Establishing the Minimum Effective Dose and Additive Effects of Depot Progestin in Suppression of Human Spermatogenesis by a Testosterone Depot*

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ABSTRACT

Hormonally induced azoospermia induced by weekly intramuscular injections of testosterone enanthate provides effective and reversible male contraception, but more practical regimens are needed. Given our previous findings that six 200-mg pellets implanted subdermally produced more stable, physiological testosterone levels and reduced the delivered testosterone dose by more than 50% while maintaining equally effective suppression of sperm output with fewer metabolic side-effects than weekly 200-mg testosterone enanthate injections, we sought in this study to determine 1) whether further dose-sparing could be achieved by lower testosterone doses while maintaining efficacy and 2) the efficacy of adding a depot progestin to a suboptimally suppressive testosterone dose as a model depot progestin/androgen combination male contraceptive. Healthy volunteers were randomized into groups (n = 10) who received either of two lower testosterone doses (two or four 200-mg T pellets) or four 200-mg T pellets plus a single intramuscular injection of 300 mg depot medroxyprogesterone acetate (DMPA). Two T pellets (400 mg, 3 mg/day) had a negligible effect on sperm output. Four T pellets (800 mg, 6 mg/day) suppressed sperm output between the second to fourth postimplant months; output returned to normal by the seventh postimplant month, although only 4 of 10 men became azoospermic or severely oligozoospermic (<3 mol/L/mL). The addition of a depot progestin markedly increased the extent, but not the rate, of sperm output suppression, with 9 of 10 becoming azoospermic and 10 of 10 becoming severely oligozoospermic. There were no serious adverse effects during the study. Plasma total and free testosterone levels remained within the eugonadal range at all times with each treatment. Plasma epitestosterone was suppressed by all 3 regimens, consistent with a dose-dependent inhibition of endogenous Leydig cell steroidogenesis. Plasma LH and FSH measured by a two-site immunoradiometric assay were suppressed in a dose-dependent fashion by T and further suppressed by the addition of DMPA. Sex hormone-binding globulin levels were decreased by DMPA, but not by either T dose. Prostate-specific antigen and lipids (total, low, or high density lipoprotein cholesterol, and triglycerides) were not significantly changed in any group. Thus, a depot testosterone preparation with zero order release must be delivered at between 6-9 mg/day to provide optimal (but not uniform) efficacy at inducing azoospermia. The addition of a single depot dose of a progestin to a suboptimal testosterone dose (6 mg/day) markedly enhances the extent, but not the rate, of spermatogenic suppression, with negligible biochemical androgenic side-effects. These findings provide a basis for the use of a progestin/androgen combination depot for hormonal male contraception. (J Clin Endocrinol Metab 81: 4113–4121, 1996)
Assays
Subjects

cording to methods described in the WHO Semen Manual (13) using a
not taking regular medication, and having normal testicular function,
mine the minimum testosterone dose
or GnRH antagonists (10). As a testosterone depot is the basis
of anterofinal suppression and 2) to determine the effects of a depot pro-
pregestin when it was added to the first suboptimally suppressive
testosterone dose (eventually identified as four 200-mg implants) that
showed partial suppression of spermatogenesis plus a single im injection of
300 mg depot medroxyprogesterone acetate (DMPA; Depo-Provera,
Upjohn, Kalamazoo, MI).

Volunteers provided two baseline sets of semen and blood samples
at least 2 weeks apart before hormone administration. Pellets of fused
crystalline testosterone (Organon, Sydney, Australia) were implanted
subdermally in the lower abdominal wall under local anesthesia as
previously described (7, 8). The pellets are composed of crystalline
testosterone without excipient, thereby being fully biodegradable and
not requiring removal. Subsequently, all volunteers provided monthly
semen and blood samples for 12 months. Recovery was defined as the
point when sperm density reached pretreatment baseline geometric
mean or consistently normal levels (>20 million sperm/mL). The study
was approved by the Central Sydney Area Health Service Ethics Review
Committee.

Subjects

Healthy men, aged 21–50 yr, who were free of chronic medical illness,
not taking regular medication, and having normal testicular function,
were recruited by advertisement on noticeboards and in the news media.
Exclusion criteria were any history of gonadal dysfunction (including
infertility), drug abuse, or abnormalities in medical screening tests.
Participants were advised to continue reliable contraception throughout
this study if they wished to avoid conception. Controls were age
matched men who were screened in an ongoing study (12) of potential
sperm donors (n = 509).

Assays

Semen collected by masturbation was analyzed within 60 min ac-
cording to methods described in the WHO Semen Manual (13) using a
Makler chamber (SEFI-Medical Instruments, Haifa, Israel).

Assays of total and free testosterone were performed as described
previously (8, 11, 14, 15). LH and FSH were measured by highly sensitive
two-site enzyme immunoradiometric assays (IMX/AXSYM, Abbott,
North Chicago, IL) with a detection limit of 0.1 U/L for both LH and
FSH. In addition, all samples were reasayed by two-site time-resolved
fluororimmunoradiometric LH and FSH assays (Delfia, Pharmacia, Pisc-
cataway, NJ). Prostate-specific antigen and sex hormone-binding glob-
ulin (SHBG) were measured by solid phase, two-site immunometric
assays (Diagnostic Products Corp., Los Angeles, CA). Inhibin was mea-
sured by double antibody RIA using the Monash antibody (no. 1989) and
recombinant inhibitin for standard and iodination material, as described
previously (16, 17). Samples were measured within a single assay when-
ever possible, and between-assay coefficients of variation ranged from
6–12% for all assays.

Eryptosterone (17α-hydroxyandrost-4-en-3-one) was measured by
an in-house liquid phase RIA using an antiserum and tritiated eryptes-
tosterone tracer (Wien Laboratories, Succasunna, NJ) with a standard
dextran-coated charcoal separation. Plasma was extracted by applying
0.4-mL plasma aliquots to a glass Pasteur pipette column filled with
~a 2-mL bed volume of Extrelut (Merck, Darmstadt, Germany). After
plasma had soaked in, extracts were eluted with 3 mL hexane-ethyl
acetate (3:2, in 0.5-mL aliquots), which were then combined, dried,
and reconstituted in assay buffer. Extraction efficiency was 89%, and results
were corrected individually for recovery. Cross-reactivity of the anti-
body with other androgens was low (testosterone, 0.36%; androstenedi-
one, <1.9%; nandrolone, <0.01%; dihydrotestosterone, 0.12%), the
detection limit was 2.7 pg/tube (equivalent to 0.09 nmol/L), and the
between-assay coefficient of variation was 8.5%. In validation studies,
the mean plasma eryptosterone concentration was 2.1 ± 0.09 nmol/L
(range, 1.4-2.8) in healthy men without known gonadal disorder (n =
42) and 0.76 ± 0.04 nmol/L (range, 0.44–0.96) in castrate men receiving
androgen replacement therapy (n = 13).

Hemoglobin, lipids (total, low density lipoprotein (LDL) and high
density lipoprotein (HDL) cholesterol, and triglycerides), renal (urea
and creatinine), and liver function tests (bilirubin, albumin, alkaline
phosphatase, and transaminases) were assayed by routine autoanalyzer
methods.

Data analysis

Results are expressed as the mean ± SEM. Data were analyzed by
multiple or repeated measures ANOVA using BMDP software (version
7 for VAX) or exact categorical analysis using StatXact software (version
3 for Windows) as appropriate. Baseline levels for each variable were
defined as the arithmetic mean of all pretreatment samples apart from
sperm variables, for which the geometric mean was used. Semen data
were cube root transformed, and hormonal data were log transformed
where required to normalize distribution and stabilize variance. Severe
oligozoospermia was defined as a sperm concentration of less than 3
mol/L/mL. The degree of suppression of sperm output was defined on
the basis of the lowest recorded monthly sperm density and related to
the geometric baseline sperm output. For time-related variables that did
not uniformly reach the end point (e.g. gonadotropin recovery), life-table
estimates of median time to the end point are reported. Results are
reported as the mean and SEM or as two-sided 95% confidence intervals
otherwise unless stated.

Results

Subjects

The men entering this study did not differ between groups
(n = 10) in age, height, weight, body surface area, body mass
index, or testis size (Table 1) and were similar to our ongoing
control group (12) of healthy men screened as potential
sperm donors (n = 509; data not shown).

Implantation of testosterone pellets was well tolerated. There
were 2 extrusion episodes among the 30 procedures in
this study, both involving a single pellet extruding from men
in the combined treatment group and occurring at weeks 11
and 14 after implantation. There were no discontinuations or
serious adverse effects reported by participants or any
changes in mood or behavior observed by study personnel.
Mild acne was reported by 3 of 10 men receiving 800 mg
testosterone and 1 of 10 receiving 800 mg testosterone plus
DMPA. None required any specific treatment for acne. Increased libido at the start of the study was reported by 5 of 10 men in the 800 mg testosterone plus DMPA group and by 1 of 10 men after 800 mg testosterone alone, but only 1 regarded this as troublesome. One man receiving 800 mg testosterone alone felt that he was transiently more aggressive. There were no adverse effects reported in men receiving 400 mg testosterone. All subjects completed the study, and 412 of 420 (98%) semen samples required for primary endpoint evaluation were obtained.

**TABLE 2. Baseline, suppression, and recovery of sperm output**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Testosterone Progestin</th>
<th>400 mg</th>
<th>800 mg</th>
<th>800 mg</th>
<th>800 mg</th>
<th>300 mg</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
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<tr>
<td>Abstinence (days)</td>
<td>2.5 ± 0.4</td>
<td>2.1 ± 0.1</td>
<td>5.0 ± 2.0</td>
<td>0.175</td>
<td></td>
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</tr>
<tr>
<td>Sperm density (mmol/L·mL)</td>
<td>3.6 ± 0.4</td>
<td>3.1 ± 0.4</td>
<td>3.4 ± 0.3</td>
<td>0.738</td>
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</tr>
<tr>
<td>Total sperm (mmol/L·ejaculate)</td>
<td>89 ± 13</td>
<td>153 ± 37</td>
<td>80 ± 11</td>
<td>0.074</td>
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</tr>
<tr>
<td>Sperm motility (%)</td>
<td>56 ± 3</td>
<td>60 ± 5</td>
<td>59 ± 5</td>
<td>0.429</td>
<td></td>
<td></td>
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<tr>
<td>Rate of suppression</td>
<td></td>
<td></td>
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<tr>
<td>Decrease at 1 month (% baseline)</td>
<td>77 ± 12</td>
<td>87 ± 20</td>
<td>16 ± 9</td>
<td>&lt;0.001</td>
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<tr>
<td>Decrease at 2nd month (% baseline)</td>
<td>99 ± 14</td>
<td>37 ± 14</td>
<td>1 ± 0</td>
<td>&lt;0.001</td>
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<tr>
<td>Nadir</td>
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<tr>
<td>Sperm density (mmol/L·mL)</td>
<td>38 ± 7</td>
<td>21 ± 8</td>
<td>0.1 ± 0.1</td>
<td>&lt;0.001</td>
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<tr>
<td>Sperm density (% baseline)</td>
<td>43 ± 6</td>
<td>23 ± 10</td>
<td>0 ± 0</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>Time (months)</td>
<td>5.1 ± 0.9</td>
<td>2.6 ± 0.2</td>
<td>2.5 ± 0.4</td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligozoospermia (&lt;3 mmol/L·mL)</td>
<td>0 (0)</td>
<td>4 (40)</td>
<td>9 (90)</td>
<td>&lt;0.001</td>
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<tr>
<td>Recovery</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to &gt;50% baseline (months)</td>
<td>2.0 ± 0</td>
<td>5.2 ± 1.1</td>
<td>7.6 ± 0.5</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to &gt;20 mmol/L·mL (months)</td>
<td>2.0 ± 0</td>
<td>3.6 ± 0.6</td>
<td>7.3 ± 0.4</td>
<td>&lt;0.001</td>
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</tr>
</tbody>
</table>

Results are expressed as the mean ± SEM. Baseline levels are defined as the arithmetic mean of two pretreatment baseline concentrations for all except the sperm variable, in which the geometric mean was used. Percentages are in parentheses.
the study (Fig. 2). There were, however, significant differences between treatments in the time course of blood total and free testosterone (treatment × time interactions, P < 0.001). Total and free testosterone increased modestly after 800 mg testosterone alone, but both decreased after 800 mg testosterone plus 300 mg DMPA, whereas 400 mg testosterone had no consistent effect on testosterone concentrations over time.

Epitestosterone concentrations were significantly reduced by all three treatments in a dose-dependent fashion (Fig. 2). Only in men receiving 800 mg T plus 300 mg DMPA were epitestosterone concentrations consistently suppressed to levels comparable to those in castrate men. Testosterone dose as well as DMPA administration significantly decreased epitestosterone concentrations. The median time to recovery to baseline epitestosterone levels did not differ significantly between groups (overall median 7.4 months, P = 0.87).

Plasma LH concentrations were significantly reduced by the first month and remained suppressed for 3 months with all three treatments (Fig. 3). Both the extent and duration of inhibition as well as rate of recovery were dose dependent (Table 3). Undetectable LH levels were observed in 0 (400 mg DMPA) of the 10 men, but the other six exhibiting only modest suppression of spermatogenesis. Among men receiving 800 mg testosterone alone, the only significant difference between those who did (n = 4) and those who did not (n = 6) become azoospermic was a lower baseline urea concentration (4.3 ± 0.5 vs. 6.0 ± 0.5; P = 0.046), but not in any other baseline anthropometric, seminal, hormonal, or biochemical variables. Men who became azoospermic had significantly lower overall total and free testosterone concentrations, but there was no difference in epitestosterone concentrations or time course of total or free testosterone, epitestosterone, LH, or FSH concentrations according to for those who did not (azoospermia × time interaction, P > 0.05).

The combination of 800 mg testosterone with 300 mg DMPA caused a striking fall in sperm output, with 9 of 10 reaching azoospermia and all reaching severe oligozoospermia (<3 mol/L/mL). In the two groups receiving 800 mg testosterone, the nadir of sperm output was reached at 2-3 months, with marked suppression lasting for 3 months followed after the 4th month by a gradual return in sperm output toward normal and reaching baseline levels in the 10th month but without overshoot. Essentially identical patterns were observed whether expressed as concentrations or total output of motile or all sperm. The study provided a power of more than 90% to reject each of the following hypotheses that 1) 800 mg testosterone alone would induce azoospermia uniformly (100%), and 2) the addition of DMPA had no effect on induction of azoospermia.

Reproductive hormones

After all treatments, blood testosterone concentrations remained within the eugonadal ranges for total (10–35 nmol/L) and free (170–510 pmol/L) testosterone throughout
T), 3 (800 mg T), and 2 (800 mg T plus 300 mg DMPA) of the blood samples taken at weeks 4 (5 samples), 8 (7 samples), 12 (6 samples), 16 (4 samples), and 20 (1 sample). There was no evidence of LH rebound during recovery. Similar findings were confirmed using the Delfia LH assay (data not shown).

Plasma FSH concentrations were significantly reduced in the first month in both groups receiving 800 mg testosterone, but not in the 400 mg testosterone group (Fig. 3). Both the extent and duration of inhibition as well as the rate of recovery were dose dependent, remaining suppressed for 3 months by testosterone alone and for 4 months with the addition of DMPA treatment (Table 3). Undetectable levels were observed in 0 (400 mg testosterone), 3 (800 mg testosterone), and 2 (800 mg testosterone plus 300 mg DMPA) of the blood samples taken at weeks 8 (one sample) and 12 (four samples). There was no evidence of FSH rebound during recovery. Similar findings were confirmed using the Delfia FSH assay (data not shown).

Inhibin concentrations were decreased in a dose-dependent manner (73 ± 10%, 51 ± 9%, and 27 ± 4% of baseline inhibin levels), with a nadir at 3 months and subsequent recovery (Fig. 4). SHBG concentrations were significantly reduced by DMPA administration, but not by either testosterone dose (Fig. 4).

To determine whether the effects of DMPA on testosterone could be explained by the reduced SHBG levels, the greater inhibition of LH levels, or other effects, we examined the effects of DMPA on total testosterone concentrations using either concurrent SHBG or LH levels as covariates. Adjustment for either covariate, however, had little influence on the DMPA effect on the time course of testosterone, which remained highly significant (treatment × time interactions, P < 0.0001).

**Discussion**

Testosterone implants provide the first opportunity to systematically test the effects of steady state administration of exogenous testosterone on normal human spermatogenesis. These implants provide near zero order release kinetics, ensuring stable dose-dependent testosterone levels within the physiological range for up to 6 months after a single subdermal implantation (8). The characteristics of the spermatogenic suppression with this true testosterone depot should reliably predict the suppression achievable with other depot testosterone formulations, such as testosterone microcapsules (6) or testosterone buciclate (5), both of which have significantly shorter durations of action. We previously showed that the implantation of six 200-mg testosterone pellets suppressed sperm output to the same extent as weekly 200-mg TE injections, whereas daily testosterone exposure was lowered by more than 50%, blood testosterone levels were reduced to remain within the physiological range, and some, but not all, metabolic effects of testosterone were reduced (11). The testosterone dose used in that first study of testosterone implants (1200 mg) was arbitrarily selected toward the upper range of doses used conventionally for androgen replacement therapy (9), and its daily delivery rate of testosterone (9 mg/day) also corresponds with the upper limits of normal endogenous testosterone daily production (3–10 mg/day). These results prompted the present downward dose-ranging study to determine the minimum testosterone dose that could maintain optimal spermatogenic suppression.

This study now identifies the limits of a testosterone depot in the suppression of human spermatogenesis when used alone. We found that a testosterone implant dose of 800 mg (four 200-mg implants), releasing 6 mg testosterone/day, when administered alone achieves inadequate suppression of spermatogenesis for a hormonal male contraceptive. A still lower dose (2 200-mg implants, 3 mg testosterone/day) has...
TABLE 3. Suppression and recovery of gonadotropins

<table>
<thead>
<tr>
<th>Variables</th>
<th>Testosterone</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prorgestin</td>
<td>400 mg</td>
<td>800 mg</td>
<td>800 mg</td>
<td>300 mg</td>
</tr>
<tr>
<td>No.</td>
<td></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline (IU/L)</td>
<td></td>
<td>4.2 ± 0.4</td>
<td>4.9 ± 0.6</td>
<td>5.0 ± 0.6</td>
<td>0.531</td>
</tr>
<tr>
<td>Nadir (IU/L)</td>
<td></td>
<td>1.8 ± 0.2</td>
<td>1.0 ± 0.3</td>
<td>0.1 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nadir (% of baseline)</td>
<td></td>
<td>44 ± 4</td>
<td>17 ± 5</td>
<td>2 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time of nadir (months)</td>
<td></td>
<td>2.5 ± 0.8</td>
<td>1.1 ± 0.1</td>
<td>1.8 ± 0.4</td>
<td>0.158</td>
</tr>
<tr>
<td>Recovery (IU/L)</td>
<td></td>
<td>4.0 ± 0.4</td>
<td>4.9 ± 1.8</td>
<td>4.7 ± 0.8</td>
<td>0.626</td>
</tr>
<tr>
<td>Recovery (% of baseline)</td>
<td></td>
<td>104 ± 12</td>
<td>101 ± 7</td>
<td>99 ± 8</td>
<td>0.679</td>
</tr>
<tr>
<td>Median time to recovery (months)</td>
<td></td>
<td>3.9 ± 1.0</td>
<td>9.0 ± 1.6</td>
<td>10.8 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FSH</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (IU/L)</td>
<td></td>
<td>3.7 ± 0.7</td>
<td>2.5 ± 0.5</td>
<td>4.5 ± 0.7</td>
<td>0.105</td>
</tr>
<tr>
<td>Nadir (IU/L)</td>
<td></td>
<td>2.4 ± 0.5</td>
<td>0.7 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nadir (% of baseline)</td>
<td></td>
<td>69 ± 4</td>
<td>26 ± 8</td>
<td>4 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time of nadir (months)</td>
<td></td>
<td>2.3 ± 0.7</td>
<td>1.4 ± 0.2</td>
<td>2.1 ± 0.3</td>
<td>0.351</td>
</tr>
<tr>
<td>Recovery (IU/L)</td>
<td></td>
<td>4.6 ± 0.6</td>
<td>3.2 ± 0.5</td>
<td>5.0 ± 0.6</td>
<td>0.096</td>
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<tr>
<td>Recovery (% of baseline)</td>
<td></td>
<td>131 ± 7</td>
<td>136 ± 8</td>
<td>121 ± 17</td>
<td>0.627</td>
</tr>
<tr>
<td>Median time to recovery (months)</td>
<td></td>
<td>3.4 ± 0.7</td>
<td>5.1 ± 0.5</td>
<td>9.5 ± 0.8</td>
<td>&lt;0.001</td>
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</tbody>
</table>

Results are expressed as the mean ± SEM. Baseline levels are defined as the arithmetic mean of two pretreatment baseline concentrations. Recovery levels are defined as the mean of observations at the last three time points (posttreatment months 10–12).

Fig. 4. Plasma SHBG (upper panel), inhibin (middle panel), and prostate-specific antigen (lower panel) before and after implantation of two 200-mg testosterone pellets (400 mg total; closed circles), four 200-mg testosterone pellets (600 mg total; closed squares), or four testosterone pellets plus depot progestin (800 mg total testosterone plus 300 mg DMPA; closed diamonds) in groups (n = 10) of healthy fertile men. Results are expressed as the mean and SEM.

Fig. 5. Plasma total (filled symbols), HDL cholesterol (open symbols; upper panel), LDL cholesterol (filled symbols), and triglycerides (open symbols; lower panel) before and after implantation of two 200 mg testosterone pellets (400 mg total; circles), four 200-mg testosterone pellets (800 mg total; squares), or four testosterone pellets plus depot progestin (800 mg total testosterone plus 300 mg DMPA; diamonds) in groups (n = 10) of healthy fertile men. Results are expressed as the mean and SEM.

mg testosterone buciclate, a novel testosterone ester containing 760 mg testosterone, which produced azoospermia in 3 of 8 healthy men, but minimal spermatogenic suppression in the remaining volunteers (7). Given the prolonged zero order testosterone release by testosterone buciclate injection (5, 7) and extrapolating our previous findings, it can be expected that higher testosterone buciclate doses would improve spermatogenic suppression, but still not provide uniform
azoospermia. Although our findings may be reliably extrapolated to other true testosterone depots (such as testosterone microspheres), they may not apply to synthetic androgens, particularly those metabolically different from testosterone by virtue of restricted activation by aromatization and/or 5α-reduction (18). Extrusions of a single implant were observed in only 2 men among 30 participants in this study, a rate consistent with that of pellet extrusions among hypogonadal men (5-7%) (Handelsman, D. J., unpublished observations). As these 2 men became azoospermic and had no evidence of androgen deficiency, it is unlikely that these extrusions materially affected our findings.

This study is the first demonstration of synergism between a depot progestin and a depot androgen in suppressing human spermatogenesis. We observed markedly greater suppression of sperm output by the addition of a depot progestin to a depot androgen. Although many previous studies have examined various combinations of oral or parenteral progestins with androgens (19), none combined depot formulations of each agent or, until recently, involved controlled prospective comparisons (20, 21). Two recent prospective controlled studies have shown that daily ingestion of an oral progestin augments the spermatogenic suppression produced by weekly injections of 100 mg 11c: (20, 21) consistent with our findings. One study claimed an acceleration of spermatogenic suppression (20) that we did not observe. The apparent acceleration observed by Bebb et al., however, was due to the use of a suboptimal TE dose (100 mg weekly) shown previously to provide slower, less durable, and less reliable spermatogenic suppression compared with the conventional TE dose of 200 mg weekly (1, 2, 22, 23). Furthermore, a difference of a few weeks in time to adequate spermatogenic suppression have minimal practical importance if a waiting period of months is still required. As with vasectomy (24), any contraceptive method that relies on clearance of sperm from the male reproductive tract will feature a delayed onset and offset of action. This may still be well suited to elective use of hormonal male contraception in circumstances such as the postpartum period, delaying vasectomy, and intolerance of female methods. The inconsistent dose-dependent findings with TE (20, 21, 25), contrasting with those of a true testosterone depot, reinforce the relegation of TE to obsolete status for further path-finding studies for hormonal male contraception. Future studies should use more practical and effective depot testosterone formulations.

Although blood testosterone concentrations are useful to monitor Leydig cell activity, during the administration of exogenous testosterone, the mixture of endogenous and exogenous testosterone negates such interpretation. To resolve this difficulty we monitored blood concentrations of epitestosterone, the natural 17-epimer of testosterone, to indicate Leydig cell secretion. Epitestosterone is a Leydig cell product cosecreted with testosterone, thereby constituting a useful indicator of endogenous testosterone production. This is the basis for the use of urinary epitestosterone to detect the administration of exogenous testosterone among athletes. To avoid the inconvenience of 24 h urine collection and the more complex quantitative gas chromatograph/mass spectrometry (GC/MS) assay, we established a RIA for epitestosterone in blood. Epitestosterone concentrations were decreased in a time- and dose-dependent fashion, such that only the combined treatment depressed epitestosterone concentrations to castrate levels, although both testosterone alone doses decreased epitestosterone concentrations proportionally to dose. Furthermore, recovery toward baseline epitestosterone coincided well with the recovery of testicular function. Our findings support the use of blood epitestosterone concentrations as a valid and sensitive marker of Leydig cell steroidogenesis during the administration of exogenous testosterone.

This study again demonstrates the between-subject heterogeneity in suppression of human spermatogenesis by sex steroids. We previously postulated that a minority of healthy men had become severely oligozoospermic, but not azoospermic, after the administration of 1200 mg testosterone (six 200-mg implants) because blood testosterone concentrations may have been high enough to support spermatogenesis, particularly as we recently demonstrated that spermatogenesis may be induced by physiological levels of testosterone in the gonadotropin-deficient hpg mouse (26). The present study, however, refutes this hypothesis, as lower testosterone doses were even less (rather than more) effective, although a subgroup of men (4 of 10) still became azoospermic with the lower (800-mg) testosterone dose. This between-subject heterogeneity was not associated with differences in pretreatment SHBG levels (7) or any other measured variable. Blood testosterone concentrations were consistently, but marginally, higher among those who remained oligozoospermic but never azoospermic, although blood epitestosterone, LH, and FSH concentrations did not differ. Whether this is related to the suggested testosterone-induced increase in 5a-reductase activity (27), although men in our study had much lower, more physiological doses of testosterone, remains to be elucidated. The recent identification of an activating mutation of the human FSH receptor leading to persistence of testosterone-independent (and presumably refractory to testosterone-induced suppression) spermatogenesis (28) raises the possibility of a widely distributed genetic polymorphism as a possible mechanism worthy of exploration. This between-subject heterogeneity within as well as between populations (29) remains unexplained, but clarification of its mechanism might explain how uniform azoospermia may be achieved with hormonal regimens for male contraception.

The mechanism of the additive suppression of spermatogenesis by injection of 300 mg DMPA appears to be multifactorial. DMPA decreased SHBG concentrations and augmented inhibition of blood gonadotropin and testosterone concentrations, presumably reflecting direct hepatic and negative feedback hypothalamic effects, respectively. The fall in blood testosterone concentrations, however, was greater than could be accounted for by the DMPA effects on LH and SHBG in the covariance analysis, suggesting the possible importance of a direct inhibitory effect of DMPA on Leydig cell steroidogenesis. Whether these effects are all due to medroxyprogesterone acetate (MPA) action via progestin receptors or also involve MPA (or metabolite) cross-reactivity with androgen receptors remains unclear. The acute lowering of SHBG levels reflects the pharmacokinetic limitations of DMPA as this older progestin depot formulation has non-
zero order release, and the biochemical effects may reflect excessive early peak blood MPA concentrations. Such transient metabolic changes might be obviated by newer depot progestins with more steady state release kinetics, such as levonorgestrel esters or depot formulations. If the DMPA effects are primarily attributable to effects mediated via progestin receptors, other depot progestins should have similar effects, whereas if effects are partly due to androgen or estrogen receptor cross-reactivity, they may not be replicated exactly by other depot progestins. These observations reinforce the view that changes in SHBG or HDL cholesterol, sometimes considered androgenic effects, are actually toxic or excessive hepatic effects of some sex steroids, notably oral 17a-alkylated androgens or progestins, or a high dose of any parenteral sex steroid. Our findings suggest that optimized depot formulations with effective spermatogenic suppression can be developed with minimal or no biochemical effects on lipids, SHBG, or other nonhormonal biochemical end points. In this respect, SHBG is an easily measured indicator suitable for routine monitoring in path-finding studies as a convenient marker of excessive hepatic steroidal effects.

The absence of significant clinical or biochemical adverse effects or discontinuations during this study confirm and extend our previous observations with a higher dose (1200 mg, 9 mg/day) of testosterone implants. These findings illustrate the advantage of using the minimum testosterone doses that still maintain adequate androgen replacement. Based on our experience with hypogonadal men, this would be 800 mg testosterone (6 mg/day), which closely replicates the normal endogenous testosterone production rate. Although such doses provide inadequate suppression of spermatogenesis when used alone, they would provide adequate androgen replacement if another gonadotropin suppressing agent, such as a progestin or GnRH antagonist, was used concurrently. Further lowering of testosterone doses would provide inadequate androgen replacement, with likely adverse consequences for structure and function of bone, muscle, and other androgen-dependent tissues, including loss of libido. In deciding the relative advantages of androgen alone vs. androgen combinations with a second agent, the key issue is the relative safety of reducing testosterone exposure from 9 to 6 mg/day vs. the addition of a second gonadotropin-suppressing agent, and the optimal approach remains to be determined (see discussion in Ref 10).

This path-finding study was not designed to resolve the issue of the long term risks and benefits of androgen usage. These considerations will require evaluation of the risks of cardiovascular or prostate disease balanced against the noncontraceptive benefits on bone, muscle, and general anabolic effects during prolonged surveillance over decades, as has been required for female hormonal contraception. Nevertheless, the short term findings in this study are reassuring. The only relevant established cardiovascular risk association in men is that lowered blood testosterone levels are associated with excess cardiovascular risk (30). The absence of lipid changes together with the dose-sparing effect of a steady state depot formulation and maintenance of completely physiological testosterone concentrations throughout the study indicate that testosterone-based male contraceptive regimens with minimal metabolic impact on biochemical variables can be developed. Further study of the influence of physiological doses of androgens and progestins on nonlipid cardiovascular risk factors, such as vascular reactivity (31, 32), also need evaluation. Similarly, the unchanged prostate-specific antigen concentrations are evidence against any change in total prostate size under the conditions pertaining to this study. This supports the strategy that maintaining adequate physiological testosterone concentrations and avoiding excessive or underreplacement dosages may minimize long term cardiovascular or prostate risk from androgen-based hormonal regimens for male contraception.

The present study demonstrates the feasibility and advantages of using a depot progestin/androgen combination for hormonal male contraception. The ongoing public interest and enthusiastic participation in such contraceptive studies signal the motivation and willingness of men to continue to share the burdens as well as the benefits of reliable contraception. If more convenient depot formulations can be made available, the promise of hormonal contraception for men indicated by the WHO studies and the clear community niche for hormonal male methods can be brought into fruitful conjunction.

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