay (Farmos Diagnostica, Oulun Salo, Findland), and ENG was determined by an in-house RIA. All serial samples from one individual were assayed in a single batch to reduce variability.

Biochemical analyses

Full blood counts, glucose, hemoglobin A_{1c}, lipids (total cholesterol, HDL-C, and triglyceride), and renal and liver functions were measured by routine autoanalyzer methods. LDL-C was derived from the other lipid measures using Friedwald's formula.

Statistical analyses

The data were analyzed by repeated measure ANOVA, paired *t* tests, one-way ANOVA with Tukey's *post-hoc* test for continuous variables, and contingency table (Fisher's) test for categorical variables, with statistical significance set at $P < 0.05$. Data that were not normally distributed were log transformed before analysis. Values were expressed as the arithmetical mean ± SEM. LH and FSH concentrations below the sensitivity of the assay were allocated a value of 0.05 U/L, the lower limit of detection.

Results

Spermatogenesis

The mean sperm densities before, during, and after DSG and T enanthate administration in the three treatment groups are shown in Fig. 1. The rates of suppression to various target sperm densities during the 24-week treatment phase and recovery after cessation of treatment are shown in Fig. 2 and Table 1. All three dose combinations suppressed sperm production significantly by week 8. Eighteen (78%) of 23 subjects who completed the suppression phase achieved azoospermia, the earliest by week 8 (n = 3) and the latest after 24 weeks (n = 1) of treatment. Some 91.7% (22 of 24) and 95.8% (23 of 24) of subjects suppressed to less than 1 million/mL and less than 3 million/mL, respectively, after 24 weeks. The most effective regimen was 300 μg DSG daily combined with 50 mg T enanthate weekly, under which all eight subjects became azoospermic after 20 weeks of treatment, although a statistically significant difference in the number or percentage of subjects achieving any of the 3 targets (azoospermia or <1 or <3 million/mL) among the three regimens could not be detected (Table 1). However, the overall decline in sperm densities with time in response to 300 μg DSG/50 mg T enanthate was significantly greater (by ANOVA: F = 6.99; $P < 0.02$) than that observed with 300 μg DSG/100 mg T enanthate but not that observed with 150 μg DSG/100 mg T enanthate (by ANOVA: F = 3.11; $P = 0.1$; Fig. 1). Sperm densities at any of the individual times during treatment from weeks 4–24 (Fig. 1) were not significantly different among the three treatment groups. One subject in the 300 μg DSG/100 mg T enanthate group was relatively unresponsive to treatment, with nadir sperm density remaining at 16.3 million/mL at week 24. His compliance with oral medication was confirmed by the ENG concentrations during the treatment phase, which were consistently above the 10th percentile of values for subjects receiving 300 μg DSG daily. Exclusion of this single outlier did not change the conclusions of the above analyses.

There was no difference in the duration of treatment required to achieve the three suppression targets among the three treatment groups (Table 1). The average times taken to suppress sperm density to azoospermia, less than 1 mil-

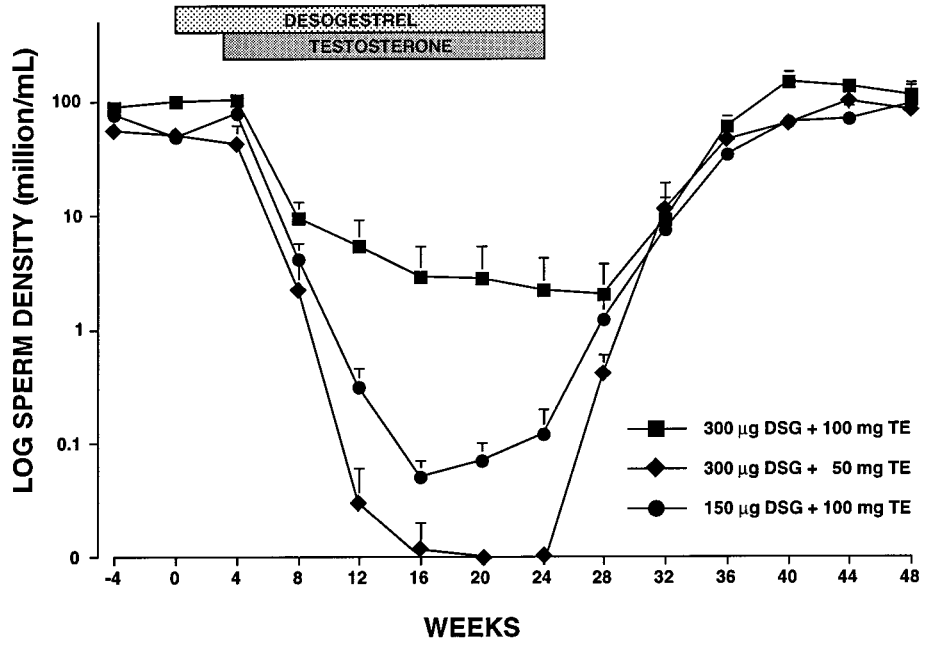

FIG. 1. Sperm density (log scale) at pretreatment baseline (−4 and 0 weeks), during treatment (0–24 weeks), and at recovery (24–28 weeks) phases in three groups of healthy men (n = 8) treated with 300 μg DSG, orally, daily and 100 mg T enanthate, im, weekly (*solid squares*); 300 μg DSG, orally, daily and 50 mg T enanthate, im, weekly (*solid rhomboids*); and 150 μg DSG, orally, daily and 100 mg T enanthate, im, weekly (*solid circles*) for 24 weeks. DSG alone was administered during the first 21 days (0–3 weeks), and T enanthate was started on day 22 and was coadministered with DSG between weeks 4–24. Values are the mean ± SEM.

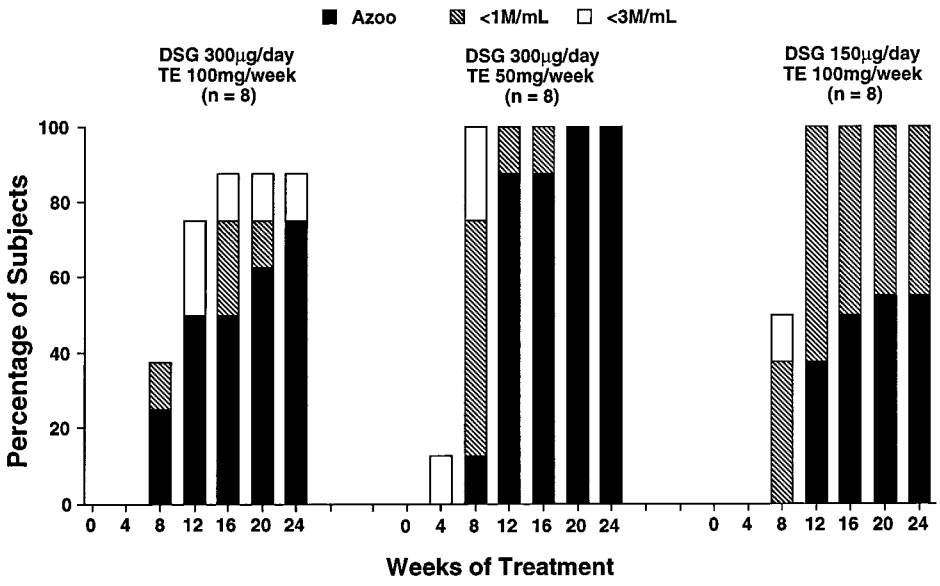

FIG. 2. Rate of suppression of spermatogenesis as indicated by the percentage of subjects attaining azoospermia or oligozoospermic targets (<3 and <1 million/mL) at 4-week intervals during 24 weeks of treatment in three groups of healthy men (n = 8) receiving 1) 300 μg DSG, orally, daily and 100 mg T enanthate, im, weekly; 2) 300 μg DSG, orally, daily and 50 mg T enanthate, im, weekly; and 3) 150 μg DSG, orally, daily and 100 mg T enanthate, im, weekly. DSG alone was administered during the first 21 days (0–3 weeks), and T enanthate was started on day 22 and was coadministered with DSG between weeks 4–24.

TABLE 1. Suppression and recovery of spermatogenesis by three combinations of oral DSG daily and im TE weekly in 24 healthy adult men

Treatment group	Response	Suppression targets			Recovery targets		
		Oligozoospermia (<3 million/mL)	Oligozoospermia (<1 million/mL)	Azoospermia	50% baseline	>20 million/mL	Baseline
(1) 300 μg DSG, 100 mg TE (n = 8)	Week to target	10.9 ± 1.1	10.7 ± 1.3	14.0 ± 2.7	12.5 ± 0.9	11.5 ± 0.9	13.5 ± 0.7
	% Responding	87.5	75	75	100	100	100
(2) 300 μg DSG, 50 mg TE (n = 8)	Week to target	8.0 ± 0.8	9.0 ± 0.7	12.5 ± 1.2	12.0 ± 0.8	12.0 ± 0.8	13.0 ± 1.0
	% Responding	100	100	100	100	100	100
(3) 150 μg DSG, 100 mg TE (n = 8)	Week to target	10.0 ± 0.8	10.5 ± 0.7	13.0 ± 1.0	14.5 ± 1.1	14.5 ± 1.1	16.0 ± 1.1
	% Responding	100	100	57[a]	100	100	100

Values are the mean ± SEM. % Responding, at week 24, end of treatment. Week to target denotes the duration of treatment required for the first of two consecutive semen samples to attain stated target.

[a] 4/7 because one subject dropped out after week 16. (See text: *Discontinuation and side-effects.*)

lion/mL, and less than 3 million/mL were 13.1 ± 1.0, 10.0 ± 0.5, and 9.6 ± 0.5 weeks, respectively.

Sperm density in all three groups started to recover within 4–8 weeks after discontinuation of treatment, and all subjects achieved the recovery criteria by week 44 (20 weeks after the end of treatment). The slower rate of recovery in group 3 was due to one individual who did not recover until 20 weeks after treatment. There was no significant difference in the posttreatment sperm densities among the three treatment groups (Fig. 1).

LH

All three dose combinations of DSG with T enanthate were highly effective in suppressing LH secretion (Fig. 3). DSG treatment alone in the first 3 weeks suppressed LH significantly ($P < 0.001$; Fig. 3, *inset*) from a baseline of 3.53 ± 0.31 to 2.16 ± 0.46 U/L (group 1) and from 3.62 ± 0.30 to 2.0 ± 0.20 (group 2) with 300 μg DSG and from 4.29 ± 0.51 to 2.81 ± 0.56 in response to 150 μg DSG (group 3) at the end of 3 weeks of treatment. Although the suppression of LH by 300 μg (groups 1 and 2) was consistently greater than that by 150 μg (group 3) DSG daily during the first 3 weeks, the difference did not reach statistical significance. The suppression of LH by DSG alone was rapid, being apparent by day 3 ($P < 0.006$ compared to day 0). Indeed, there was no further decline in LH from days 3–21. Addition of T enanthate to DSG from weeks 4–24 induced a further striking fall in LH ($P < 0.0001$ compared with the first 3 weeks) in all three treatment groups (Fig. 3). The decrease in LH continued until week 6 (0.06 ± 0.01, 0.13 ± 0.03, and 0.11 ± 0.04 U/L, groups 1, 2, and 3, respectively), after which the LH concentrations were maintained at or below the lower limit of assay detection. From week 16 to the end of treatment, all subjects had undetectable levels of LH, except for one man (LH, 0.2–0.6 U/L) in the 300 μg DSG/50 mg T enanthate group. LH recovered rapidly, so that levels were not significantly different from baseline within 4 weeks after the cessation of treatment in all three groups. There was no significant difference in LH among the three treatment groups in the baseline, treatment, or recovery phases of the study.

FSH

All three dose combinations of DSG with T enanthate were highly effective in suppressing FSH (Fig. 4). DSG alone suppressed FSH significantly ($P < 0.0001$) from the baseline of 3.58 ± 0.59 progressively to a nadir of 1.61 ± 0.32 U/L (group 1), from 3.15 ± 0.42 to 1.44 ± 0.26 U/L with 300 μg (group 2), and from 3.95 ± 0.45 to 2.10 ± 0.26 U/L with 150 μg DSG

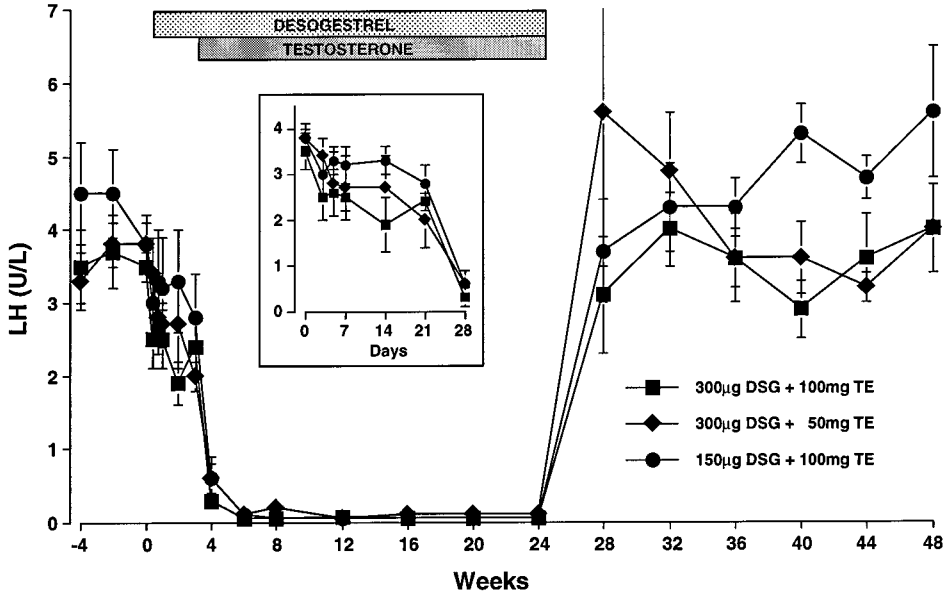

FIG. 3. Plasma LH concentrations at pretreatment baseline (−4 and 0 weeks), during treatment (0–24 weeks), and at recovery (24–28 weeks) phases in three groups of healthy men (n = 8) treated with 300 μg DSG, orally, daily and 100 mg T enanthate, im, weekly (*solid squares*); 300 μg DSG, orally, daily and 50 mg T enanthate, im, weekly (*solid rhomboids*); and 150 μg DSG, orally, daily and 100 mg T enanthate, im, weekly (*solid circles*) for 24 weeks. DSG alone was administered during the first 21 days (0–3 weeks), and T enanthate was started on day 22 and was coadministered with DSG between weeks 4–24. The *inset* shows changes in LH during the first 28 days of treatment. Values are the mean ± SEM.

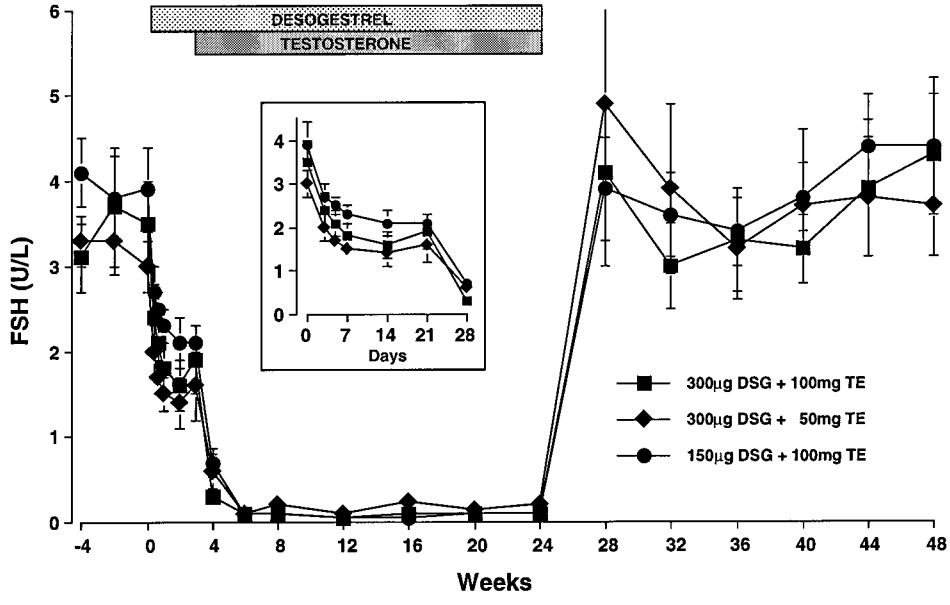

FIG. 4. Plasma FSH concentrations at pretreatment baseline (−4 and 0 weeks), during treatment (0–24 weeks), and at recovery (24–28 weeks) phases in three groups of healthy men (n = 8) treated with 300 μg DSG, orally, daily and 100 mg T enanthate, im, weekly (*solid squares*); 300 μg DSG, orally, daily and 50 mg T enanthate, im, weekly (*solid rhomboids*); and 150 μg DSG, orally, daily and 100 mg T enanthate, im, weekly (*solid circles*) for 24 weeks. DSG alone was administered during the first 21 days (0–3 weeks), and T enanthate was started on day 22 and was coadministered with DSG between weeks 4–24. The *inset* shows changes in FSH during the first 28 days of treatment. Values are the mean ± SEM.

(group 3) at the end of week 2; there was no further decline between weeks 2–3 (Fig. 4, *inset*). FSH was consistently suppressed to a lower level by 300 μg (groups 1 and 2) than by 150 μg (group 3) DSG daily during the first 3 weeks; this difference reached statistical significance on day 7 (1.7 ± 0.2 *vs.* 2.3 ± 0.2; $P < 0.05$). Addition of T enanthate to DSG induced a further decrease ($P < 0.0001$) in FSH from sweek 3–6. Between weeks 8 and 24, FSH was maintained around the lower limit of assay detection (0.07 ± 0.01, 0.18 ± 0.06, and 0.06 ± 0.01 U/L for groups 1, 2, and 3, respectively). Although there was no significant difference in FSH concentrations among the three groups during the combination treatment, the number of samples with detectable FSH was significantly higher in the group receiving 300 μg DSG/50 mg T enanthate (25 of 40) than in the other two groups receiving 300 μg DSG/100 mg T enanthate (13 of 40; $P < 0.02$) and 150 μg DSG/100 mg T enanthate (9 of 40; $P < 0.001$). After treatment, FSH recovered to baseline concentrations within 4 weeks similarly in the three groups.

T

In response to DSG, T decreased from the baseline of 19.7 ± 1.0 to a nadir on day 14 of 6.6 ± 1.9 nmol/L with 300 μg (group 1; $P < 0.0001$), 19.5 ± 1.2 to 6.1 ± 0.6 nmol/L with 300 μg (group 2; $P < 0.0001$), and 21.2 ± 2.0 to 8.8 ± 1.4 nmol/L with 150 μg DSG (group 3; $P < 0.0001$; Fig. 5, *inset*). A small, but significant, rise ($P < 0.05$) in T was observed between days 14 and 21. Lower T levels were consistently observed during the first 3 weeks with the 300-μg DSG dose in groups 1 and 2; this approached statistical significance on day 7 (7.8 ± 1.0 *vs.* 10.2 ± 1.7 nmol/L; $P = 0.074$; Fig. 5, *inset*).

Addition of 100 mg T enanthate weekly in groups 1 and 3 significantly raised ($P < 0.0001$) plasma T from the end of

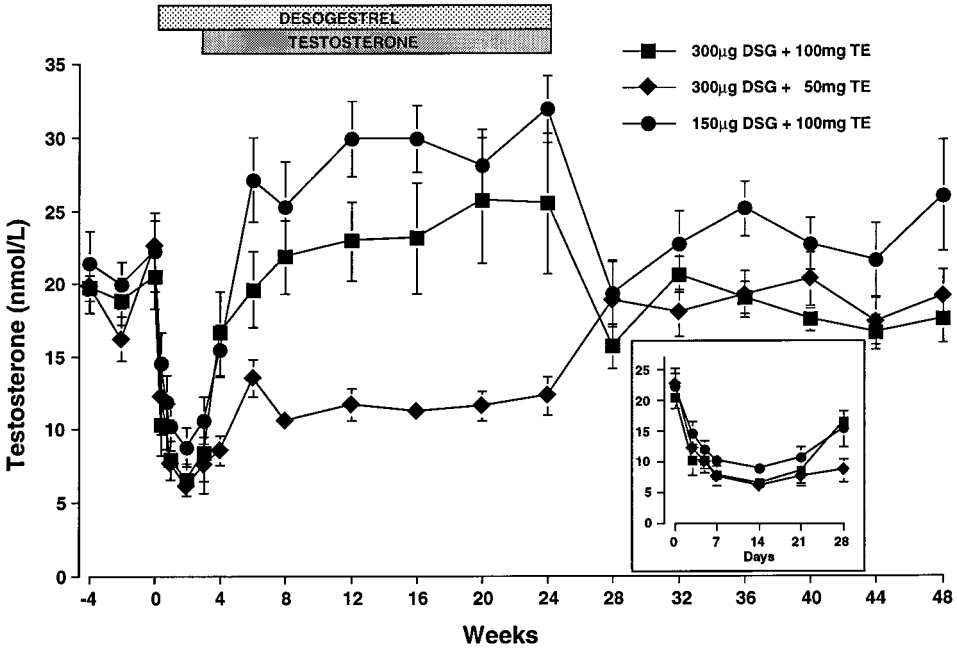

FIG. 5. Plasma T concentrations at pretreatment baseline (−4 and 0 weeks), during treatment (0–24 weeks), and at recovery (24–28 weeks) phases in three groups of healthy men (n = 8) treated with 300 μg DSG, orally, daily and 100 mg T enanthate, im, weekly (*solid squares*); 300 μg DSG, orally, daily and 50 mg T enanthate, im, weekly (*solid rhomboids*); and 150 μg DSG, orally, daily and 100 mg T enanthate, im, weekly (*solid circles*) for 24 weeks. DSG alone was administered during the first 21 days (0–3 weeks), and T enanthate was started on day 22 and was coadministered with DSG between weeks 4–24. The *inset* shows changes in T during the first 28 days of treatment. Values are the mean ± SEM.

week 3 to mean levels of 22.1 ± 2.9 and 26.7 ± 2.9 nmol/L (P = NS between the two groups), respectively, and to 11.5 ± 0.6 in group 2 ($P < 0.0001$ compared with the other two groups) with 50 mg T enanthate (Fig. 5). T levels returned to baseline 4 weeks after treatment terminated in all three groups.

ENG levels

ENG concentrations increased within 1 week to stable levels that did not change subsequently during the 24-week treatment phase. The mean ENG concentrations were 1056 ± 103, 1176 ± 167, and 592 ± 107 pg/mL in the 300 μg DSG/100 mg T enanthate, 300 μg DSG/50 mg T enanthate, and 150 μg DSG/100 mg T enanthate groups, respectively. The 150-μg dose produced ENG concentrations significantly lower ($P < 0.02$) than the 300-μg dose.

SHBG

DSG alone decreased SHBG concentrations significantly ($P < 0.0001$) from 28.2 ± 4.0 to 21.3 ± 3.7, 28.1 ± 3.9 to 20.7 ± 2.1, and 29.3 ± 4.5 to 21.7 ± 3.1 nmol/L in groups 1, 2, and 3, respectively, after 3 weeks of treatment (Table 2). Addition of T enanthate at either 100 or 50 mg weekly further decreased SHBG to 17.7 ± 3.0, 16.8 ± 1.9, and 17.0 ± 3.2 nmol/L in groups 1, 2, and 3, respectively, at week 24 ($P < 0.0001$ compared to week 3). These recovered to the pretreatment levels by 4 weeks after cessation of treatment. There was no significant difference in SHBG concentrations among the three treatment groups in the baseline, treatment, or recovery phases of the study. However, the SHBG decline after introduction of T enanthate was apparent by weeks 6 and 12 in groups 1 ($P < 0.03$) and 3 ($P < 0.005$), respectively, whereas in group 2, this only became significant at week 24 ($P < 0.02$).

Lipids and biochemical and hematological parameters

The lipid results from the three treatment groups are shown in Fig. 6 and Table 2. In the first 3 weeks, DSG alone significantly suppressed total cholesterol (−9.3 ± 1.7%; $P < 0.0001$), HDL-C (−10.3 ± 2.6%; $P < 0.0001$), and LDL-C (−7.7 ± 2.8%; $P < 0.005$), with no difference between the daily doses of 300 and 150 μg. However, with the addition of T enanthate at the end of week 3, only HDL-C, but not total or LDL-C, decreased further until week 12 (−18.8 ± 2.4%; $P < 0.005$). There was no significant difference in the extent of suppression of HDL-C among the three dose combinations compared to that at week 3 or baseline. In particular, 50 and 100 mg T enanthate induced similar degrees of HDL-C suppression when combined with DSG. The overall decrease in HDL-C during treatment with DSG combined with T enanthate (all three groups) was 22.6 ± 3.7% compared to baseline. Triglyceride levels were unaffected by DSG alone or by DSG with T enanthate. All lipid parameters returned to baseline after treatment.

There were no changes in plasma glucose, hemoglobin A_{1c}, hematocrit, or creatinine (Table 2). No changes in routine biochemistry (data not shown) or liver function were observed (Table 2).

Physical changes

No significant changes in body weight or blood pressure were observed during treatment (Table 2). Testicular volume decreased ($P < 0.001$) during treatment, but recovered by week 40 (Table 2).

Discontinuations and side-effects

There were four discontinuations, all at the end of week 16. One subject (group 2) discontinued because of headaches and sweating. Another subject (group 3) had to withdraw

TABLE 2. Biochemical and clinical parameters at baseline, during treatment (weeks 3, 12, 24), and recovery (12 weeks after stopping treatment)

	Group 1				
	Control baseline	Treatment			Recovery week 36
		Week 3	Week 12	Week 24	
SHBG (nmol/L)[a]	28.2 ± 4.2	21.3 ± 3.7	17.3 ± 2.8	17.7 ± 3.0	24.6 ± 3.9
Total cholesterol (mmol/L)[a]	4.90 ± 0.30	4.45 ± 0.26	4.30 ± 0.26	4.28 ± 0.15	5.25 ± 0.38
HDL cholesterol (mmol/L)[a]	1.14 ± 0.7	1.01 ± 0.07	0.96 ± 0.05	0.94 ± 0.04	1.16 ± 0.06
Apo A1 (mg/dL)[a]	115.8 ± 3.8	106.1 ± 4.3	106.3 ± 3.2	104.1 ± 4.9	119.0 ± 2.9
LDL cholesterol (mmol/L)[a]	3.51 ± 0.25	3.24 ± 0.25	3.15 ± 0.23	3.09 ± 0.16	3.73 ± 0.37
Triglyceride (mmol/L)	1.25 ± 0.13	1.01 ± 0.10	1.03 ± 0.19	1.20 ± 0.14	1.81 ± 0.32
Glucose (mmol/L)	5.4 ± 0.1	5.2 ± 0.2	5.2 ± 0.2	5.0 ± 0.2	4.9 ± 0.1
HbA_{1c} (%)	5.1 ± 0.1	5.0 ± 0.1	4.7 ± 0.2	4.9 ± 0.1	4.9 ± 0.2
ALT (U/L)	23.1 ± 3.8	17.8 ± 1.7	17.8 ± 1.0	18.1 ± 1.3	28.4 ± 4.9
AST (U/L)	20.1 ± 1.6	18.0 ± 1.4	20.9 ± 1.8	22.8 ± 2.1	23.8 ± 2.3
Alk phosphatase (U/L)	159.3 ± 14.9	148.8 ± 17.7	155.0 ± 16.3	147.8 ± 13.8	163.4 ± 12.6
Creatinine (μmol/L)	101.9 ± 2.5	97.9 ± 2.8	99.9 ± 2.9	102.5 ± 3.1	102.8 ± 3.1
Hematocrit	0.41 ± 0.01	0.41 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	0.42 ± 0.01
BW (kg)	83.4 ± 4.1	83.0 ± 3.8	83.9 ± 3.3	84.3 ± 4.3	83.7 ± 4.9
Blood pressure, systolic (mm Hg)	125.0 ± 4.2	123.8 ± 4.2	120.0 ± 3.3	118.8 ± 4.8	117.5 ± 4.5
Blood pressure diastolic (mm Hg)	74.8 ± 3.6	75.0 ± 1.9	79.8 ± 3.2	75.0 ± 1.9	73.8 ± 1.8
Testis volume (mL)[a]	22.5 ± 1.1	21.4 ± 1.3	17.3 ± 1.1	15.7 ± 1.0	22.5 ± 1.1

[a] Significant changes described in text.
Values are the mean ± SEM. DSG and TE treatment for 24 weeks in groups 1–3. For details, see *Subjects and Methods.* n = 8 in each group.

from the study because his 16-month-old son died from a congenital abnormality. One subject (group 3) withdrew for social reasons. The fourth subject (group 2) discontinued because his partner conceived (condom failure at a time when sperm densities were between 0.1–6.0 million/mL, but achieved azoospermia 4 weeks later) and subsequently miscarried at 6 weeks. There were no other pregnancies during this study. All prematurely discontinued subjects were followed until recovery was complete.

During the first 3 weeks of treatment (DSG without T), 5 subjects experienced side-effects, including decreased sex drive (n = 4), tiredness (n = 1), and feeling depressed (n = 1). Between weeks 4 and 24, side-effects reported included mild acne (10 subjects; but only 1 from group 2), short temper (n = 5), increased sexual interest (n = 3), emotional lability (n = 2), tiredness (n = 2), night sweat (n = 1), and headache (n = 1).

Discussion

The half-life of ENG is 20–24 h, and the peak concentration was achieved within 1–2 h after oral administration of DSG in females (31). Our data in men showed that steady state levels were reached within 1 week. The pharmacokinetics were dose proportional and remained constant on continued treatment for 24 weeks. Coadministration of T enanthate did not have any effect on ENG pharmacokinetics. Mean ENG levels of 592 ± 107 pg/mL (150 μg DSG daily) and 1056 ± 103 or 1176 ± 187 pg/mL (300 μg DSG daily) in our subjects were in the range of that achieved in women (982 ± 502 pg/mL) receiving 150 μg DSG with 30 μg ethinyl estradiol daily (32), indicating similar oral bioavailability. Although no posttreatment measurements were undertaken, the rapidity of recovery of gonadotropins and T after the cessation of treatment suggests that significant tissue accumulation of ENG is unlikely.

The most effective suppression of spermatogenesis was found in the group receiving the lowest T enanthate dose of 50 mg weekly with 300 μg DSG daily, with which all subjects became azoospermic. In a study complementary to ours (Anawalt, B. D., *et al.*, 1998, unpublished), men receiving 50 mg T enanthate with 150 μg DSG showed suboptimal spermatogenesis suppression with 57% azoospermia, 75% with less than 1 million/mL, and 78% with less than 3 million/mL, whereas both LH and FSH remained detectable throughout treatment. Our results show that an increase in dose of either DSG or T will achieve significantly greater suppression. Thus, either 300 μg DSG daily with 50 mg T enanthate weekly or 150 μg DSG daily with 100 mg T enanthate weekly suppressed spermatogenesis to less than 1 million/mL in all subjects. However, increasing the dose of both agents, DSG to 300 μg daily and T enanthate to 100 mg weekly, did not bring about any additional suppression. Indeed, the suppression of spermatogenesis with the highest doses (300 μg DSG and 100 mg T enanthate) was marginally less effective. As gonadotropin suppression in groups 1 and 3 was greater than that in group 2, it is possible that higher circulating testosterone in groups 1 and 3 or its 5α-reduced metabolites (33) may prevent the achievement of azoospermia by direct stimulatory action of the testis. This supports the use of the lowest possible doses of testosterone in male contraception, provided gonadotropin suppression is adequate and physiological androgen-dependent functions can be maintained.

The efficacy of spermatogenesis suppression achieved by DSG and T enanthate (78% azoospermia overall and 100% in group 2) is superior to those obtained in earlier studies of progestogen/androgen combinations that used suboptimal doses and longer dosing intervals of T (2–5). The efficacy and rate of spermatogenic suppression in the present study also compared favorably with more recent work investigating the oral progestogens levonorgestrel (500 μg daily; 67% azoospermic) and cyproterone acetate (100 mg daily; 100% azoospermic) combined with 100 mg T enanthate weekly (19,

TABLE 2. *Continued*

Group 2					Group 3				
Control baseline	Treatment			Recovery week 36	Control baseline	Treatment			Recovery week 36
	Week 3	Week 12	Week 24			Week 3	Week 12	Week 24	
28.1 ± 3.9	20.7 ± 2.1	19.8 ± 3.4	16.8 ± 1.9	24.7 ± 2.1	29.3 ± 4.5	21.7 ± 3.1	18.3 ± 2.7	17.0 ± 3.2	27.4 ± 4.8
4.75 ± 0.35	4.30 ± 0.35	4.28 ± 0.27	4.23 ± 0.24	5.1 ± 0.32	5.01 ± 0.24	4.49 ± 0.22	4.50 ± 0.22	4.43 ± 0.22	5.16 ± 0.26
1.59 ± 0.20	1.40 ± 0.15	1.30 ± 0.17	1.15 ± 0.08	1.58 ± 0.16	1.41 ± 0.14	1.26 ± 0.12	1.05 ± 0.09	0.98 ± 0.08	1.40 ± 0.11
132.3 ± 11.4	131.1 ± 7.1	125.8 ± 9.4	114.5 ± 5.3	132.6 ± 6.2	137.1 ± 6.4	123.5 ± 5.7	118.5 ± 5.6	117.8 ± 5.8	131.0
2.99 ± 0.41	2.73 ± 0.33	2.87 ± 0.28	2.97 ± 0.30	3.15 ± 0.30	3.41 ± 0.32	3.05 ± 0.29	3.21 ± 0.25	3.21 ± 0.23	3.50 ± 0.32
0.86 ± 0.11	0.86 ± 0.13	1.10 ± 0.24	0.65 ± 0.06	1.21 ± 0.30	0.98 ± 0.20	0.89 ± 0.09	1.23 ± 0.22	1.20 ± 0.18	1.30 ± 0.03
5.1 ± 0.1	4.9 ± 0.2	5.0 ± 0.2	5.2 ± 0.2	5.3 ± 0.2	5.3 ± 0.2	5.1 ± 0.2	5.3 ± 0.3	5.0 ± 0.2	5.1 ± 0.2
4.9 ± 0.2	4.8 ± 0.2	4.9 ± 0.1	4.7 ± 0.1	4.8 ± 0.1	5.0 ± 0.2	5.0 ± 0.1	4.8 ± 0.1	5.0 ± 0.2	5.0 ± 0.1
23.3 ± 2.9	22.9 ± 5.3	20.1 ± 2.9	19.6 ± 1.8	24.4 ± 4.1	25.3 ± 3.1	21.3 ± 2.6	22.5 ± 2.8	21.3 ± 3.4	27.6 ± 4.7
22.0 ± 1.6	23.0 ± 2.7	19.4 ± 1.5	20.3 ± 1.2	21.9 ± 2.0	24.8 ± 2.8	21.4 ± 1.8	22.7 ± 2.7	21.7 ± 2.0	22.4 ± 2.1
138.6 ± 8.6	126.0 ± 9.3	128.1 ± 10.5	121.1 ± 6.4	139.0 ± 7.7	155.4 ± 8.3	148.9 ± 10.0	146.0 ± 10.7	150.4 ± 15.9	154.8 ± 14.9
102.5 ± 3.4	105.1 ± 4.0	103.1 ± 2.8	99.9 ± 3.2	101.6 ± 4.1	103.9 ± 3.4	107.0 ± 3.7	110.9 ± 5.0	112.2 ± 2.9	112.3 ± 1.9
0.40 ± 0.01	0.38 ± 0.00	0.40 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	0.45 ± 0.01	0.43 ± 0.01
79.7 ± 3.5	79.2 ± 3.4	81.7 ± 3.4	77.4 ± 2.1	82.5 ± 2.8	76.0 ± 3.5	75.9 ± 3.2	78.4 ± 3.3	81.9 ± 2.3	75.9 ± 3.6
122.5 ± 3.7	118.8 ± 3.0	123.8 ± 3.8	121.5 ± 4.3	120.0 ± 4.4	123.8 ± 4.6	127.5 ± 2.5	127.5 ± 3.7	128.3 ± 4.0	120.0 ± 2.2
73.3 ± 3.9	70.0 ± 2.7	74.5 ± 4.1	77.1 ± 2.9	77.1 ± 1.8	79.3 ± 3.0	79.8 ± 1.7	80.8 ± 2.8	80.0 ± 2.2	78.6 ± 2.6
20.9 ± 1.3	19.6 ± 1.6	15.1 ± 1.2	14.9 ± 1.5	20.6 ± 1.4	19.6 ± 1.9	18.7 ± 1.7	17.4 ± 1.6	17.7 ± 1.2	19.6 ± 1.6

20) and 250 mg depot-MPA, im, combined with 800-mg T implants (90% azoospermic) (21). The minimal effective doses of these combination regimens are as yet unknown. The present results are also superior to those obtained with androgen-only regimens, including 200 mg T enanthate weekly alone (65% azoospermia), 1200-mg T implants (56% azoospermia), and 1200 mg T buciclate (37.5% azoospermia) (11, 18, 34). The speed of suppression to azoospermia (13.1 ± 1.0 weeks) with DSG and T enanthate, however, was not faster. Apart from using lower doses of T, the improved efficacy of progestogen-containing regimens may be due to an antispermatogenic or antiandrogenic effect (20, 35) or inhibition of 5α-reductase activity (36).

DSG treatment alone decreased LH, FSH, and T within 3 days, and all subjects became profoundly hypogonadal by day 7, reaching a nadir on day 14. This dramatic and acute effect is similar to that observed with the Nal-Glu GnRH antagonist (37). DSG alone suppressed T to a greater extent than LH and FSH. This reflects the simultaneous decline in SHBG so that bioavailable T is proportionately higher than total T levels. The bioactivity of LH is not preferentially lowered during DSG treatment, and the bioactive/immunoactive ratio is unchanged (30). The greater suppression of LH, FSH, and T by 300 μg compared to 150 μg DSG during the first 3 weeks suggests that the latter is the first submaximal dose. However, oral DSG on its own, even at the maximal dose, suppressed the hypothalamic-pituitary-testicular axis only partially. T is therefore essential not only for maintenance of androgen-dependent physiological functions but also for the crucial additional suppression of gonadotropins.

By deferring the introduction of T enanthate after DSG, this study has provided the first demonstration of the individual action of an oral progestogen as well as the subsequent additive effects of T on the pituitary-testicular axis. It is clear that the addition of T to DSG was required to further suppress gonadotropins toward the limits of assay detection. This was achieved even at doses of T enanthate (50 or 100 mg weekly) that, when administered on their own, are relatively ineffective (19). Thus, combining two agents at doses that are submaximal when used individually produced a clear additive effect in suppressing gonadotropins to levels compatible with maximal abrogation of spermatogenesis. Although plasma T in group 2 (50 mg T enanthate weekly) was half that in groups 1 and 3 (100 mg T enanthate weekly), there was no difference in gonadotropin suppression. As the dose of 300 μg DSG daily was more effective in suppressing gonadotropins and T than 150 μg, this might have compensated for the lower dose of T in group 2. This is supported by the findings that increasing the dose of T to 100 mg T enanthate weekly allowed a reduction in the dose of DSG from 300 to 150 μg (*i.e.* group 3) without losing efficacy, whereas 150 μg DSG and 50 mg T enanthate were ineffective (Anawalt, B. D., *et al.*, 1998, unpublished). These results not only show an additive effect, but also demonstrate an interchangeable action between T and DSG in inhibiting pituitary-testicular endocrine function and accord well with the results of spermatogenesis suppression (see above).

The present results show that a dose of 50 mg T enanthate weekly was sufficient for maintaining physiological circulating T (attaining a consistent predose trough level of 11 nmol/L) and normal sexual function during DSG treatment for 24 weeks. This has also been reported in normal men rendered hypogonadal by Nal-Glu GnRH antagonist treatment for 6 weeks (38). The higher dose of 100 mg T enanthate weekly raised preinjection trough plasma T above the pretreatment baseline and is therefore a supraphysiological dose (10.3 mg T daily). On its own, however, 100 mg T enanthate weekly was unable to induce maximal suppression of spermatogenesis (19), which required an even higher dose of 200 mg weekly. Our results therefore indicate that introducing a second gonadotropin-suppressing agent permitted a major (75%) reduction in the dose of T, which was not only sufficient to support secondary sexual functions but was also essential for suppression of spermatogenesis, with efficacy surpassing that obtained with higher dose androgen-only regimens. This is similar to using 300 mg depot-MPA monthly with a 800-mg T implant (6 mg T daily), which was suboptimal on its own, yet was fully effective when admin-

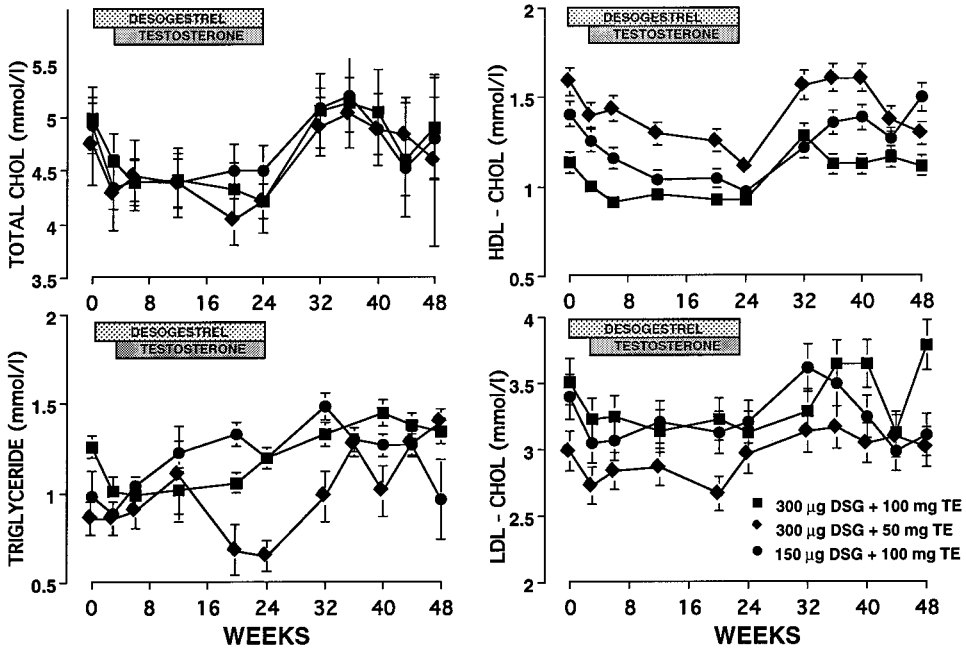

FIG. 6. Serum total cholesterol, HDL-C, and LDL-C and triglyceride at pretreatment baseline (−4 and 0 weeks), during treatment (0–24 weeks), and recovery (24–28 weeks) phases in three groups of healthy men (n = 8) treated with 300 μg DSG, orally, daily and 100 mg T enanthate, im, weekly (*solid squares*); 300 μg DSG, orally, daily and 50 mg T enanthate, im, weekly (*solid rhomboids*); and 150 μg DSG, orally, daily and 100 mg T enanthate, im, weekly (*solid circles*) for 24 weeks. DSG alone was administered during the first 21 days (0–3 weeks), and T enanthate was started on day 22 and coadministered with DSG between weeks 4–24. The *inset* shows changes in T during the first 28 days of treatment. Values are the mean ± SEM.

istered in combination (21). We have not extended the T dose range further downward because it is likely that lower doses than 50 mg T enanthate weekly would produce symptoms of androgen deficiency. The T production rate in young men determined by the stable isotope dilution technique is 3.7 ± 2.2 mg/day (39). Thus, 50 mg T enanthate weekly, or the equivalent of 5 mg of free T daily, is probably the minimally effective and optimal dose when combined with progestogens for male contraception. However, even these mandatory minimum replacement doses of T contribute substantially to the suppression of spermatogenesis, thereby permitting the use of the second antigonadotropic agent at relatively low or submaximal doses. This mutual dose-sparing effect and interchangeable additive action between T and progestogens are the key findings in the present study.

DSG treatment alone (either 150 or 300 μg daily) decreased HDL-C, apolipoprotein A1 lipoprotein, SHBG, and, to a lesser extent, total cholesterol and LDL-C. These metabolic effects on the liver had a rapid onset, being apparent within 3 weeks. It should be noted that at the time when these metabolic changes occurred, T was also declining into the hypogonadal range. When T is acutely lowered, as in men receiving GnRH antagonist (37), HDL-C and SHBG would be expected to rise. This anticipated increase in HDL-C or SHBG not only did not occur, but was actually reversed during oral DSG treatment in the first 3 weeks. Although DSG is a 19-nortestosterone-derived progestogen, it is noted for its low androgenic activity compared with other members of this family (40), and its relative binding affinity for the androgen receptor is only 0.12 compared to that of dihydroxytestosterone (23). It is therefore unlikely that the acute metabolic effects of oral DSG were mediated through its cross-reactivity with the androgen receptor. The simultaneous, albeit minor, suppression of total and LDL-C in addition to HDL-C was also uncharacteristic of an androgenic effect.

The addition of T enanthate in week 4 returned T into the normal range, but further decreases in HDL-C and SHBG were also induced that were similar whether 100 or 50 mg weekly was administered. As 100 mg T enanthate (and, by inference, 50 mg also) weekly did not alter lipids when administered in men rendered hypogonadal experimentally (37), but 200 mg T enanthate weekly decreased HDL-C only with no change in total cholesterol or LDL-C (16, 41, 42), the observed overall changes in lipid metabolism during weeks 4–24 (DSG plus T) were probably predominantly due to the effects of DSG rather than T. The magnitude of the fall of 22.6% in HDL-C during combined DSG and T enanthate treatment was greater than that observed with 200 mg T enanthate weekly alone (16, 41, 42) [it was typically 13–18%], similar to the 23% fall observed in men treated with 500 μg levonorgestrel daily plus 100 mg T enanthate weekly (19) and was much greater than that when DSG was coadministered with estrogen in the female pill (43).

It is accepted that 19-nortestosterone-derived progestogens such as DSG and levonorgestrel stimulate hepatic lipoprotein lipase and decrease HDL-C (44, 45). This is generally believed to be due to their inherent androgenic properties. However, supposedly nonandrogenic 17-hydroxyprogesterone-derived progestogens such as MPA also decreased HDL-C (46) even when administered parentally. This would be more in keeping with a class effect of progestogens rather than a reflection of androgenicity or route of administration. It is interesting to note that the antigonadotropic effects of 19-norprogesterone-derived progestogens are not mediated by the androgen receptor (47). Whether the metabolic effects of progestogens are mediated via the progesterone receptor or other alternative receptor pathways is currently unclear.

Side-effects were relatively uncommon, and oral DSG was well tolerated. Sexual function in particular was not significantly impaired even with the lowest dose of T enanthate

combined with DSG. Subjects in groups 1 and 3 noticed a modest increase in acne and skin greasiness. This supports the view that 100 mg T enanthate weekly may give rise to a net increase in androgenic activity in normal men.

In summary, this study has clearly demonstrated the individual contributions to the additive actions of an oral progestogen combined with T on the hypothalamic-pituitary testicular axis. The downward dose-ranging showed that DSG at either 300 or 150 μg daily combined with low doses of T enanthate (50 or 100 mg weekly) can augment each other's action interchangeably, thereby creating an extremely effective regimen for suppressing spermatogenesis with moderate doses of both steroid. The optimal combination that induced azoospermia in all subjects was 300 μg DSG daily with 50 mg T enanthate weekly. Oral DSG, however, exerts discernible metabolic effects, the clinical significance of which is currently unclear. We conclude that the use of an oral progestogen for male contraception allows a substantial reduction in the dose of T with greater efficacy than androgen only regimens. Combination of DSG with T is a promising approach for reversible male contraception that is worthy of further investigation.

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