

Intramuscular Testosterone Enanthate Plus Very Low Dosage Oral Levonorgestrel Suppresses Spermatogenesis Without Causing Weight Gain in Normal Young Men: A Randomized Clinical Trial

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ABSTRACT: The development of a safe, well-tolerated, effective, and reversible male hormonal contraceptive would be a major clinical advance for couples planning their family size and for control of population growth. High-dosage parenteral testosterone (T) esters alone or in combination with a progestogen (eg, depot medroxyprogesterone) have been shown to confer effective and reversible male contraception in clinical trials, but these regimens are associated with weight gain and suppression of serum high-density lipoprotein cholesterol (HDL) levels. We have previously demonstrated that intramuscular T enanthate 100 mg weekly plus oral levonorgestrel (LNG) 125, 250, or 500 μg daily suppresses spermatogenesis to levels associated with effective contraception, but there is a LNG-dosage-dependent effect of weight gain and HDL suppression. We hypothesized that intramuscular T enanthate 100 mg weekly plus a very low dosage of oral LNG would effectively suppress spermatogenesis in normal men without inducing weight gain or HDL suppression. We conducted a randomized trial comparing 6 months of intramuscular T enanthate (100 mg weekly) plus 31.25 μg of oral LNG daily (T+LNG 31; $n = 20$) or 62.5 μg of oral LNG daily (T+LNG 62; $n = 21$). The 2 regimens were equally effective in suppressing spermatogenesis to azoospermia, fewer than 1 million sperm/mL and fewer than 3 million sperm/mL (T+LNG 31 [60%, 85%, and 90%] vs T+LNG 62 [62%, 91%, and 95%] for azoospermia, fewer

than 1 million and fewer than 3 million, respectively; $P = \text{NS}$). The T+LNG 31 group did not gain weight (0.25 ± 1.08 kg; $P = \text{NS}$ compared with baseline), but the T+LNG 62 group gained 2.5 ± 0.77 kg ($P < .05$ compared with baseline). Serum HDL cholesterol levels declined significantly in both groups (percentage decline month 6 of treatment vs baseline: $12.0\% \pm 2.6\%$ and $15.1\% \pm 3.0\%$; $P < .05$ for T+LNG 31 and 62 respectively). Serum low-density lipoprotein cholesterol levels also declined in both groups (percentage decline month 6 of treatment vs baseline: 6.9 ± 3.9 and $6.0\% \pm 4.1\%$; $P < .05$ for T+LNG 31 and $P = \text{NS}$ for T+LNG 62). There were no clinically significant adverse events or significant changes in hematology or chemistry profiles in either group during the study. We conclude that 1) intramuscular T plus oral LNG has a very potent synergistic effect in suppressing spermatogenesis at LNG dosages equal to or lower than dosages used in common female oral contraceptive regimens and 2) large, long-term contraceptive efficacy trials should be conducted with a variety of androgen-progestogen combinations including long-acting T formulations such as depot T pellets or intramuscular T undecanoate plus depot LNG or very low dosage oral LNG.

Key words: Contraception, gonadotropins, azoospermia, oligospermia, oligozoospermia, free testosterone.

J Androl 2005;26:405–413

The development of a safe, well-tolerated, effective, and reversible male hormonal contraceptive would be a major clinical advance. Results of multicenter clinical trials supported by the World Health Organization

(WHO)/Contraceptive Research and Development (CONRAD) program showed that an androgen-based male contraceptive regimen provided effective contraception (World Health Organization Task Force, 1990, 1996). In those studies, supraphysiological dosages of testosterone (T) enanthate (200 mg intramuscular weekly) induced azoospermia in 60%–70% and oligozoospermia (fewer than 3×10^6 spermatozoa/mL) in 98% of healthy young men. Those studies also demonstrated that suppression to persistent azoospermia or oligozoospermia resulted in effective contraception; suppression to azoospermia and fewer than 3×10^6 spermatozoa resulted in pregnancy rates of less than 1 per 100 person-years and less than 2 per 100 person-years, respectively. A more recent study of a longer acting intramuscular testosterone ester (tes-

Supported by grants from Contraceptive Research and Development (CONRAD) Program of East Virginia Medical School CSA-94-143 and NICHD/NIH through Cooperative Agreement U54 HD12629 as part of the Specialized Cooperative Centers Program in Reproduction Research and by NICHD Cooperative Agreement U54 HD 42454.

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Received for publication August 18, 2004; accepted for publication January 7, 2005.

DOI: 10.2164/jandrol.04135

tosterone undecanoate 500 mg monthly after an initial loading dose of 1000 mg) showed similar contraceptive efficacy in healthy Chinese men (Gu et al, 2003). However, exogenous supraphysiological T does not uniformly suppress spermatogenesis and causes significant weight gain and suppression of high-density lipoprotein cholesterol (HDL) levels (World Health Organization Task Force, 1990; Gu et al, 2003).

It has long been recognized that progesterone and synthetic progestogens suppress spermatogenesis (Heller et al, 1958). Because the administration of progestogens alone suppresses endogenous testosterone secretion and results in iatrogenic hypogonadism, progestogens are not viable as single agents for male contraception. However, the combination of androgens plus progestogens may act additively to uniformly suppress spermatogenesis. The additive effect of the progestogen might allow a lower dosage of the androgen and result in less androgenic-induced weight gain or HDL suppression. Several androgen plus progestogen regimens have been studied as potential male contraceptive regimens (Merigiola et al, 2003). The most extensively studied progestogens for male hormonal contraception include medroxyprogesterone acetate, cyproterone acetate, norethisterone acetate, desogestrel and its active metabolite etonogestrel, and levonorgestrel (LNG) (Anderson and Baird, 2002).

We have previously demonstrated that 6 months of T enanthate (100 mg intramuscularly [IM] weekly) plus LNG (125, 250, or 500 µg per os [PO] daily) induced oligozoospermia (fewer than 3×10^6 spermatozoa/mL) in healthy American men significantly more than the same dosage of T enanthate alone (89%–94% vs 61%; $P < .05$) (Bebb et al, 1996; Anawalt et al, 1999). Although there were no significant differences in suppression of circulating gonadotropins or achievement of azoospermia or oligozoospermia between the 3 regimens of T enanthate plus levonorgestrel, there was a dosage-dependent effect of LNG on weight gain and serum HDL suppression.

We hypothesized that intramuscular T enanthate plus a very low dosage of oral LNG would effectively suppress circulating gonadotropins and spermatogenesis without causing weight gain or HDL suppression. We therefore compared the effects of 6 months of T enanthate (100 mg IM weekly) plus LNG (31.25 or 62.5 µg PO daily) in healthy men.

Materials and Methods

Subjects

Normal men, ages 18–51 years, were recruited by advertisement on bulletin boards, in newspapers, and on the radio. Inclusion criteria were the following: a normal medical history and phys-

ical examination, normal basal serum total and calculated free T, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels, 3 successive normal seminal fluid analyses (sperm count greater than 20 million/mL and motility and oval forms greater than 50% after 48 hours of ejaculatory abstinence) on specimens collected at 2-week intervals, and normal basal hematology, chemistry, and fasting lipid profiles. Exclusion criteria included any history of significant medical illnesses, current use of prescription medications, alcohol abuse, anabolic steroid use, or medications known to affect gonadal steroid metabolism or reproductive dysfunction.

Sixty-six men were screened for the study. Eighteen of the 66 men were excluded from the study before entering the treatment phase. Ten men were excluded for medical reasons (8 men for low sperm counts, 1 man for abnormal liver function tests, and 1 man for newly diagnosed Klinefelter syndrome). Prior to the treatment phase of the study, 6 men were removed from the study because they did not keep their follow-up appointments, and 2 men opted to discontinue the study for personal reasons.

Experimental Design

After meeting the screening criteria, subjects were entered into a 3-month control period. During this control period, monthly baseline hormone levels and biweekly seminal fluid analyses were performed. At the end of the control period, 48 subjects were assigned to 1 of 2 groups: 6 months of T+LNG 31 (T enanthate [Schein Pharmaceuticals, Florham Park, NJ] 100 mg IM weekly plus LNG 31.25 µg PO daily; $n = 24$) or T+LNG 62 (T enanthate 100 mg IM weekly plus LNG 62.5 µg PO daily; $n = 24$). To ensure unbiased balance between the 2 intervention groups, the subjects were allocated in a restricted randomization using the minimization method (ie, “randomization with a balanced design”) with a random-number generator computer program (Altman et al, 2001). A statistician created the randomization list, which was then distributed to the research coordinator and pharmacist. The allocation was concealed from the subjects and investigators until the end of treatment. The LNG (Wyeth Ayerst, Philadelphia, Pa) was formulated in identical capsules that ensured that the dosage was concealed from the subjects and researchers. A research nurse or one of the investigators administered all of the T injections. We recorded weekly pill counts and injections. Subjects who missed more than 1 weekly injection or more than 10% of pills in any month were counted as protocol violations and were discontinued from the treatment phase. Following the 6-month treatment period, all subjects entered a recovery period that extended until 2 consecutive sperm concentrations were within the individual’s baseline (pretreatment) range.

The University of Washington Human Subjects Review Committee and the Veterans Affairs Puget Sound Health Care System Research and Development Committee reviewed and approved our study. All subjects signed a consent form reviewing potential side effects before enrollment in the study.

Measurements

Physicians interviewed and examined all subjects monthly throughout the study. Seminal fluid analysis was performed every 2 weeks on samples obtained by masturbation after 48 hours

of ejaculatory abstinence. Throughout the study, monthly blood samples were obtained for measurement of serum total and calculated free T, sex hormone binding globulin (SHBG), FSH, and LH levels. During the treatment phase, blood samples were drawn immediately before the administration of intramuscular T enanthate. During the treatment phase, additional blood samples were drawn monthly immediately before and 1 hour after ingestion of LNG to measure nadir and peak LNG levels. During control, months 3 and 6 of treatment, and during recovery, we measured complete blood counts, serum electrolytes, creatinine, and albumin and administered liver function tests and a fasting lipid panel. The fasting lipid panel was performed on serum obtained after a 12-hour fast. The lipid panel included measurement of serum total, HDL, HDL₂, HDL₃, and low-density lipoprotein (LDL) cholesterol levels and serum triglyceride levels.

Hormone and Lipid Assays

We measured serum SHBG, total T, FSH, and LH levels by immunofluorometric assay (Delfia, Wallac Inc, Turku, Finland). The lower limit of quantification of SHBG was determined to be 0.2 nmol/L for SHBG; the intra-assay and interassay coefficients of variation (CV) were 1.7% and 10.6% for midrange pooled values, respectively. The lower limit of quantification of total T was 0.35 nmol/L; intra-assay and interassay coefficient of variation were 4.5% and 9.5% for midrange pools, respectively. The lower limit of quantification of FSH was 0.016 IU/L; the intra-assay and interassay coefficient of variation were 12% and 22.3%, respectively, for a low-range pooled value of 0.045 IU/L, and these coefficients were 2.9% and 6.1% for a midrange pooled value of 0.96 IU/L. Free T was calculated based on serum total T, SHBG, and albumin levels (Vermeulen et al, 1999). The lower limit of quantification of LH was 0.019 IU/L; the intra-assay and interassay coefficient of variation were 6.5% and 17.7% for a low-range pooled LH value of 0.074 IU/L, and these coefficients were 3.2% and 12.5% for a midrange pooled value of 1.15 IU/L. LNG levels were determined by radioimmunoassay at the California Regional Primate Center (courtesy of Dr Lisa Laughlin, University of California, Davis) (Ahsan et al, 1988). Samples from each participant were run in duplicate in the same assay to avoid interassay variability. We determined the lower limit of quantification of hormone assays by the first point discernible from zero on standard curves.

Lipid analyses were performed on freshly prepared (never frozen) plasma samples that were processed within hours of collection. Total plasma cholesterol and triglycerides were measured enzymatically at the Northwest Lipid Research Clinic (Seattle, Wash) on the Roche Hitachi 917 (Roche Laboratories, Indianapolis, Ind). Cholesterol was measured by a Trinder-type method, and triglycerides were measured by an ultraviolet light method (Warnick, 1986). Total HDL and HDL₂ cholesterol was measured directly after separation from plasma by precipitation with a modified dextran-sulfate technique (Warnick et al, 1982). HDL₃ cholesterol and LDL cholesterol levels were calculated (Warnick et al, 1982).

Seminal Fluid Analysis

We determined sperm counts by Coulter counter (Coulter Electronics Inc, Hialeah, Fla), and concentrations below 15 million/

mL were confirmed by direct determination using a hemocytometer (Gordon et al, 1965; Bremner et al, 1981). Sperm motility assessment was performed according to the WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction (WHO, 1999). We defined azoospermia, severe oligozoospermia, and oligozoospermia as 2 or more consecutive sperm counts of zero, fewer than 1 million/mL, and fewer than 3 million/mL, respectively. We defined sperm recovery as the first of 3 normal sperm counts (more than 20 million/mL) with at least 1 value greater than or equal to the subject's mean baseline concentration.

Statistical Analysis

Our primary outcomes were comparing the effects of the 2 regimens on effectiveness of spermatogenic suppression, weight gain, and HDL suppression. Secondary outcomes included change in serum hormones, change in hematocrit, and the incidence of gynecomastia or acne. The study was powered to detect a significant difference between the 2 groups if 60% of 1 group and 90% of the other became azoospermic or oligospermic. Comparisons between groups at the end of control, treatment, and recovery were made with paired *t* tests. Comparison within groups over time was analyzed by ANOVA with a Duncan post hoc test. For the total cholesterol data where there were baseline differences between the 2 groups, we performed a comparison by analysis of covariance (ANCOVA). Two-sided *P* values of less than .05 were considered significant. All results are expressed as mean values \pm SEM.

Results

Baseline Characteristics

The 2 groups were similar in all of their baseline clinical and biochemical characteristics except for serum total cholesterol level, which was significantly higher in the T+LNG 31 group (Table 1). Although baseline sperm concentrations appeared to be higher in the T+LNG 62 group, this difference did not achieve statistical significance (*P* = .11; Figure 1). Of the 48 men who entered the study, 44 were white, 3 were Native Americans, and 1 was African American. Forty men completed the entire study. One subject in the T+LNG 62 group completed 5 months of treatment and then moved out of the state, but he completed the recovery period. He became azoospermic by 7 weeks in the treatment period, and his data were included in the analysis. Seven men discontinued early in the treatment period (less than 4 months), and they were excluded from the analysis. The reasons for discontinuation of these 7 men included the following: 1 man reported scrotal pain from a pre-existing epididymal cyst, 1 man developed very mild acne, 2 men discontinued for personal reasons, and 3 men were removed from the study because of noncompliance with clinic visits and medication administration.

Table 1. Baseline clinical and biochemical parameters (mean \pm SEM)*

Characteristic	T+LNG 31 (n = 20)	T+LNG 62 (n = 21)
Age, y	34 \pm 2	32 \pm 2
Weight, kg	82.7 \pm 2.3	84.2 \pm 2.7
BMI	26.6 \pm 0.7	26.6 \pm 0.7
Total testosterone, nmol/L	18.0 \pm 1.3	16.3 \pm 1.3
Free testosterone, nmol/L	0.4 \pm 0.03	0.4 \pm 0.03
SHBG, nmol/L	27.2 \pm 2.6	24.4 \pm 2.3
FSH, IU/L	3.4 \pm 0.4	3.5 \pm 0.4
LH, IU/L	3.7 \pm 0.3	3.8 \pm 0.5
Total cholesterol, mg/dL	198.4 \pm 5.7†	176.3 \pm 6.8
LDL cholesterol, mg/dL	127.4 \pm 5.7	110.7 \pm 6.3
HDL cholesterol, mg/dL	51.7 \pm 2.4	45.6 \pm 2.7
Triglycerides, mg/dL	94.3 \pm 10.5	102.7 \pm 12.8

* T indicates testosterone; LNG, levonorgestrel; BMI, body mass index; SHBG, sex hormone binding globulin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; LDL, low-density lipoprotein; and HDL, high-density lipoprotein.

† $P < .05$ compared to T+LNG 62; conversion factor to SI units for total, LDL, HDL cholesterol = 0.259 and for triglycerides = 0.0113.

Response to Treatment

Sperm Counts—By 4 weeks of therapy, sperm concentrations were suppressed significantly in both groups (Figure 1). There were no significant differences in overall rates or rapidity of onset of azoospermia, severe oligozoospermia, or oligozoospermia between the T+LNG 31 and T+LNG 62 groups (Table 2). There was no significant difference in the rate of recovery between the 2 groups. Sperm counts returned to baseline during the recovery period in 40 out of 41 men. The 1 subject whose sperm counts did not return to baseline suffered testicular trauma (unrelated to the study) during the recovery period and underwent unilateral orchidectomy. His sperm concentration peaked at 40 million/mL during recovery compared with a pretreatment baseline range of 50–120 million/mL.

Hormones—During treatment, serum total and calculated free T rose significantly in both groups while SHBG levels declined significantly (Figure 2). In both groups, serum FSH and LH levels were significantly suppressed by the end of the first month of treatment and remained suppressed throughout the treatment period (Figure 3). During treatment, serum LH levels were suppressed to or below the lower limit of quantification in the majority of

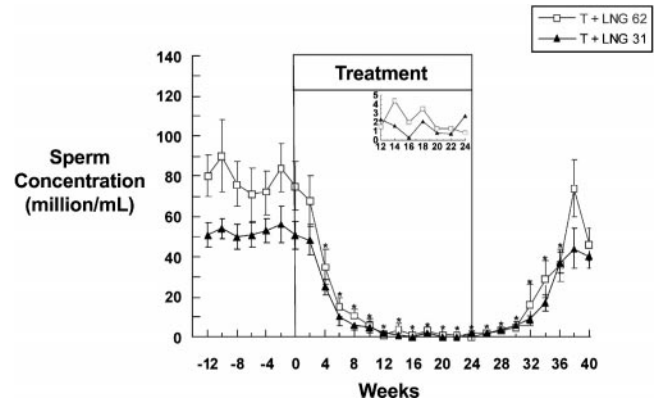


Figure 1. Intramuscular testosterone (T) enanthate plus very low dosage oral levonorgestrel (LNG) regimens suppressed spermatogenesis rapidly ($P < .05$ by 4 weeks compared with baseline). There were no differences in rapidity or completeness of spermatogenic suppression between the T+LNG 31 (n = 20) and T+LNG 62 (n = 21) groups. Insert depicts sperm concentrations during the last 12 weeks of treatment; a different scale is used for the ordinate (0–5 million/mL) to clearly depict the absolute concentrations when sperm concentrations are very low during the last half of the treatment period.

subjects in both groups, but FSH levels were detectable in all but 5 subjects (2 in the T+LNG 31 group and 3 in the T+LNG 62 group) at the end of the treatment period. Serum total and calculated free T and serum FSH and LH levels were not significantly different between T+LNG 31 and 62 groups during control, treatment, or recovery ($P = NS$).

Nadir and peak LNG levels were significantly higher in the T+LNG 62 group than the T+LNG 31 group at month 6 of treatment (T+LNG 62 vs T+LNG 31 nadir and peak at treatment month 6: 0.08 ± 0.02 and 0.25 ± 0.04 $\mu\text{g/L}$ vs 0.06 ± 0.08 and 0.13 ± 0.14 $\mu\text{g/L}$; $P < .05$).

We compared men who became azoospermic vs those who did not during treatment with either regimen and found no significant differences in total and free T, SHBG, FSH, and LH levels at baseline, end of treatment, or percentage change from baseline.

Lipids—During treatment and recovery, there were no significant differences in lipid parameters between the T+LNG 31 and 62 groups. Total cholesterol declined significantly by 8%–9% during treatment in both groups (percentage decline month 6 of treatment vs control: 9.1%

Table 2. Effectiveness in suppressing sperm counts to levels associated with effective male contraception

Outcome (mean \pm SEM)	T+LNG 31 (n = 20)*	T+LNG 62 (n = 21)
% Azoospermia	60	62
Weeks to azoospermia	13.2 \pm 0.9	13.5 \pm 1.5
% Fewer than 3 million sperm/mL	85	91
Weeks to fewer than 3 million sperm/mL	8.9 \pm 0.7	10.1 \pm 1.0
% Oligozoospermia	90	95
Weeks to fewer than 1 million sperm/mL	8.2 \pm 0.8	9.1 \pm 1.0

* T indicates testosterone; LNG, levonorgestrel.

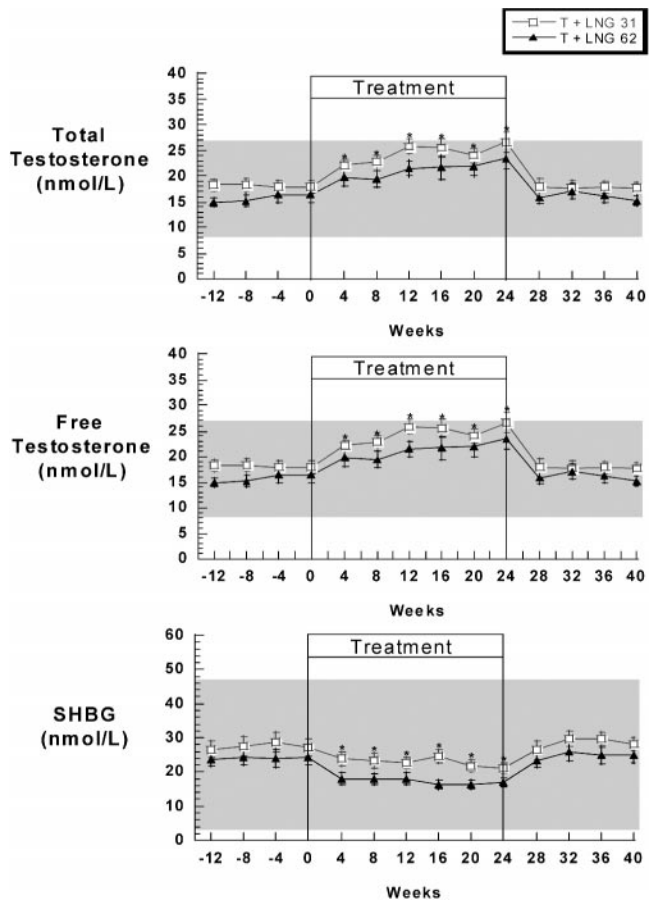


Figure 2. Intramuscular testosterone (T) enanthate plus very low dosage oral levonorgestrel (LNG) raised total testosterone levels significantly and suppressed sex hormone binding globulin (SHBG) levels significantly by 4 weeks of treatment ($P < .05$ compared with baseline for both groups). Calculated free testosterone levels were significantly higher than baseline by the end of 4 weeks of treatment and nearly doubled by the end of 24 weeks of treatment ($P < .05$ for both groups). There were no significant differences between the T+LNG 31 ($n = 20$) and T+LNG 62 ($n = 21$) groups. The shaded area represents the normal range of the hormone assays.

$\pm 2.6\%$ and $8.4\% \pm 2.9\%$; $P < .05$ for T+LNG 31 and 62 respectively). More than half of the absolute decrease in total cholesterol was due to a decline in LDL cholesterol (Table 3); LDL declined by 6%–7% in both groups (percentage decline month 6 of treatment vs control: $6.9\% \pm 3.9\%$ and $6.0\% \pm 4.1\%$; $P < .05$ for T+LNG 31 and $P = NS$ for T+LNG 62). HDL cholesterol declined significantly by 12%–15% during treatment in both groups (percentage decline month 6 of treatment vs control: $12.0\% \pm 2.6\%$ and $15.1\% \pm 3.0\%$; $P < .05$ for T+LNG 31 and 62). Most of the absolute decrease in HDL cholesterol was due to a decline in HDL₃ (Table 3). Although HDL₂ was unchanged in the T+LNG 31 group and tended to decline in the T+LNG 62 group (percentage decline month 6 of treatment vs control: $0.1\% \pm 8.9\%$ and $13.6\% \pm 6.6\%$; $P = NS$ for T+LNG 31 and $P = .06$ for T+LNG

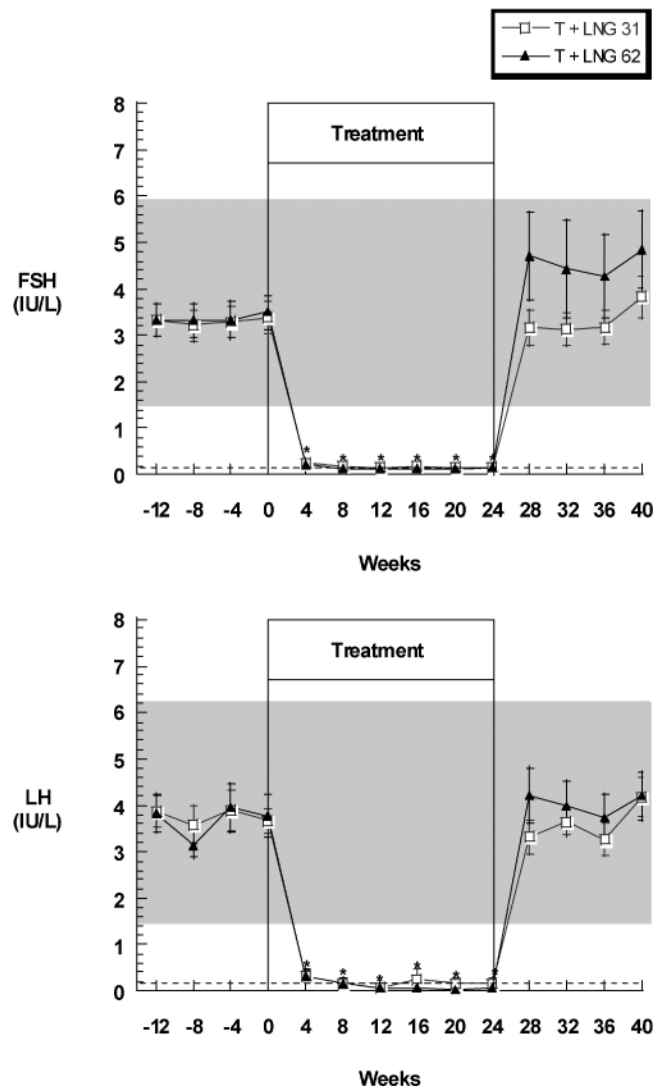


Figure 3. Intramuscular testosterone (T) enanthate plus very low dosage oral levonorgestrel (LNG) rapidly suppressed circulating gonadotropins by the end of 4 weeks of therapy ($P < .05$ compared with baseline). There were no significant differences between the T+LNG 31 ($n = 20$) and T+LNG 62 ($n = 21$) groups. Dashed line indicates limit of quantification of each assay, and the shaded area represents the normal range of each assay.

62), HDL₃ levels decreased significantly in both groups (percentage decline month 6 of treatment vs control: $12.7\% \pm 2.9\%$ and $13.1\% \pm 2.7\%$; $P < .05$ for T+LNG 31 and T+LNG 62). Apoprotein A-1 levels declined significantly in both groups (percentage decline month 6 of treatment vs control: $11.0\% \pm 2.5\%$ and $13.8\% \pm 2.5\%$; $P < .05$ for T+LNG 31 and T+LNG 62), but serum triglyceride levels were unchanged in both groups (percentage increase month 6 of treatment vs control: $7.3\% \pm 13.2\%$ and $19.1\% \pm 11.6\%$; $P = NS$ for T+LNG 31 and T+LNG 62). All lipid parameters normalized within 3 months during the recovery period.

Hematological and Chemistry Profiles—Hematological

Table 3. Absolute concentrations in fasting lipid parameters, mg/dL*

Group/Lipid Parameter	Control Month	Treatment (Month 6)
LNG 31		
Total cholesterol†	198.4 ± 5.7‡	179.8 ± 6.7§
LDL cholesterol	127.4 ± 5.7	117.3 ± 5.9§
HDL cholesterol	51.7 ± 2.4	45.0 ± 2.1§
HDL ₂	8.6 ± 1.0	7.7 ± 0.8
HDL ₃	43.1 ± 1.6	37.3 ± 1.7§
Apo-AI	141.4 ± 3.6	125.8 ± 4.9
Triglycerides	94.3 ± 10.5	85.3 ± 7.9
LNG 62		
Total cholesterol	176.3 ± 6.8	161.5 ± 8.0§
LDL cholesterol	110.7 ± 6.3	101.8 ± 6.4
HDL cholesterol	45.6 ± 2.7	37.7 ± 1.8§
HDL ₂	7.1 ± 1.0	5.1 ± 0.4§
HDL ₃	38.5 ± 1.9	32.95 ± 1.5§
Apo-AI	129.4 ± 4.5	110.4 ± 3.7
Triglycerides	98.5 ± 14.6	102.7 ± 12.8

* LNG indicates levonorgestrel; LDL, low-density lipoprotein; and HDL, high-density lipoprotein.

† Conversion factor to SI units for total, LDL, HDL cholesterol = 0.259 and for triglycerides = 0.0113.

‡ $P < .05$ compared with T+LNG 62.

§ $P < .05$ compared with baseline.

and chemistry profiles including liver function tests did not change significantly from baseline during the treatment and recovery periods.

Weight, Testicular Size, Digital Rectal Prostate Exam, Gynecomastia, and Acne—Although subjects in the T+LNG 62 group gained a significant amount of weight during treatment ($2.50 \text{ kg} \pm 0.77$; $P < .05$), subjects in the T+LNG 31 group did not ($0.25 \pm 1.08 \text{ kg}$; $P = \text{NS}$). By the end of the recovery phase, mean weights were not significantly different from baseline for either group. Testicular size shrank significantly in both groups, but shrank more in the T+LNG 62 group (percentage decrease month 6 of treatment vs control: T+LNG 31; left testis $15.6\% \pm 4.4\%$ and right testis $23.4\% \pm 3.0\%$ vs T+LNG 62 left testis $29.0\% \pm 2.9\%$ and right testis $32.1\% \pm 2.6\%$; $P < .05$ vs baseline and between groups). None of the subjects spontaneously commented on or complained about testicular shrinkage during the study.

We found no significant abnormalities in the digital rectal examination at baseline, the end of treatment, or control periods. There was no clear relationship between the study drugs and development of acne. Four subjects in the T+LNG 31 group, and 6 subjects in the T+LNG 62 group developed acne during the treatment phase. Three subjects in the T+LNG 31 and 5 subjects in the T+LNG 62 had mild baseline acne that resolved during the treatment phase. No subjects requested or received treatment for acne during the study. As previously noted, 1 subject discontinued early in the study due to very mild acne. No subjects complained of sleep disturbances during the study. No subjects developed symptomatic gynecomastia

during the study; 1 subject in the T+LNG 62 group developed asymptomatic gynecomastia (defined as an increase of breast tissue diameter greater than 2 cm) that was detected by one of the investigators, but the subject had not noticed any abnormalities.

Discussion

In this study, we demonstrated that the combination of weekly intramuscular T enanthate plus very low dosages of daily oral LNG suppressed spermatogenesis to levels associated with effective contraception (World Health Organization Task Force, 1990, 1996). Both regimens caused similar rates of oligoazoospermia, severe oligoazoospermia, and azoospermia compared with the same dosage of T enanthate (100 mg weekly) plus higher dosages up to 500 μg of LNG daily in studies of young healthy men that we performed using similar protocols to the present study (Bebb et al, 1996; Anawalt et al, 1999). In the current study, the combinations of T enanthate 100 mg weekly plus 31.25 or 62.5 μg of LNG daily were also significantly superior in achieving oligoazoospermia, severe oligoazoospermia, and azoospermia than the historical control of healthy young men administered the same dosage of T enanthate (100 mg weekly) alone in a similar protocol (Bebb et al, 1996) to the current study (T+LNG 31 or 62 vs T alone: oligoazoospermia 90%–95% vs 60%; severe oligoazoospermia 85% vs 56%; azoospermia 61% vs 33%; $P < .05$). In addition, the rates of spermatogenic suppression compare favorably with the results seen in the WHO efficacy trials that used a dosage of T enanthate that was twice the dosage in our current study (World Health Organization Task Force, 1990, 1996).

In the current study, we reduced the dosage of levonorgestrel to a dosage equal to or less than those used in common female oral combination contraceptives (Boonstra et al, 2000; Audet et al, 2001), but still induced oligoazoospermia (fewer than 3 million/mL) in 90%–95% of normal healthy young men. Our data showing spermatogenic suppression that is greater than the same dosage of T enanthate alone and equivalent to spermatogenic suppression with twice the dosage of T enanthate demonstrate the potent synergistic effect of intramuscular T plus oral LNG in suppressing spermatogenesis with very low dosages of oral LNG (31.25 or 62.5 μg of LNG daily) being equally effective as dosages more than 10-fold greater (125–500 μg of LNG daily).

Although there was no difference in spermatogenic suppression between the 2 groups, the T+LNG 62 group gained 2.5 kg ($P < .05$ compared with baseline) while the T+LNG 31 did not gain weight during the 6-month treatment period. This dose-dependent effect of oral LNG on weight gain in a LNG-T contraceptive regimen is con-

sistent with our earlier findings (Anawalt et al, 1999). Most male hormonal contraceptive regimens have been associated with adverse metabolic effects on weight and lipid profiles (Anderson and Baird, 2002). Our findings showing that the combination of T plus very low dosage of LNG is as effective as T plus higher dosages of LNG while causing less weight gain may apply to other androgen-progestogen combinations. Our study highlights the importance of careful dose-finding studies of promising androgen-progestogen male contraceptive regimens to ensure the safest dosage while maintaining effective spermatogenic suppression.

Both intramuscular T enanthate plus 31.25 μg and 62.5 μg oral LNG regimens suppressed serum HDL cholesterol significantly by 12%–15% ($P = \text{NS}$ between groups). Most of the suppression of serum HDL cholesterol was due to a decline in HDL₃. Both serum HDL₂ and HDL₃ cholesterol levels are inversely associated with atherosclerotic disease, but HDL₃ cholesterol may be particularly important for reverse cholesterol transport (Salter and Brindley, 1988; Stampfer et al, 1991). The potentially unfavorable suppression of serum HDL levels was at least partially offset by a decline in LDL cholesterol. Both dosages of oral daily LNG plus intramuscular T enanthate were otherwise well tolerated. No clinically significant hematological or biochemical abnormalities occurred during treatment. No subjects developed symptomatic sleep disturbance or sleep apnea. There were no adverse effects on prostate or skin, and only 1 subject developed mild, asymptomatic gynecomastia.

The route of administration of testosterone significantly affects the degree of spermatogenic suppression induced by T+LNG regimens. A study of a transdermal testosterone patch system plus a higher dosage of oral LNG (250–500 μg daily) yielded much lower rates of oligoazoospermia (44%) than our study (Buchter et al, 1999). It is likely that this difference in spermatogenic suppression is due to the greater amount of testosterone delivered by intramuscular T enanthate with higher peak and more sustained total and free T levels. Another male contraceptive study that included a comparison of intramuscular T or T patch plus LNG implants confirmed that intramuscular T+LNG more effectively induces azoospermia (93% vs 35%) and oligoazoospermia (100% vs 50%) compared with T patch plus LNG (Gonzalo et al, 2002). Finally, a study of a long-acting formulation of intramuscular T undecanoate plus oral LNG 250 μg daily caused oligoazoospermia in 14 out of 14 men (Kamischke et al, 2000). Thus, sustained high or high-normal levels of T achieved by intramuscular T esters such as T enanthate or T undecanoate are superior to T patch systems for spermatogenic suppression.

It is less clear that the route of administration of LNG has important effects on the degree of spermatogenic sup-

pression achieved by T+LNG regimens although oral administration of LNG might cause more HDL suppression by a first-pass hepatic effect. A study of monthly intramuscular T undecanoate plus levonorgestrel implants (Sino-implant) at a dosage of 150 mg for 3 months yielded disappointing results with less than 50% (7/16) of the Chinese subjects becoming oligoazoospermic (Gao et al, 1999). There were no significant changes in serum lipid parameters including HDL cholesterol in the Sino-implant study. However, the study was very short and the dosage of intramuscular T undecanoate (250 mg monthly) was probably suboptimal. In a study by Gonzalo et al, T enanthate 100 mg IM weekly plus subcutaneous LNG (Norplant II) implants 300 mg for 6 months caused severe oligoazoospermia (less than 1 million sperm/mL) in 14 out of 14 men (Gonzalo et al, 2002). As with our results with intramuscular T enanthate plus very low dosage oral LNG, there was no weight gain, but there was approximately 12% suppression of serum HDL cholesterol in subjects administered intramuscular T enanthate plus Norplant II. The LNG levels (approximately 0.4 $\mu\text{g/L}$) were much higher in the study of men administered subcutaneous Norplant II than the LNG levels (peak approximately 0.13 $\mu\text{g/L}$ and nadir approximately 0.06 $\mu\text{g/L}$) in the group of men receiving 31.25 μg of daily oral LNG in our study. In summary, the data from our studies and others suggest that there is a dosage-dependent effect of LNG on weight and to a lesser degree on HDL suppression, but there is also likely to be an important first-pass hepatic effect of oral LNG on HDL suppression. In our study, we have shown that this first-pass hepatic effect of HDL suppression occurs at very low dosages of oral LNG. It is possible that a lower dosage of LNG implant plus intramuscular testosterone ester could result in less HDL suppression (because of lower levels of intrahepatic LNG) while still effectively inducing uniform suppression of spermatogenesis and no weight gain.

There are several potential explanations for nonuniform suppression of spermatogenesis in our study. In 1 study of men administered transdermal T by patch plus oral LNG, men with higher baseline T and SHBG levels and higher sperm counts appeared to be less likely to develop oligoazoospermia (Buchter et al, 1999). In another study of men administered intramuscular T undecanoate plus LNG implant (Sino-implant), men who suppressed to severe oligoazoospermia had higher baseline LH levels and lower FSH levels than nonresponders (Liu et al, 2004). However, we did not find any differences in total or calculated free T, SHBG, FSH, or LH at baseline or treatment between those men who suppressed to azoospermia or oligoazoospermia and those who did not. One likely explanation of nonuniform achievement of azoospermia in our study of intramuscular T enanthate plus oral LNG is inadequate suppression of serum FSH (McLachlan et

al, 2004). While treatment serum LH levels were suppressed to or below the lower limit of quantification in the majority of subjects, treatment FSH levels were detectable in all but 5 subjects.

Several studies have demonstrated the synergistic effects of androgens plus progestogens for the suppression of circulating gonadotropins and spermatogenesis. Many androgen-progestogen regimens have been shown to achieve very high rates of severe oligozoospermia or azoospermia in small, short trials, and these combination regimens could provide safe, effective, and reversible male hormonal contraception. These regimens include the combination of subcutaneous T pellets plus either depot medroxyprogesterone, depot etonogestrel, or oral desogestrel or the combinations of intramuscular T undecanoate plus oral cyproterone acetate or norethindrone (oral or injectable) (Anderson et al, 2002a,b; Kamischke et al, 2002; Meriggiola et al, 2003b; Turner et al, 2003). Testosterone enanthate, the injectable T formulation used in our study, is an unattractive androgen formulation because it requires weekly injections. However, injectable T undecanoate, a long-acting T ester formulation that was not available in the United States when we initiated our study, may be injected intramuscularly at intervals as long as every 6–12 weeks and is thus convenient enough to be feasible in an androgen-based male hormonal contraceptive. Long-acting injectable formulations of an androgen plus a progestogen are attractive first candidates for a male hormonal contraceptive in part because these formulations are more likely to ensure greater compliance and greater contraceptive efficacy. The data from our study and the previous study of Norplant plus T enanthate suggest that a long-acting T formulation such as subcutaneous T pellets or intramuscular T undecanoate plus a lower dosage of LNG implant (eg, 150 mg q 6 months) might provide safe and effective contraception.

Although long-acting depot formulations of T plus progestogens appear to be the most likely male hormonal contraceptive regimens to be available soon, depot implantable formulations such as T pellets, Norplant II, or Sino-implant require a trained operator to implant. Therefore, regimens that could be self-administered or administered without special training will also be desirable. Like the combinations of long-acting intramuscular T undecanoate plus oral desogestrel, norethindrone, or cyproterone acetate, long-acting intramuscular T undecanoate plus very low dosage oral LNG appears to be an attractive contraceptive option that could be easily self-administered.

Although androgen-progestogen combinations show promise as safe, effective, and reversible contraceptive regimens, the only androgen-progestogen combination to be studied in a male contraceptive efficacy trial is implantable, long-acting T pellets plus depot medroxypro-

gesterone. This combination has recently been shown to provide safe and effective short-term contraception but is associated with weight gain (Turner et al, 2003). Women currently have many hormonal contraceptive options that offer them freedom of choice, convenience, and alternative when 1 option causes intolerable side effects. The time has come for men to have a similar broad range of hormonal contraceptive choices. Large, long-term clinical contraceptive efficacy trials should be conducted with a variety of androgen-progestogen combinations including long-acting T formulations such as depot T pellets or intramuscular T undecanoate plus depot LNG or very low dosage oral LNG.

Acknowledgments

We gratefully acknowledge the gift of levonorgestrel from Wyeth Ayerst, technical assistance from Dorothy McGuinness and Arlen Sarkissian, and administrative assistance from Connie Nosbisch, Rebecca Brotman, Jenny Bullock, Elaine Rost, Connie Pete, and Kathy Winters.

References

- Ahsan R, Sufi SB, Cekan S. *Laboratory Method Manual for the Radioimmunoassay of Levonorgestrel, Norethisterone, Norethisterone and Medroxyprogesterone Acetate*. London: World Health Organization Collaborating Centre for Research and Reference Services in the Immunoassay for Hormones in Human Reproduction; 1988.
- Altman DG, Schulz KF, Moher D, Egger M, Davidoff F, Elbourne D, Gøtzsche PC, Lang T. The revised CONSORT statement for reporting randomized trials: explanation and elaboration. *Ann Intern Med*. 2001; 134:663–694.
- Anawalt BD, Bebb RA, Bremner WJ, Matsumoto AM. A lower dosage levonorgestrel and testosterone combination effectively suppresses spermatogenesis and circulating gonadotropin levels with fewer metabolic effects than higher dosage combinations. *J Androl*. 1999;20: 407–414.
- Anderson RA, Baird DT. Male contraception. *Endocrine Rev*. 2002;23: 735–762.
- Anderson RA, Kinniburgh D, Baird DT. Suppression of spermatogenesis by etonogestrel implants with depot testosterone: potential for long-acting contraception. *J Clin Endocrinol Metab*. 2002a;87:3640–3649.
- Anderson RA, Van Der Spuy ZM, Dada OA, Tregoning SK, Zinn PM, Adenjii OA, Fakoya TA, Smith KB, Baird DT. Investigation of hormonal male contraception in African men: suppression of spermatogenesis by oral desogestrel with depot testosterone. *Hum Reprod*. 2002b;17:2869–2877.
- Audet MC, Moreau M, Kotun WD, Waldbaum AS, Shangold G, Fisher AC, Creasy GW. Evaluation of contraceptive efficacy and cycle control of a transdermal contraceptive patch vs an oral contraceptive. *JAMA*. 2001;285:2347–2354.
- Bebb RA, Anawalt BD, Christensen RB, Paulsen CA, Bremner WJ, Matsumoto AM. Combined administration of levonorgestrel and testosterone induces more rapid and effective suppression of spermatogenesis than testosterone alone: a promising male contraceptive approach. *J Clin Endocrinol Metab*. 1996;81:757–762.
- Boonstra H, Duran V, Northington Gamble V, Blumenthal P, Dominguez L, Pies C. The “Boom or Bust Phenomenon”: the hopes, dreams and broken promises of the contraceptive revolution. *Contraception*. 2000; 61:9–25.

- Bremner WJ, Matsumoto AM, Sussman AM, Paulsen CA. Follicle-stimulating hormone and human spermatogenesis. *J Clin Invest*. 1981;68:1044–1052.
- Buchter D, von Eckardstein S, von Eckardstein A, Kamischke A, Simoni M, Behre H, Nieschlag E. Clinical trial of transdermal testosterone and oral levonorgestrel for male contraception. *J Clin Endocrinol Metab*. 1999;84:1244–1249.
- Gao E, Lin C, Gui Y, Li L, He C. Inhibiting effects of Sino-implant plus testosterone undecanoate on spermatogenesis in Chinese men. *Reprod Contracept*. 1999;10:98–105.
- Gonzalo IT, Swerdloff RS, Nelson AL, Clevenger B, Garcia R, Berman N, Wang C. Levonorgestrel implants (Norplant II) for male contraception clinical trials: combination with transdermal and injectable testosterone. *J Clin Endocrinol Metab*. 2002;87:3562–3572.
- Gordon DL, Moore DL, Thorslund T, Paulsen CA. The determination of size and concentration of human sperm with an electronic particle counter. *J Lab Clin Med*. 1965;65:506–512.
- Gu Y-Q, Wang X-H, Xu D, Peng L, Cheng L-F, Huang M-K, Huang Z-J, Zhang G-Y. A multicenter contraceptive efficacy study of injectable testosterone undecanoate in healthy Chinese men. *J Clin Endocrinol Metab*. 2003;88:562–568.
- Heller CG, Laidlaw WM, Harvey HT, Nelson WO. Effects of progestational compounds on the reproductive processes of the human male. *Ann NY Acad Sci*. 1958;71:649–665.
- Kamischke A, Heuermann T, Krüger K, von Eckardstein S, Schellschmidt I, Rübiger A, Nieschlag E. An effective hormonal male contraceptive using testosterone undecanoate with oral or injectable norethisterone preparations. *J Clin Endocrinol Metab*. 2002;87:530–539.
- Kamischke A, Plöger D, Venherm S, von Eckardstein S, von Eckardstein A, Nieschlag E. Im testosterone undecanoate with or without oral levonorgestrel: a randomized placebo-controlled feasibility study for male contraception. *Clin Endocrinol*. 2000;53:43–52.
- Liu ST, Gui YL, Lin CH, He CH. Hormonal contraception in Chinese men: variations in suppression of spermatogenesis with injectable testosterone undecanoate and levonorgestrel implants. *Asian J Androl*. 2004;6:41–46.
- McLachlan RI, Robertson DM, Pruyers E, Ugoni A, Matsumoto AM, Anawalt BD, Bremner WJ, Meriggiola C. Relationship between serum gonadotropins and spermatogenic suppression in men undergoing steroidal contraceptive treatment. *J Clin Endocrinol Metab*. 2004;89:142–149.
- Meriggiola MC, Farley TMM, Mbizvo MT. A review of androgen-progestin regimens for male contraception. *J Androl*. 2003a;24:466–482.
- Meriggiola MC, Costantino A, Cerpolini S, et al. Testosterone undecanoate maintains spermatogenic suppression induced by cyproterone acetate plus testosterone undecanoate in normal men. *J Clin Endocrinol Metab*. 2003b;88:5818–5826.
- Salter AM, Brindley DN. The biochemistry of lipoproteins. *J Inherit Metab Dis*. 1988;11(suppl 1):4–17.
- Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N Engl J Med*. 1991;325:373–381.
- Turner L, Conway AJ, Jimenez M, Liu PY, Forbes E, McLachlan RI, Handelsman DJ. Contraceptive efficacy of a depot progestin and androgen combination in men. *J Clin Endocrinol Metab*. 2003;88:4659–4667.
- Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab*. 1999;84:3666–3672.
- Warnick GR. Enzymatic methods for the quantification of lipoprotein lipids. *Methods Enzymol*. 1986;129:101–123.
- Warnick GR, Benderson J, Albers JJ. Dextran sulfate Mg^{2+} precipitation procedure for quantification of high-density lipoprotein cholesterol. *Clin Chem*. 1982;28:1379–1388.
- World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*. 4th ed. Cambridge, United Kingdom: Cambridge University Press; 1999.
- World Health Organization Task Force on Methods for the Regulation of Male Fertility. Contraceptive efficacy of testosterone-induced azoospermia in normal men. *Lancet*. 1990;336:955–959.
- World Health Organization Task Force on Methods for the Regulation of Male Fertility. Contraceptive efficacy of testosterone-induced azoospermia and oligozoospermia in normal men. *Fertil Steril*. 1996;65:821–829.