Comparison between testosterone oenanthate-induced azoospermia and oligozoospermia in a male contraceptive study. IV. Suppression of endogenous testicular and adrenal androgens

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Administration of supraphysiological doses of testosterone to normal men causes inhibition of spermatogenesis, but while most become azoospermic, 30–55% maintain a low rate of spermatogenesis. We have investigated whether there are differences in endogenous androgen production, of testicular and adrenal origin, which may be related to the degree of suppression of spermatogenesis. Thirty-three healthy Caucasian men were given weekly i.m. injections of 200 mg testosterone oenanthate (TE), 18 became azoospermic, while 15 remained oligozoospermic. Urinary excretion of epitestosterone, a specific testicular product, was reduced to <10% of pretreatment values, with no differences between the groups. Similar results were obtained for other markers of testicular steroidogenesis. Urinary and plasma adrenal androgens were also reduced during TE treatment: a statistically significant decrease in both ($P < 0.001$ and $P < 0.05$ respectively) was seen in the azoospermic but not oligozoospermic responders. These results suggest that testicular steroidogenesis is decreased to <10% by the administration of supraphysiological doses of exogenous testosterone. Differences in the degree of ongoing steroidogenesis in the testis do not appear to account for incomplete suppression of spermatogenesis, thus differences in androgen metabolism may underlie this heterogeneous response. A small but significant reduction in secretion of adrenal androgens was also detectable, the relevance of which is unclear.

Key words: adrenal androgens/epitestosterone/male contraception/spermatogenesis

Introduction

Abolition of pituitary gonadotrophin secretion by exogenous sex steroids results in testicular atrophy characterized by cessation of Leydig cell steroidogenesis, intratesticular testosterone depletion and arrest of spermatogenesis. This approach is currently under evaluation as a method of hormonal male contraception (World Health Organization, 1990, 1996). A consistent but perplexing finding of studies using testosterone alone or in combination with a progestogen or gonadotrophin releasing hormone (GnRH) antagonist is that azoospermia is not uniformly achieved, with 30–55% of Caucasian men maintaining a low rate of spermatogenesis (World Health Organization, 1995). However, in Chinese and Indonesian men, the incidence of azoospermia is significantly higher than their Caucasian counterparts (World Health Organization, 1995, 1996). The basis for this heterogeneity in spermatogetic suppression remains an unexplained but critical question, the answer to which could optimize the efficacy and acceptability of hormonal male contraception. One possible mechanism may be differences in residual testicular steroidogenesis despite suppression of gonadotrophins to undetectable levels (Anderson and Wu, 1996). The degree to which testicular steroidogenesis is reduced in the azoospermic and oligozoospermic subjects is currently unknown. Unfortunately, measurement of peripheral blood testosterone concentrations does not reflect endogenous testicular steroidogenesis during administration of exogenous testosterone.

Epitestosterone (17α-hydroxyandrost-4-en-3-one) is a natural epimer of testosterone secreted predominantly by the testis (Dehennin, 1993) and excreted in urine in similar amounts to testosterone (Cowan et al., 1991). Excretion of epitestosterone increases following chorionic gonadotrophin administration (Cowan et al., 1991) and during puberty (Raynaud et al., 1993). Excretion is drastically suppressed by exogenous testosterone administration (Dehennin and Matsumoto, 1993) and an increased urinary testosterone/epitestosterone ratio is used for the detection of surreptitious testosterone administration by the International Olympic Committee (Wheeler, 1993). Since little, if any, epitestosterone is derived from peripheral metabolism of testosterone, androstenedione, or dehydroepiandrosterone (DHA) (Wilson and Lipssett, 1966), this steroid can therefore serve as a valid index of endogenous testicular steroidogenesis during administration of exogenous testosterone.

In this study, we have investigated the possibility that differences in suppression of endogenous steroidogenesis in response to supraphysiological doses of exogenous testosterone may be responsible for the heterogeneity in the degree of suppression of spermatogenesis observed in healthy Caucasian subjects. Urinary epitestosterone, 17-hydroxyprogesterone (17OH-progesterone) and urinary pregnanetriol were used as markers of testicular steroidogenesis.

Adrenal androgens may be converted to more potent androgens such as dehydrotestosterone (DHT) within the prostate in vivo and in vitro (Farnsworth, 1973; Harper et al., 1974), and also in skin (Kaufman et al., 1990). Such mechanisms may contribute to the overall androgen precursor pool in the
androstenedione were increased from 6.8% in the hypogonadotropic (intratesticular) testosterone-depleted state. While there are some data suggesting an effect of testosterone on adrenal androgens in women (Azziz et al., 1991; Polderman et al., 1995), the effect of exogenous testosterone on adrenal androgen secretion in men has not previously been extensively studied (Dehennin and Matsumoto, 1993). We have therefore investigated adrenal androgen secretion by our subjects before and during TE suppression of spermatogenesis.

Materials and methods

Subjects and design
Thirty-three healthy Caucasian men aged 21–41 years (mean 31 ± 1 SEM) were recruited into a clinical efficacy trial of hormonal male contraception after screening medical examination and biochemical and haematological analyses. This study was part of a World Health Organization multicentre trial investigating the contraceptive efficacy of testosterone-induced severe oligozoospermia. Further details concerning volunteer recruitment can be found in the World Health Organization (1996) publication. All subjects produced two baseline pretreatment semen samples with sperm concentrations of >20 × 10^6/ml, and blood samples with concentrations of gonadotrophins and testosterone within the reference range for adult males. Each subject received 200 mg testosterone enanthate (TE, ‘Testoviron’; Schering AG, Berlin, Germany) i.m. weekly, and were required to use this as their only method of contraception for one year once their sperm concentration had fallen to below 5 × 10^6/ml.

Subjects were classified into azoospermic and oligozoospermic responders on the basis of three consecutive semen analyses at twice weekly intervals after 20 weeks of TE treatment. From that time, none of the azoospermic subjects had detectable spermatozoa in subsequent ejaculates during TE treatment, and none of the oligozoospermic responders became azoospermic with up to 40 weeks continuing TE treatment. This study received the approval of the Lothian Paediatric/Reproductive Medicine Ethics Committee.

Plasma and urine sampling
Plasma samples were obtained and 24 h urine collections taken at baseline before TE treatment and repeated after 16 weeks of treatment. All blood samples were taken between 9 a.m. and 12.30 a.m. The repeat samples were taken 7 days after the last TE injection, i.e. when plasma testosterone concentrations are at their nadir at the time the next injection was due.

Semen analysis
Semen analyses were carried out according to the World Health Organization (1987) after 3 days of ejaculatory abstinence at 2–4 week intervals. Azoospermia was confirmed by examination of the pellet after centrifugation.

Plasma steroid assays
Plasma concentrations of testosterone, androstenedione and 170H-progestrone were measured by established in-house radioimmunoassays (Corker and Davidson, 1978; Wallace and Wood, 1984), and dehydroepiandrosterone sulphate (DHAS) by radioimmunoassay using a commercial kit (Diagnostic Products Corporation). Cross-reactivity with testosterone was <0.5% in the androsterone assay and <1% in the 170H-progesterone assay. Epitestosterone was known not to cross-react with testosterone or androstenedione. In all cases, intra-and interassay coefficients of variation were <10%.

Urinary androgen and glucocorticoid metabolite measurements
The selected urinary steroids were measured by a method based on that of Bevan et al. (1986), except for urinary testosterone and epitestosterone which were measured according to the method of Cowan et al. (1991). In brief, all urinary steroid conjugates were hydrolysed using the enzyme extracted from Helix pomatia, which has predominantly glucuronidase activity combined with selective sulphatase activity. Following ether extraction, testosterone and epitestosterone were converted to their bis-trimethylsilyl ether derivatives for analysis by capillary column gas chromatography–mass spectrometry. Other steroids were converted to methyloxime-trimethylsilyl ether derivatives for analysis on a model 438 gas chromatograph (Packer-Becker BV, Delft, Netherlands) fitted with a ‘Flexsil’ 25 m × 0.32 mm capillary column coated with OV-1 (Phase-Sep, Queensferry, Clywd) and quantified by comparison with peak height of the internal standards. Methoxime formation prior to trimethylsilylation is important for the thermal stability of the pregnane type structures, e.g. pregnanetriol, but is not necessary for the analysis of androgens. Between-batch coefficients of variation for both methods were <10%.

Statistical analysis
Statistical analyses were performed by analysis of variance, with P < 0.05 regarded as significant. Results are expressed as mean ± SEM.

Results

Sperm concentration
Administration of TE 200 mg i.m. weekly induced a profound suppression of spermatogenesis in all men. There were clear differences (P < 0.001; Anderson and Wu, 1996) in the rate of decline between those who subsequently became azoospermic and those who remained oligozoospermic which was apparent after only 4 weeks of treatment. Eighteen men became azoospermic by 20 weeks of treatment, at which time the oligozoospermic responders had a mean sperm concentration of 2.0 ± 0.8 × 10^6/ml. Although sperm concentration continued to fall in the latter group, none became azoospermic at up to 40 weeks of continued TE administration.

Plasma and urinary testicular androgens
The administration of TE increased plasma testosterone concentrations from 15.6 ± 1.2 to 35.2 ± 4.2 nmol/l in the azoospermic group and from 15.8 ± 2.0 to 36.8 ± 3.4 nmol/l in the oligozoospermic group (both P < 0.001, Figure 1a). Urinary excretion of testosterone was increased from 193 ± 30 to 802 ± 147 nmol/24 h in the azoospermic group and from 159 ± 24 to 685 ± 142 nmol/24 h in the oligozoospermic group (both P < 0.001, Figure 1b). Plasma concentrations of androstenedione were increased from 6.8 ± 0.5 to 9.3 ± 0.5 nmol/l (P < 0.001) in the azoospermic group, and from 6.9 ± 0.5 to 8.5 ± 0.7 nmol/l (P < 0.005) in the oligozoospermic group (data not shown). There were no differences between the azoospermic and oligozoospermic groups either at baseline or after 16 weeks TE treatment in either plasma or urinary testosterone.

TE treatment greatly reduced urinary excretion of epitestosterone in all subjects, from 212 ± 36 to 18.3 ± 2.1 nmol/24 h.
Exogenous testosterone inhibits testicular and adrenal androgens

Figure 1. (a) Plasma testosterone concentration, (b) 24 h urinary testosterone excretion, (c) 24 h urinary excretion of epitestosterone, (d) plasma 17OH-progesterone concentrations and (e) 24 h urinary excretion of pregnanetriol in men becoming azoospermic (n = 18) and those remaining oligozoospermic (n = 15) pretreatment (open columns) and after 16 weeks testosterone enanthate treatment (filled columns). Values given are mean ± SEM. *P < 0.01, **P < 0.005, ***P < 0.001 versus pretreatment.

(P < 0.001) in the azoospermic responders and from 175 ± 31 to 161 ± 2.1 nmol/24 h (P < 0.005) in the oligozoospermic responders (Figure 1c). The mean urinary testosterone/epitestosterone ratio increased from 1.5 to 47.2 in the azoospermic responders (range of individual values during TE treatment 4.4–183) and from 1.0 to 43.2 in the oligozoospermic responders (range 4.9–76). There were no differences between the azoospermic and oligozoospermic groups either at baseline or after 16 weeks TE treatment in these measures.

Plasma concentrations of 17OH-progesterone were reduced in both groups: from 4.6 ± 0.4 to 2.5 ± 0.2 nmol/l in the azoospermic group (P < 0.001), and from 5.4 ± 0.3 to 2.4 ± 0.2 nmol/l in the oligozoospermic group (P < 0.001) after 16 weeks of TE treatment (Figure 1d). Urinary pregnanetriol excretion was also significantly reduced in both groups after 16 weeks of TE treatment. In the azoospermic group, pregnantriol excretion was reduced from 2.8 ± 0.4 to 1.8 ± 0.3 µmol/24 h (P < 0.005), and in the oligozoospermic group from 3.0 ± 0.4 to 1.6 ± 0.4 µmol/24 h (P < 0.01) (Figure 1e). There were no significant differences between the azoospermic and oligozoospermic groups either at baseline or after 16 weeks TE treatment.

Plasma and urinary adrenal androgens

After 16 weeks of TE treatment, plasma concentrations of DHAS and urinary excretion of DHA were reduced from 10.4 ± 1.1 to 9.3 ± 0.8 µmol/l (P < 0.05) and 3.1 ± 0.5 to 1.8 ± 0.5 µmol/24 h (P < 0.001) respectively. The decrease in plasma DHAS was observed in the azoospermic group (from 11.5 ± 1.6 to 9.4 ± 1.0 µmol/l, P < 0.02) but there was no change in the oligozoospermic group (9.1 ± 1.3 to 9.1 ± 1.4 µmol/l) (Figure 2). Urinary excretion of DHA showed similar changes in the azoospermic group, falling from 3.2 ± 0.7 to 1.3 ± 0.4 µmol/24 h (P < 0.002), but was unchanged in the oligozoospermic group (2.9 ± 0.9 to 2.5 ± 1.1 µmol/24 h) (Figure 2). There were, however, no significant differences in plasma DHAS or urinary DHA between the azoospermic and oligozoospermic groups either pretreatment or during TE treatment.

Changes in the excretion of the androgen metabolites 16-hydroxydehydroepiandrosterone (16OH-DHA), 11β-hydroxyandrostosterone (11β-OHA) and 11β-hydroxyaetiocholanolone (11β-OHE) are shown in Table I. The decrease in excretion of 11β-OHE in the azoospermic group was at the level of statistical significance (P = 0.05).

Urinary adrenal glucocorticoid metabolites

Values for the urinary excretion of the glucocorticosteroid metabolites tetrahydrocortisol (THF), allo-tetrahydrocortisol (αTHF), and tetrahydrocorticosterone (THE), and α- and β-cortolone pretreatment and after 16 weeks TE treatment are
Figure 2. (a) Plasma dehydroepiandrosterone sulphate (DHAS) levels and (b) 24 h urinary excretion of dehydroepiandrosterone (DHA) in men becoming azoospermic (n = 18) and those remaining oligozoospermic (n = 15) pretreatment (open columns) and after 16 weeks TE treatment (filled columns). Values given are mean ± SEM. *P < 0.02, **P < 0.002 versus pretreatment.

shown in Table I. There were no significant changes in any of these metabolites during TE treatment.

Discussion
We have previously found no difference in the pharmacokinetics and pharmacodynamics of testosterone enanthate in azoospermic compared to oligozoospermic responders during TE treatment, nor in the rate or degree of suppression of gonadotrophin secretion which fall to undetectable levels in all men (Anderson and Wu, 1996). However, higher 5α-reductase activity in the oligozoospermic compared to the azoospermic responders was found during TE treatment (Anderson et al., 1996). We therefore postulated that increased 5α-reductase activity during gonadotrophin suppression, serving to preserve some androgenic stimulus to the seminiferous tubules in the oligozoospermic but not the azoospermic responders, may account for the maintenance of a low level of spermatogenesis. To further investigate this hypothesis, we have now explored whether there are differences in the extent of suppression of endogenous testicular or adrenal androgens, precursors for 5α-reductase, which could differentiate azoospermic and oligozoospermic response groups following TE suppression.

Exogenous testosterone is able to maintain spermatogenesis directly in some animal species (see review by Weinbauer and Nieschlag, 1990). Plasma concentrations and urinary excretion of testosterone were increased by exogenous TE treatment, but the extent of this increase was similar between azoospermic and oligozoospermic responders. There was also an increase in the plasma concentration of androstenedione after 16 weeks of TE treatment in both groups of men. This elevation in plasma androstenedione concentration probably reflects shown in Table I. Twenty-four hour urinary excretion of adrenal androgens and glucocorticosteroid metabolites by azoospermic (n = 18) and oligozoospermic (n = 15) responders pretreatment and after 16 weeks testosterone enanthate treatment. Values are mean ± SEM

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Group</th>
<th>Pretreatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>16OH-DHA</td>
<td>Azoosperm</td>
<td>2.96 ± 0.56</td>
<td>2.60 ± 0.61</td>
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<td></td>
<td>Oligozoosperm</td>
<td>3.07 ± 0.66</td>
<td>3.04 ± 0.99</td>
</tr>
<tr>
<td>11β-OHA</td>
<td>Azoosperm</td>
<td>3.01 ± 0.47</td>
<td>2.36 ± 0.36</td>
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<tr>
<td></td>
<td>Oligozoosperm</td>
<td>3.41 ± 0.40</td>
<td>2.97 ± 0.55</td>
</tr>
<tr>
<td>11β-OHE</td>
<td>Azoosperm</td>
<td>1.22 ± 0.18</td>
<td>0.93 ± 0.14*</td>
</tr>
<tr>
<td></td>
<td>Oligozoosperm</td>
<td>1.07 ± 0.16</td>
<td>1.11 ± 0.14</td>
</tr>
<tr>
<td>THF</td>
<td>Azoosperm</td>
<td>5.99 ± 0.75</td>
<td>6.57 ± 0.78</td>
</tr>
<tr>
<td></td>
<td>Oligozoosperm</td>
<td>6.40 ± 0.85</td>
<td>6.33 ± 0.82</td>
</tr>
<tr>
<td>αTHF</td>
<td>Azoosperm</td>
<td>5.48 ± 0.81</td>
<td>5.51 ± 0.69</td>
</tr>
<tr>
<td></td>
<td>Oligozoosperm</td>
<td>6.93 ± 1.38</td>
<td>6.17 ± 1.40</td>
</tr>
<tr>
<td>THE</td>
<td>Azoosperm</td>
<td>9.87 ± 1.42</td>
<td>10.52 ± 1.69</td>
</tr>
<tr>
<td></td>
<td>Oligozoosperm</td>
<td>12.21 ± 1.30</td>
<td>10.81 ± 1.55</td>
</tr>
<tr>
<td>α-Cortolone</td>
<td>Azoosperm</td>
<td>2.54 ± 0.35</td>
<td>2.94 ± 0.53</td>
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<tr>
<td></td>
<td>Oligozoosperm</td>
<td>3.14 ± 0.47</td>
<td>3.02 ± 0.45</td>
</tr>
<tr>
<td>β-Cortolone</td>
<td>Azoosperm</td>
<td>1.82 ± 0.26</td>
<td>1.99 ± 0.24</td>
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<tr>
<td></td>
<td>Oligozoosperm</td>
<td>2.00 ± 0.21</td>
<td>1.85 ± 0.20</td>
</tr>
</tbody>
</table>

All values µmol/24 h; n = 18, azoospermic group; n = 15, oligozoospermic group.

*P = 0.05 versus pretreatment. 16OH-DHA = 16-hydroxydehydroepiandrosterone, 11β-OHA = 11β-hydroxyandrostenedione, 11β-OHE = 11β-hydroxyaetiocholanolone, THF = tetrahydrocortisol, αTHF = allo-tetrahydrocortisol, THE = tetrahydrocortistosterone. 

Epitestosterone is secreted by the testis (Dehennin, 1993), although there may also be a minor adrenocortical contribution (Wilson and Lipsett, 1966; Tamm et al., 1966). After 16 weeks TE treatment, urinary epitestosterone excretion in both azoospermic and oligozoospermic responders was reduced to <10% of baseline. Our results are consistent with those obtained using short-duration urine collections (Dehennin and Matsumoto, 1993) and plasma levels (Handelsman et al., 1996) and confirm that the testes are the major source of epitestosterone. Since epitestosterone is an indicator of Leydig cell activity, the present results also suggest that endogenous testicular steroidogenesis has been drastically curtailed as a result of luteinizing hormone inhibition by exogenous TE (Anderson and Wu, 1996; Handelsman et al., 1996). This is compatible with the 90% reduction in directly-measured intratesticular testosterone levels reported in normal men treated with testosterone propionate (Morse et al., 1973). The decrease in markers of other secreted testicular intermediate
steroidogenic products, 17OH-progesterone and its urinary metabolite pregnanetriol (Strott et al., 1969; Laatikainen et al., 1971; Hammond et al., 1977; Nankin et al., 1980) is a further sign of suppressed Leydig cell function. Thus, using these testicular steroids as markers of endogenous testicular steroidogenesis, we found no difference between azoospermic and oligozoospermic responders. This indicates that residual testicular steroidogenesis, and by implication intratesticular testosterone concentration, is unlikely to be a critical determinant in the spermaticogenic response in testosterone-induced hypogonadotrophism. These findings therefore lend further support to our hypothesis that differences in 5α-reductase activity, rather than its peripheral or testicular steroid substrates, determine the degree of residual spermaticogenic during gonadotrophin suppression.

Previous studies of the effects of exogenous sex steroids on the adrenals have produced conflicting results (Azziz et al., 1991; Polderman et al., 1995) and any specific effects of testosterone treatment on adrenal steroidogenesis in normal men has not been previously reported. Our results, showing a decrease in both plasma DHAS concentration and urinary DHA excretion during TE treatment in men, with no effect on glucocorticoid metabolites, are consistent with the findings in male baboons (Goncharov et al., 1995). Although conversion of adrenal androgens to more potent androgens has been demonstrated in tissues such as the skin and prostate (Farnsworth, 1973; Thomas and Oake, 1974; Harper et al., 1974; Kaufman et al., 1990), the existence of such pathways in the testis has not been demonstrated. A role for adrenal androgens as precursors in androgen metabolism in hypogonadogenic states is supported by the limited effect of castration on prostatic DHT concentrations (Geller et al., 1978) compared to the profound reduction in prostatic DHT concentration after administration of finasteride, a 5α-reductase inhibitor (Geller, 1990; McConnell et al., 1992). Thus a decline in adrenal androgen levels may possibly contribute to the complete cessation of spermatogenesis or spermiogenesis in the presence of very low intratesticular testosterone concentrations (as demonstrated by the dramatic fall in epitestosterone excretion). In the context of this study, however, the exact reason for the falls in DHAS and DHA after TE treatment in the azoospermic group only is at present unclear, since there were not statistically significant differences in the levels of these hormones between the azoospermic and oligozoospermic subjects either before or during TE treatment.

In conclusion, our results demonstrate that exogenous TE causes suppression of endogenous testicular steroidogenesis to <10% of pretreatment levels, but this could not account for differences in the degree of suppression of spermatogenesis. This finding further highlights the importance of the postulated role of 5α-reductase in maintaining a low level of spermatogenesi in the presence of gonadotrophin suppression and intratesticular testosterone depletion. Epitestosterone, 17OH-progesterone and pregnanetriol are useful markers of testicular steroidogenesis during exogenous testosterone administration. A reduction in adrenal androgen secretion during TE treatment was observed but the concentrations of DHAS and DHA did not differ between the azoospermic and oligozoospermic groups. The implications of this finding require further investigation.

Acknowledgement

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References


Harper, M.E., Pike, A., Peeling, W.B. et al. (1974) Steroids of adrenal origin and their metabolic activity, rather than its peripheral or testicular steroid substrates, of very low intratesticular testosterone concentrations (as demonstrated by the dramatic fall in epitestosterone excretion). In the context of this study, however, the exact reason for the falls in DHAS and DHA after TE treatment in the azoospermic group only is at present unclear, since there were not statistically significant differences in the levels of these hormones between the azoospermic and oligozoospermic subjects either before or during TE treatment.

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