

Oral desogestrel with testosterone pellets induces consistent suppression of spermatogenesis to azoospermia in both Caucasian and Chinese men

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BACKGROUND: Effective hormonal male contraception requires a high prevalence of spermatogenic suppression, which has proved particularly difficult in Caucasian populations. We have investigated the combination of oral desogestrel with depot testosterone in Caucasian and Chinese men. **METHOD:** Thirty men in Edinburgh and 36 men in Shanghai received 150 or 300 µg desogestrel p.o. daily for 24 weeks with 400 mg testosterone pellets s.c. on day 1 and at 12 weeks. **RESULTS:** Eight men withdrew before completing 24 weeks treatment. Testosterone concentrations remained within the normal range. Spermatogenesis was profoundly suppressed in all men. Azoospermia was achieved by a higher proportion of men in the 300 µg desogestrel group: 28/28 men versus 22/31 men ($P < 0.05$). All Caucasian men in the 150 µg group achieved sperm concentrations of $<1 \times 10^6/\text{ml}$ whereas three men in the Shanghai group maintained sperm concentrations of $>3 \times 10^6/\text{ml}$. Fifteen men continued on this regimen for a subsequent 24 weeks: all remained azoospermic for the duration of treatment. High-density lipoprotein cholesterol fell by 15% in Caucasian men, but was unchanged in the Chinese men; both groups showed some weight gain. **CONCLUSION:** This combination of oral desogestrel with depot testosterone maintains physiological testosterone concentrations with consistent suppression of spermatogenesis to azoospermia in both Caucasian and Chinese men and therefore has many of the properties necessary for a contraceptive preparation for men.

Key words: desogestrel/male contraception/progestogen/spermatogenesis/testosterone

Introduction

Hormonal contraceptive regimes for the male are based on the suppression of pituitary gonadotrophin secretion to achieve maximal spermatogenic suppression while minimizing exogenous steroid administration. Administration of testosterone alone results in marked suppression of spermatogenesis, but approximately one-third of Caucasian men maintain low rates of spermatogenesis that result in risk of pregnancy (Barfield *et al.*, 1979; World Health Organization Task Force on Methods for the Regulation of Male Fertility, 1990, 1996). Supraphysiological testosterone concentrations also induce changes in non-reproductive androgen-sensitive target organs, with effects on skin, haemopoiesis, and serum lipoproteins, particularly high-density lipoprotein cholesterol (HDL-C) (Wu *et al.*, 1996). The co-administration of a progestogen with testosterone has been suggested to improve the degree of suppression of spermatogenesis compared with testosterone only (Bebb *et al.*, 1996; Handelsman *et al.*, 1996; Meriggiola *et al.*, 1996), and the administration of a second agent with gonadotrophin-suppressing activity allows the dose of testos-

terone to be lowered towards that of physiological replacement (Büchter *et al.*, 1999; Wu *et al.*, 1999; Martin *et al.*, 2000b). GnRH antagonists also appear promising but have been less widely investigated (Nieschlag and Behre, 1998).

Progress towards an acceptable contraceptive formulation is also hampered by the characteristics of the testosterone preparations available. Ideally, testosterone should be administered as a zero-order release preparation with a long duration of action. Many studies investigating the hormonal approach to male contraception have used relatively short-acting injections of testosterone esters, administered weekly. Testosterone pellets are an alternative preparation, providing a closer approximation to the ideal and which have been shown to be effective both alone (Handelsman *et al.*, 1992) and in combination with a progestogen (Handelsman *et al.*, 1996; Martin *et al.*, 2000b).

Therefore the major problem remains the need to suppress spermatogenesis completely, particularly in Caucasian populations, which appear relatively resistant compared with Chinese and Asian men (World Health Organization Task

Force on Methods for the Regulation of Male Fertility, 1995; Handelsman *et al.*, 1995), without adversely affecting non-reproductive androgen-dependent processes. A third aspect is the acceptability to the user of the method: we have recently demonstrated that an oral preparation is more acceptable than an injectable form among a wide range of men surveyed (Martin *et al.*, 2000a).

We have recently investigated the dose-dependency of gonadotrophin suppression using the oral progestogen desogestrel with testosterone pellets (Martin *et al.*, 2000b) and have demonstrated that repeated administration of testosterone pellets in such a regimen could maintain testosterone concentrations in the physiological range in normal men with significant suppression of spermatogenesis (Kinniburgh *et al.*, 2001). The primary objective of the present study was to investigate the degree of suppression of spermatogenesis, particularly the prevalence and maintenance of azoospermia with this regimen in two different ethnic populations.

Materials and methods

Subjects

Thirty Caucasian men aged 22–39 years (mean 31 years) were recruited in Edinburgh, Scotland and 36 Chinese men aged 25–41 years (mean 33 years) in Shanghai, People's Republic of China. All men gave informed written consent and the study received ethical approval from the appropriate local ethical review committees. The study was carried out according to International Conferences on Harmonization of Good Clinical Practice (ICH GCP) guidelines. Subjects received only reimbursement of travel expenses. None had a history of significant medical disease or abnormality on examination and screening haematological and biochemical measures were within the normal range. Subjects submitted pretreatment semen samples on two occasions at least 2 weeks apart which were assessed using WHO methodology (World Health Organization, 1992) and were regarded as normal for the local population. All men had sperm concentration of $>20 \times 10^6/\text{ml}$.

Study design and medication

The study was a prospective randomized trial comparing two doses of oral desogestrel in combination with testosterone pellets, using the same treatment protocol in two centres and designed to have sufficient power to detect differences in the prevalence of azoospermia between the desogestrel doses. Subjects were randomized equally in each centre into two treatment groups to receive either 150 µg or 300 µg desogestrel (NV Organon, Oss, The Netherlands) p.o. daily for 24 weeks. Additionally all men received 400 mg testosterone pellets (2 × 200 mg; NV Organon) inserted s.c. under local anaesthetic into the anterior abdominal wall on the first day of desogestrel treatment. This was repeated 12 weeks later. All insertions in each centre were performed by the same investigator (D.K. and H.Z.) following appropriate training.

Subjects were reviewed and examined 2 weeks after commencing medication and subsequently at 4-week intervals during the treatment phase and the recovery phase of 16 weeks after finishing desogestrel. At each visit, subjects were examined and adverse events or other health problems recorded, semen samples produced and venepuncture performed. First-void urine specimens were collected from the Edinburgh cohort pretreatment, at 12 and 24 weeks treatment, and after 16 weeks recovery. Compliance was assessed by pill returns and direct questioning of subjects. Compliance in the Shanghai cohort

was additionally assured for the first 12 weeks treatment by daily supervision by medical staff, which was discontinued thereafter by which time the reliability of the subjects had been established. Direct supervision was not possible in the Edinburgh cohort. Subjects were required to continue their current method of contraception.

After 24 weeks of treatment, subjects who were azoospermic at that time were given a choice of terminating treatment or continuing for a further 24 weeks. The aim of the extension phase was to investigate the maintenance of azoospermia over a longer time period. During this extension phase, subjects continued with oral desogestrel at the same dosage as they had taken up to that point and had further 400 mg testosterone pellets inserted at 24 weeks and 36 weeks of treatment. Clinical review, semen analysis and blood sampling continued at 4 weekly intervals throughout the extension phase. The men entering the extension phase were counselled regarding discontinuation of other forms of contraception for this phase of the study and, if they chose to do so, this was recorded. After completing a total of 48 weeks treatment these subjects entered the recovery phase. Non-azoospermic subjects and those choosing not to continue treatment commenced the 16 week recovery phase immediately after 24 weeks treatment.

Assays

Semen samples were submitted after 3–7 days abstinence. Each semen sample was assessed for sperm concentration using WHO methodology (World Health Organization, 1992). Azoospermia was confirmed by examination of the pellet following centrifugation of the ejaculate; samples with sperm seen only in the pellet were given a nominal concentration of the limit of quantification ($1 \times 10^6/\text{ml}$).

Blood samples were obtained between 0700 and 1200 h at every visit. Every 12 weeks, blood was analysed for haematological and biochemical values by routine autoanalyser. Further samples were separated by centrifugation and serum stored at -20°C until assay. Hormone assays in Edinburgh were performed as previously described (Anderson and Wu, 1996): testosterone was measured by radioimmunoassay (Corker and Davidson, 1978), and LH and FSH by time-resolved immunofluorometric assay (Delfia, Wallac, Turku, Finland). Assay sensitivities were 0.15 and 0.125 IU/l for LH and FSH respectively, and coefficients of variation (CV) were 6.9% (testosterone), 8.3% (LH) and 9.8% (FSH). Samples from individual subjects were measured in the same assay. Etonogestrel, the active metabolite of desogestrel, was measured by in-house radioimmunoassay (Organon NV). In Shanghai, testosterone was measured by radioimmunoassay (Spectria; Orion Diagnostica, Espoo, Finland) as were LH and FSH (Sino-American Joint Venture Tianjin Jiuding Medical Bioengineering Ltd, China); gonadotrophin assay sensitivities were 1.2 IU/l, assay CV were 5.8% (testosterone), 6.7% (LH) and 7.3% (FSH).

Other serum and urinary analytes were measured in Edinburgh only. Sex hormone binding globulin (SHBG) was measured by immunoradiometric assay (DPC, Los Angeles, CA, USA). Urinary epitestosterone (EpiT) was measured by capillary column gas chromatography–mass spectrophotometry (GC-MS) as previously described (Cowan *et al.*, 1991; Kicman *et al.*, 1999). Inhibin B and inhibin forms containing pro and αC (pro- αC) immunoreactivity were measured as previously described (Groome *et al.*, 1995, 1996). Assay sensitivities for inhibin B and pro- αC were 15 and 3 pg/ml respectively, and CV 15% (inhibin B) and 7.3% (pro- αC).

Serum concentrations of both total and bone-specific alkaline phosphatase (T-ALP and B-ALP) were assayed. T-ALP was measured as previously described (Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, 1974). B-ALP was measured by electrophoresis in agarose gel

following brief incubation with neuraminidase (Helena Laboratories, Sunderland, UK). Pyridinium cross-links were measured in urine by isocratic reverse phase high performance liquid chromatography (Pratt *et al.*, 1992; Crofton *et al.*, 1998). Deoxypyridinoline and pyridinoline results were expressed in relation to creatinine measured on the same urine sample.

Behavioural assessment

Sexual interest and activity were investigated pretreatment and at 12 week intervals during treatment and recovery. A structured interview was used to quantify sexual activity over the preceding 2 weeks in both centres, and the Frenken Sexual Experience Scales (SES) 2 and 3 were used to provide measures of psychosexual arousability and sexual motivation (Martin *et al.*, 2000b; Anderson *et al.*, 1992). As these scales were developed for a Caucasian population and no adequate translation or controls exist for Chinese men, the Frenken scales were not performed in Shanghai. Subjects' partners also returned a simple questionnaire at the same time points, which recorded any perceived changes in subject's mood, sexual interest and sexual activity (Martin *et al.*, 2000b).

Data analysis

Results are presented as mean \pm SEM. Hormonal data were log-transformed to correct non-equality of variance before analysis of variance (ANOVA) and sperm concentrations were cube root-transformed prior to ANOVA. Paired *t*-tests were used to investigate at what time points a significant treatment effect was seen for each group. Analysis of co-variance (ANCOVA) was used to test for an effect of dose after adjusting for baseline values if appropriate. Categorical data were analysed by Fisher's exact test.

Results

Adverse events and withdrawals

Eight men withdrew before completing 24 weeks treatment. In Edinburgh, two men in the 150 μ g group withdrew, one at 6 weeks treatment due to self-reported labile moods and one at 12 weeks due to worsening acne. In the 300 μ g group, one man withdrew at 1 week of treatment when he left the local area at short notice. In Shanghai, all five men who withdrew were in the 300 μ g group. One individual developed hypertension during the study: his blood pressure rose from 140/80 mmHg pretreatment to 170/110 after 12 weeks treatment at which point he was withdrawn from the study. His hypertension persisted following discontinuation of study medication. Three men stopped the study due to discomfort with ($n = 1$, at 12 weeks) or expulsion of ($n = 2$, at 4 and 8 weeks) the testosterone pellets. The fifth man withdrew at 12 weeks treatment due to weight gain (5 kg).

Minor adverse events included mood changes ($n = 9$), weight gain or increased appetite ($n = 5$), acne ($n = 4$), libido change with both decreases ($n = 4$) and increases ($n = 3$) reported, and implant-related ($n = 8$) which included two pellet expulsions, both in the Shanghai cohort. Other implant problems were of discomfort, with no episodes of haemorrhage or infection. There were no testosterone pellet expulsions in the Edinburgh cohort. Most minor side effects were of <1 week duration. Experience of minor side-effects was comparable between the two treatment groups. Subjects in both Edinburgh and Shanghai gained weight during the study.

Median change was +0.3 kg (range -6 to +7.3 kg) in Edinburgh and +2.5 kg (range -4 to +11.5 kg) in Shanghai. There was no significant difference in weight gained between the two treatment groups in either centre.

Fifteen men entered the extension phase of the study, four men in the 150 μ g group and four men in the 300 μ g group in Edinburgh, and six from the 150 μ g group and one from the 300 μ g group in Shanghai. Two men withdrew for personal reasons after 36 weeks treatment (both in the 150 μ g group in Edinburgh), the remaining 13 subjects completed 48 weeks treatment in total.

Compliance with oral desogestrel use was high. In the 150 μ g group in Edinburgh, subjects reported missing a median of one dose (range 0-5), and a median of 1.5 doses (range 0-11) in the 300 μ g group. Numbers of pills which were returned confirmed high levels of self-reported compliance with medication with only 1.1% of pills being returned with the exception of one subject in the 300 μ g group who returned medication amounting to 14 doses at week 20, and who admitted to a period of poor compliance between weeks 16 and 20. Etonogestrel concentrations measured in the Edinburgh centre after 20 weeks treatment were 771 ± 119 pg/ml in the 150 μ g group and 1282 ± 192 pg/ml in the 300 μ g group. Compliance in the Shanghai cohort was assured for the first 12 weeks treatment by daily supervision by medical staff. Thereafter medication was administered as in Edinburgh, and no individuals reported non-compliance of more than two doses.

Sperm concentration

Both treatment groups showed a rapid and profound suppression of sperm concentration ($P < 0.001$, Figure 1). This reduction was comparable in both ethnic groups. In particular, all men in the 300 μ g group ($n = 28$, 95% CI 86.3-100.0%) from both ethnic groups achieved azoospermia at some stage within 16 weeks of commencing treatment. While there were no significant differences in sperm concentrations between the two treatment groups within either centre at any time point, the proportion of men in Edinburgh with sperm concentrations $< 3 \times 10^6$ /ml was significantly greater in the higher dose group at 8 weeks (13/14 versus 8/15, $P = 0.03$). Thus there was some evidence that suppression was faster in the 300 μ g group than in the 150 μ g group in Edinburgh but not in Shanghai. Figure 2 shows the number of subjects with sperm concentrations of $< 3 \times 10^6$ /ml, $< 1 \times 10^6$ /ml and azoospermia during treatment in both centres. Within 12 weeks of treatment, azoospermia was achieved in 23/28 (82.1%) of the 300 μ g group and 17/32 (53.1%) of the 150 μ g group. There was a significant difference between the two treatment groups in the number of subjects achieving azoospermia ($P < 0.05$). In the 150 μ g group, 22/31 men achieved azoospermia (71.0%; 95% CI 52.0-85.8%) during the course of the study, 11/13 (84.6%) in the Caucasian group and 11/18 (61.1%) in the Chinese group, all 22 men submitting azoospermic semen samples on at least two occasions. In the 150 μ g group, all Caucasian men achieved sperm concentrations of $< 1 \times 10^6$ /ml at some stage during treatment whereas three Chinese men consistently maintained sperm concentrations $> 3 \times 10^6$ /ml. While non-

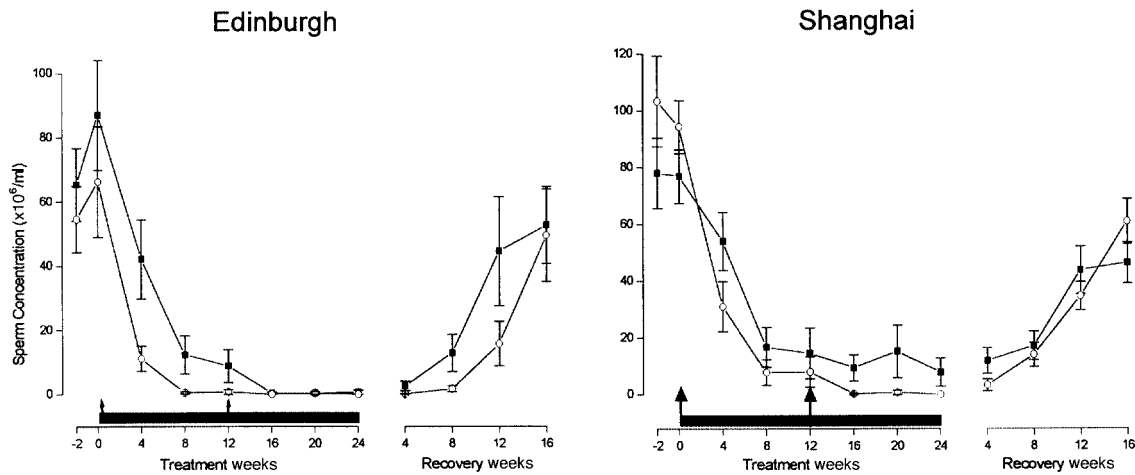


Figure 1. Sperm concentrations at pretreatment baseline, during desogestrel and testosterone administration (weeks 0–24, with bar) and following withdrawal of desogestrel treatment (subsequent weeks 4–16) in Edinburgh ($n = 14$, 150 µg group; $n = 15$, 300 µg group) and Shanghai ($n = 18$ per group). The arrows indicate the time points at which testosterone was administered, 0 and 12 weeks. ■, 150 µg group; ○, 300 µg group. Values are mean \pm SEM.

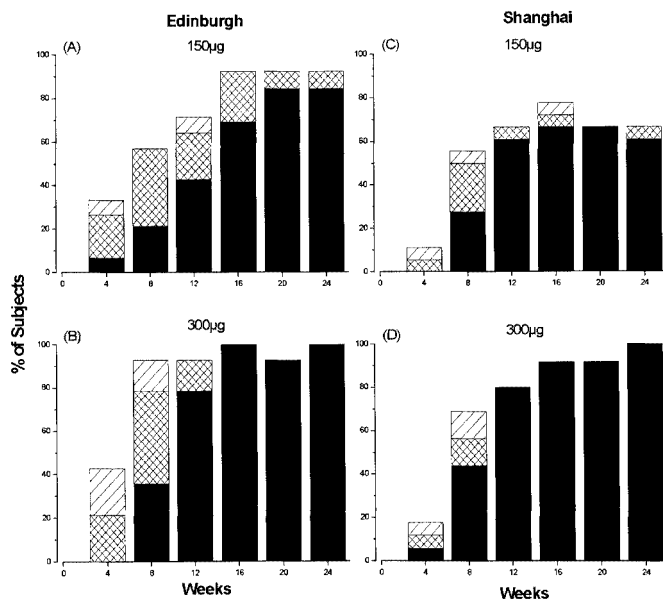


Figure 2. Suppression of spermatogenesis during desogestrel and testosterone administration for 24 weeks in Edinburgh (A and B; $n = 14$, 150 µg group; $n = 15$, 300 µg group) and Shanghai (C and D; $n = 18$ per group). Data are percentages of men achieving azoospermia (solid column) or oligozoospermia (<3 and $<1 \times 10^6$ /ml; single hatching and cross-hatching respectively) at 4 week intervals.

compliance was possible during the second 12 weeks of treatment in these three individuals, all complied fully during the first, supervised, 12 weeks. Sperm concentration remained in the normal range in these men at 12 weeks, whereas it was suppressed to $<7 \times 10^6$ /ml in all other men. Only one man in the Edinburgh cohort showed temporary recovery from azoospermia during treatment, the subject who admitted very poor compliance between weeks 16 and 20. His sperm concentration was 6.2×10^6 /ml at 20 weeks, and following improved compliance he was again azoospermic at 24 weeks. Similar results were seen in two men in the 300 µg group in Shanghai, who had sperm concentrations of 4 and 7×10^6 /ml at single

time points having been azoospermic 4 weeks previously, and who became azoospermic again thereafter.

The 15 men in the extension phase were all azoospermic at 24 weeks treatment and remained so until they ceased treatment: two men did so after 36 weeks, the remaining 13 completed 48 weeks treatment. As not all couples discontinued other forms of contraception, total exposure to risk of pregnancy was 70 months, and no pregnancies occurred. Sperm concentrations increased following discontinuation of desogestrel, and normal sperm concentrations were restored in all subjects within 16 weeks.

Testosterone

Testosterone concentrations remained within the physiological range throughout the treatment period in all groups (Figure 3). There was an initial fall at the start of treatment ($P < 0.001$ at 4 weeks versus pretreatment), with a later rise at 16 weeks following administration of the second testosterone dose at 12 weeks. Those subjects who entered the extension phase had similar testosterone concentrations in the second 24 weeks of treatment, with transient peaks at 28 and 40 weeks treatment following further testosterone placements.

Gonadotrophins

There was marked suppression in the concentrations of both LH and FSH during desogestrel administration ($P < 0.001$, Figure 4). The Edinburgh and Shanghai data are shown separately, as different assays were used in each centre. Overall there was no significant difference in either LH or FSH concentrations between the two treatment groups, but there were minor fluctuations in the 150 µg group in parallel to changes in testosterone concentrations in the Edinburgh cohort, which were not seen in the higher dose group. Thus there was a small rise in both FSH and LH at weeks 12 and 24, at the time of the nadir of testosterone concentrations. Following readministration of testosterone at 12 weeks, gonadotrophin suppression was more complete at 16 weeks ($P < 0.001$, FSH; $P = 0.01$, LH). A small rise in FSH concentrations at 12

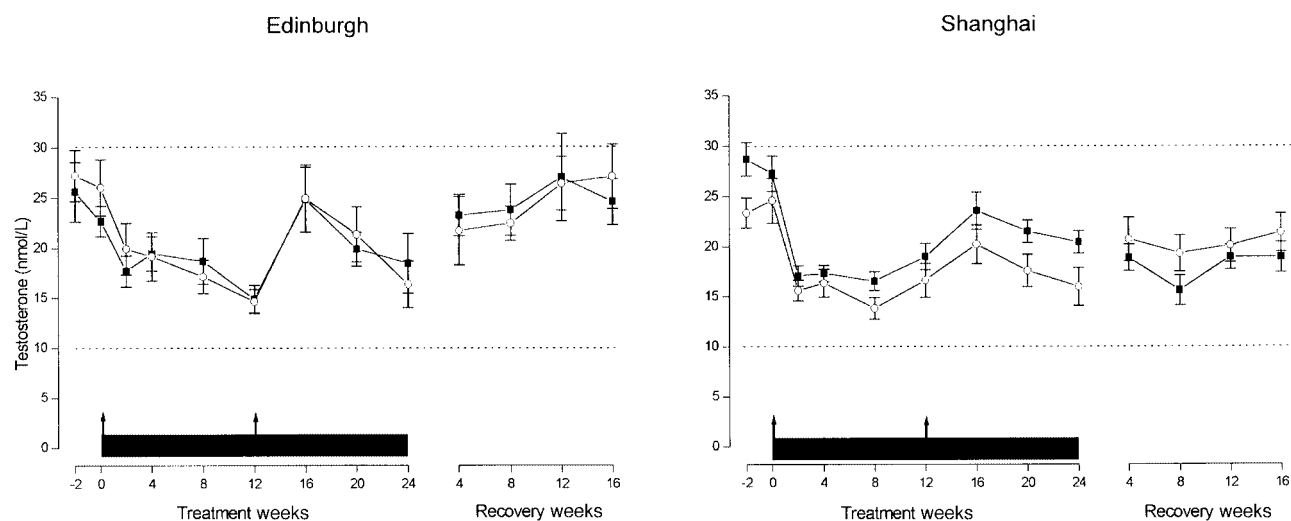


Figure 3. Testosterone concentrations in Edinburgh ($n = 14$, 150 μg group; $n = 15$, 300 μg group) and Shanghai ($n = 18$ per group) cohorts at pretreatment baseline, during desogestrel and testosterone administration (weeks 0–24 with bar) and following withdrawal of desogestrel treatment (subsequent weeks 4–16). The arrows indicate the time points at which testosterone was administered, 0 and 12 weeks and the dotted lines indicate the normal range. ■, 150 μg group; ○, 300 μg group. Values are mean \pm SEM.

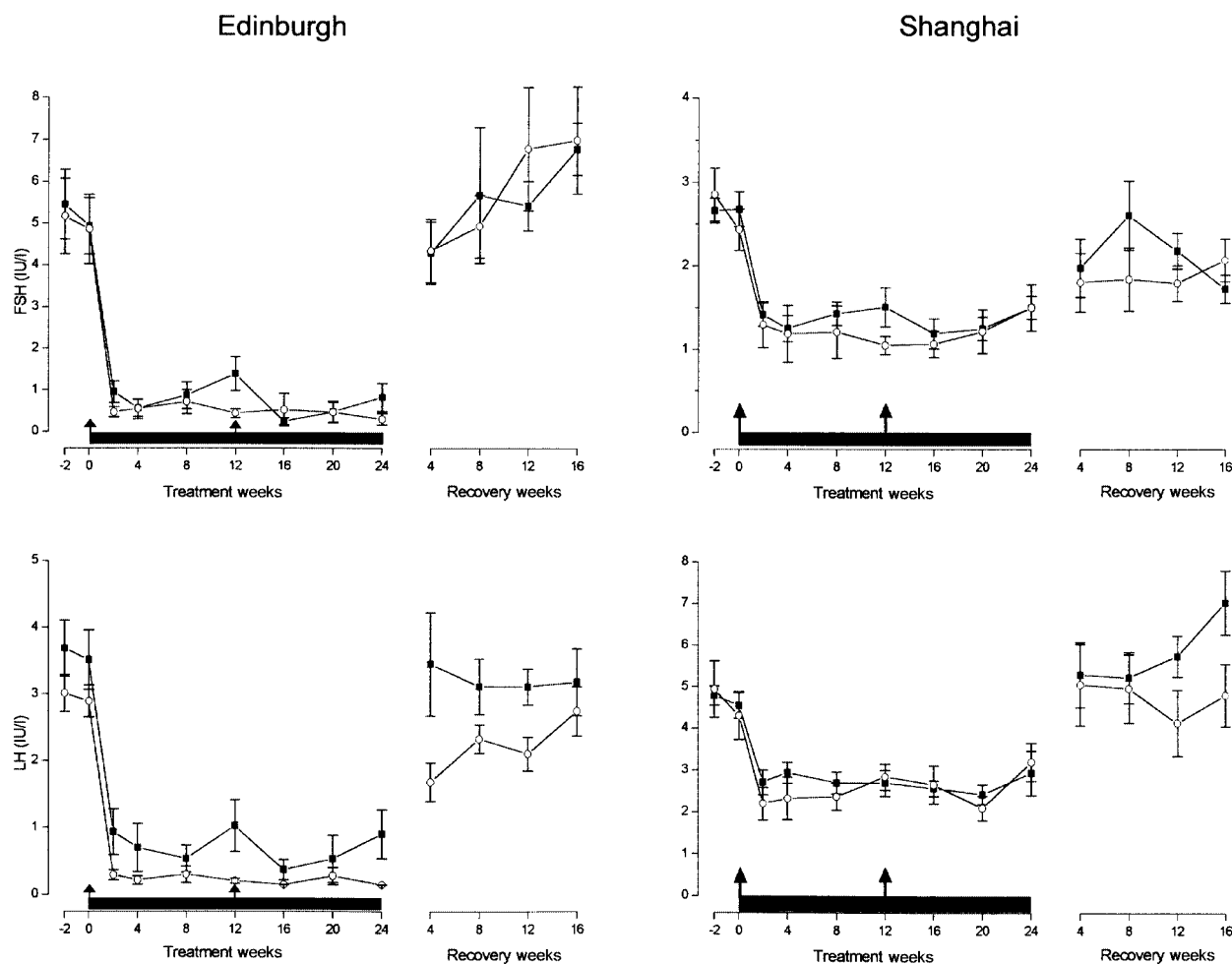


Figure 4. Gonadotrophin concentrations in Edinburgh ($n = 14$, 150 μg group; $n = 15$, 300 μg group) and Shanghai ($n = 18$ per group) cohorts at pretreatment baseline, during desogestrel and testosterone administration (weeks 0–24, with bar) and 16 weeks following withdrawal of desogestrel treatment (subsequent weeks 4–16). The arrows indicate the time points at which testosterone was administered, 0 and 12 weeks. ■, 150 μg group; ○, 300 μg group. Values are mean \pm SEM.

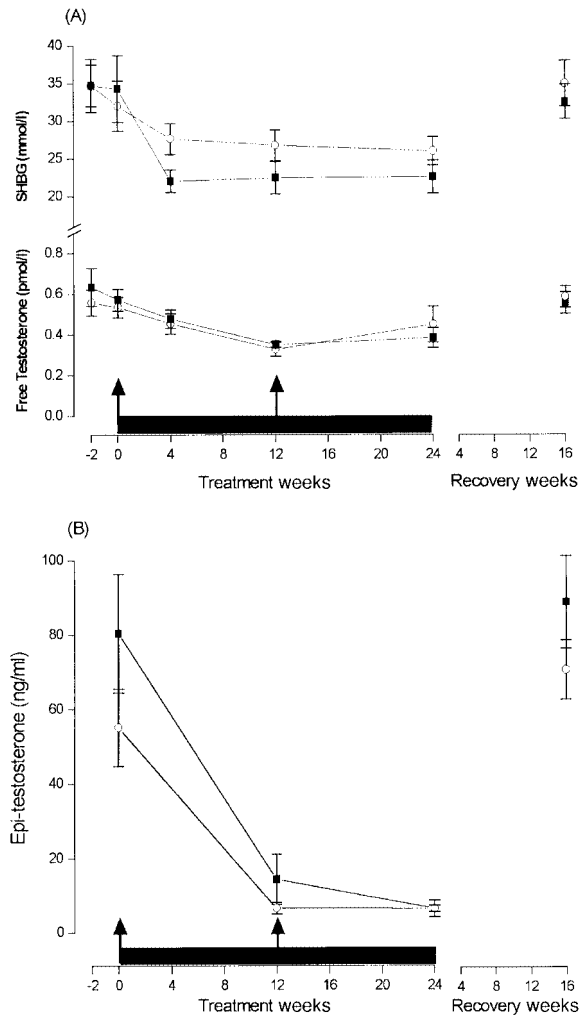


Figure 5. (A) Sex hormone binding globulin (SHBG) concentrations and calculated free testosterone concentrations and (B) Epi-testosterone concentrations in the Edinburgh cohort at pretreatment baseline, during desogestrel and testosterone administration (weeks 0–24, with bar) and following withdrawal of desogestrel treatment (subsequent weeks 4–16). The arrows indicate the time points at which testosterone was administered, 0 and 12 weeks. ■, 150 µg group, $n = 14$; ○, 300 µg group, $n = 15$. Values are mean \pm SEM.

weeks was also observed in the Shanghai cohort but this did not reach statistical significance, and no fluctuation in LH was observed. Incomplete suppression of spermatogenesis was associated with lesser suppression of gonadotrophins, particularly in the Edinburgh cohort as a result of the more sensitive assays used. Thus concentrations of both LH and FSH were significantly higher in those subjects with sperm concentrations of $>1 \times 10^6/\text{ml}$ than those with greater suppression at 12 weeks of treatment (LH 2.68 ± 0.71 versus 0.84 ± 0.30 IU/l, $P = 0.016$; FSH 2.40 ± 0.62 versus 0.17 ± 0.02 IU/l, $P < 0.001$). The two subjects who were not azoospermic at the end of treatment had readily detectable LH (1.84 and 0.84 IU/l) and FSH (both >2.4 IU/l) concentrations.

In view of the differences in sensitivity between the assays used in the two centres, Shanghai samples from pretreatment

and 12 and 24 weeks treatment were shipped to Edinburgh for repeat assay. The main objective of this was to investigate whether those samples reported as 'undetectable' remained so using the more sensitive Delfia assays. There was a very high correlation between LH and FSH concentrations as determined by the two assay methodologies ($r = 0.88$ for LH; $r = 0.91$ for FSH) using pretreatment samples. Both LH and FSH were consistently suppressed to undetectable concentrations in the 300 µg group, thus demonstrating that the degree of suppression of LH and FSH in Shanghai was similar to that in Edinburgh. This also allowed confirmation that gonadotrophin suppression was less complete in those three men in the Shanghai cohort who maintained significant sperm concentrations during treatment (LH >0.6 IU/l, FSH >2 IU/l, median value of rest of the group at the limit of sensitivity for both gonadotrophins). Non-compliance with study medication was unlikely for the initial 12 weeks of treatment as it was directly supervised, and was denied by the subjects during the later stages of the study.

Sex hormone binding globulin (SHBG)

SHBG concentrations in the Edinburgh cohort fell significantly during treatment ($P = 0.042$, 150 µg group; $P = 0.001$, 300 µg group) followed by recovery to initial levels 16 weeks after end of treatment (Figure 5). There was no significant difference in SHBG concentrations between the two groups. Despite the fall in SHBG, calculated free testosterone concentrations (Vermeulen *et al.*, 1999) at 12 and 24 weeks treatment were significantly lower than pretreatment concentrations in both groups ($P < 0.003$, 150 µg group; $P < 0.001$, 300 µg group; Figure 5). There were no differences between groups and free testosterone concentrations at 16 weeks recovery were not significantly different from pretreatment.

Epi-testosterone

EpiT concentrations in urine showed a marked reduction in both treatment groups during treatment ($P < 0.001$ in both groups), but remained detectable in all men at both 12 and 24 weeks treatment (Figure 5). There were no differences between groups, nor were EpiT concentrations higher in the small number of men with continuing detectable spermatogenesis. EpiT concentrations at 16 weeks recovery were not significantly different to pretreatment concentrations.

Inhibin

Concentrations of pro- α C inhibin forms and dimeric inhibin B in both serum and seminal plasma for the Edinburgh centre are shown in Figure 6. Inhibin B in peripheral blood showed no significant changes over the study period in either group. Pro- α C inhibin fell markedly during treatment with concentrations dropping to approximately half of pretreatment levels after 4 weeks treatment in both groups ($P < 0.001$) with no differences between groups, which was maintained for the duration of treatment. There was a prompt recovery of pro- α C concentrations on discontinuing desogestrel. Seminal plasma inhibin B concentrations showed a marked variation between individual subjects, ranging from 45 pg/ml to 10 500 pg/ml pretreatment. During treatment, concentrations fell ($P = 0.018$, 150 µg group; $P = 0.011$, 300 µg group). Although inhibin B

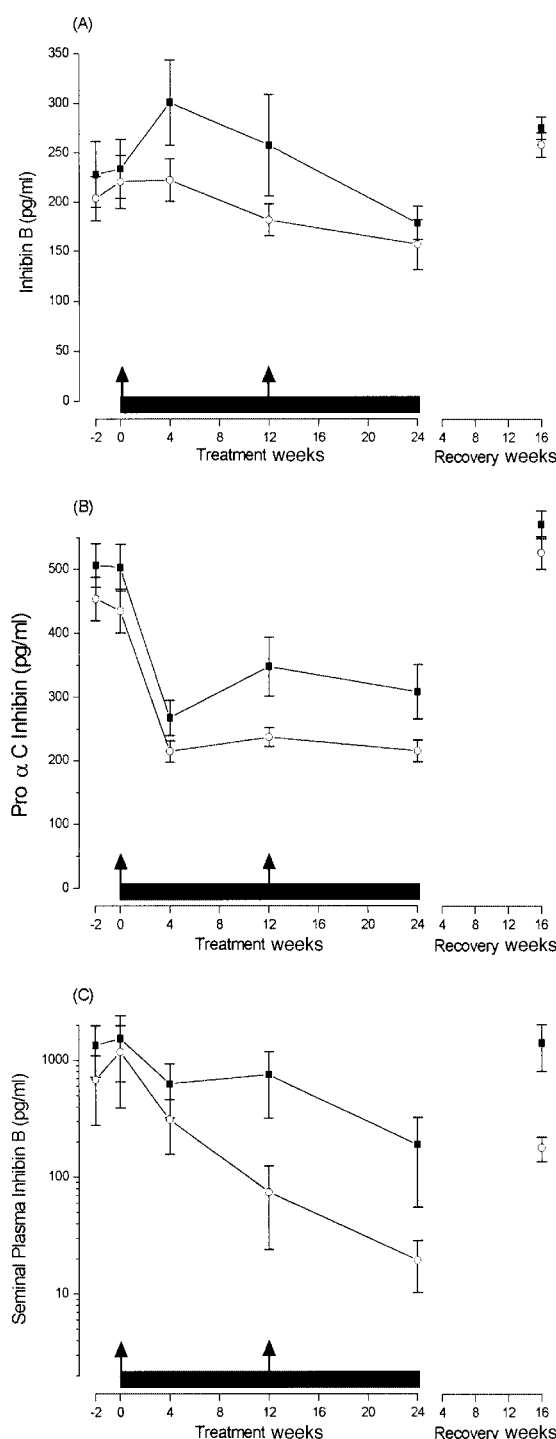


Figure 6. (A) Peripheral plasma inhibin B, (B) pro- α C, and (C) seminal plasma inhibin B concentrations in the Edinburgh cohort at pretreatment baseline, during drug administration (weeks 0–24, with bar) and following withdrawal of desogestrel treatment (subsequent weeks 4–16). The arrows indicate the time points at which testosterone was administered, 0 and 12 weeks. ■, 150 µg group, $n = 14$; ○, 300 µg group, $n = 15$. Values are mean \pm SEM.

concentrations in seminal plasma did not differ statistically between the groups during treatment, it became undetectable in 7/13 men in the 150 µg group and 10/12 men in the 300 µg group by 24 weeks: samples were not available for assay from the remaining men.

Lipids and haematological analytes

There were no differences between the 150 µg and 300 µg groups in total cholesterol or HDL-C concentrations within each centre, therefore the data were analysed as a single group for each centre. Caucasian subjects showed a significant decline in both HDL-C ($P = 0.026$) and total cholesterol ($P = 0.027$) during treatment; these then recovered to pre-treatment concentrations following cessation of desogestrel administration (Table I). Chinese men did not show these patterns: both total cholesterol and HDL-C did not change significantly during the course of the study (Table I). LDL-C and triglyceride concentrations were unchanged during treatment in both centres (data not shown).

There was no significant change in haemoglobin concentrations and haematocrit values through the duration of the study (Table I). There were no significant alterations or abnormalities in any of the clinical biochemical analytes.

Bone markers

Both treatment groups (150 and 300 µg desogestrel) showed similar significant reductions in the concentrations of both total and bone-specific alkaline phosphatase concentrations and have therefore been analysed together (T-ALP, $P < 0.001$; B-ALP $P = 0.006$; Table I). These falls were evident by 4 weeks of treatment and recovered to pretreatment levels following discontinuation of desogestrel. Urinary deoxypyridinoline and pyridinoline concentrations were unchanged throughout the study period (Table I).

Behavioural assessments

There were no differences in frequency of sexual intercourse or SES scores between the two treatment groups pretreatment or during treatment, and the results are therefore presented grouped together for each centre (Table II). There was no significant change in the reported frequency of sexual intercourse or in the behavioural questionnaires during the study.

Eighteen partners returned questionnaires describing possible effects on subjects' moods at the end of 24 weeks of treatment. Eight partners reported that the subjects had increased interest in sex; none had a fall in interest in sex. The majority of partners reported no change in subject's mood states, the exception being six partners reporting increases in irritability and four reporting increased tenseness, whilst two partners reported a reduction in both mood states. These two mood states were highly correlated, with all those reporting an increase in tenseness also recording an increase in irritability. Overall 15/18 (83%) partners responded that they were happy with the use of this regime.

Discussion

This study reports the effects of repeated administration of testosterone pellets with oral desogestrel for up to 12 months as a prototype hormonal male contraceptive. Both desogestrel dosage groups showed profound suppression of sperm concentration, and azoospermia was achieved in all subjects taking 300 µg desogestrel. The prevalence of azoospermia was 71% in the 150 µg desogestrel group, with all men in the Edinburgh cohort and 15/18 men in the Shanghai cohort achieving sperm

Table I. Metabolic parameters in the Edinburgh and Shanghai cohorts pretreatment, during treatment (12 and 24 weeks), and 16 weeks following discontinuation of desogestrel

	Pretreatment	12 weeks	24 weeks	Post-treatment
Shanghai				
Haemoglobin (g/l)	143 ± 3	140 ± 3	143 ± 3	146 ± 4
Total cholesterol (mmol/l)	3.55 ± 0.16	4.20 ± 0.14	4.21 ± 0.14	4.17 ± 0.12
HDL-cholesterol (mmol/l)	1.24 ± 0.07	1.32 ± 0.05	1.20 ± 0.08	1.31 ± 0.05
Edinburgh				
Haemoglobin (g/l)	156 ± 2	151 ± 2	155 ± 3	155 ± 2
Haematocrit	43.7 ± 0.6	42.3 ± 0.5	44.4 ± 0.8	43.8 ± 0.7
Bone ALP (mmol/l)	70 ± 5	59 ± 4**	66 ± 5	84 ± 6
Total ALP (mmol/l)	147 ± 7	116 ± 6**	124 ± 7**	157 ± 8
Pyridinoline (nmol/nmol)	25.6 ± 2.3	26.2 ± 2.5	23.5 ± 2.0	26.4 ± 2.5
Deoxypyridinoline (nmol/nmol creatinine)	3.2 ± 0.5	4.5 ± 0.7	3.6 ± 0.4	4.7 ± 1.1
Total cholesterol (mmol/l)	4.76 ± 0.12	4.50 ± 0.17**	4.41 ± 0.16**	5.08 ± 0.17
HDL-cholesterol (mmol/l)	1.33 ± 0.05	1.20 ± 0.05*	1.11 ± 0.06**	1.28 ± 0.04

Edinburgh, $n = 14$, 150 µg group; $n = 15$, 300 µg group. Shanghai, $n = 18$ per group. Values are mean ± SEM.

Bone markers were not measured in the Shanghai cohort.

* $P < 0.01$, ** $P < 0.001$ versus pretreatment.

HDL = high-density lipoprotein; ALP = alkaline phosphatase.

Table II. Behavioural data in the Edinburgh and Shanghai cohorts pretreatment, during treatment (12 and 24 weeks), and 16 weeks following discontinuation of desogestrel

	Pretreatment	12 weeks	24 weeks	Post-treatment
Shanghai				
Sexual intercourse	2.7 ± 0.4	2.5 ± 0.3	2.7 ± 0.3	2.6 ± 0.3
Edinburgh				
Sexual intercourse	2.1 ± 0.4	3.4 ± 0.5	2.6 ± 0.6	3.4 ± 1.0
SES-2	-0.85 ± 0.08	-0.88 ± 0.14	-0.90 ± 0.08	-0.93 ± 0.22
SES-3	-0.07 ± 0.13	-0.26 ± 0.07	-0.20 ± 0.13	-0.29 ± 0.19

Values are mean ± SEM.

The two treatment groups are reported together as there were no significant differences between them.

Sexual intercourse is number of occasions reported in the preceding 2 weeks.

SES = Frenken Sexual Experience Scales.

concentrations of $<1 \times 10^6$ /ml. The higher dose of progestogen also resulted in faster suppression in the Edinburgh cohort. All men entering the extension phase of the study maintained azoospermia for the duration of treatment. While these findings require to be confirmed in larger numbers of men, the demonstration here of consistent induction and maintenance of azoospermia as required for a feasible contraceptive method provides considerable support for the further investigation of oral desogestrel with depot testosterone. The rapidity of onset of azoospermia observed here is consistent with defects in spermiogenesis having an important role during the early stages of treatment (Saito *et al.*, 2000) and may also underlie the rapid reappearance and subsequent loss of sperm from the ejaculate of the individual with temporary poor compliance. Weight gain has been previously reported in one study of desogestrel with testosterone (Anawalt *et al.*, 2000) but was not found in another (Wu *et al.*, 1999) and was also noted in a recent study of testosterone undecanoate with norethisterone enanthate (Kamischke *et al.*, 2001). In the present study, weight gain was more consistently found in the Chinese group, independent of dose of desogestrel. Whether this is a differential response to the progestogen is uncertain.

The administration of injectable testosterone esters results in azoospermia in approximately two-thirds of Caucasian men, and in a greater proportion of Chinese men (World Health Organization Task Force on Methods for the Regulation of Male Fertility, 1995). The co-administration of a progestogen allows the dose of testosterone to be reduced (Foegh *et al.*, 1980; Bebb *et al.*, 1996; Handelsman *et al.*, 1996; Meriggiola and Bremner, 1997). It remains necessary to avoid the supra-physiological concentrations resulting from the currently available testosterone esters (Behre and Nieschlag, 1998) and to increase the prevalence of azoospermia particularly in Caucasian populations. Testosterone pellets provide reproducible plasma testosterone concentrations without the need for frequent administration (Handelsman, 1998) and our preliminary data using testosterone pellets with oral desogestrel suggested that this combination might result in the necessary degree of suppression of spermatogenesis (Martin *et al.*, 2000b). The main limitations of testosterone pellets are the need for a minor procedure for insertion, and occasional pellet extrusion. Extrusion was infrequent in the present study, within the range reported by other experienced groups (Handelsman, 1998). While it is likely that long-acting injectable testosterone

preparations (Nieschlag *et al.*, 1999; Zhang *et al.*, 1999) may be preferred, testosterone pellets are a valuable prototype of a depot preparation.

The ethnic variability in response to hormonal male contraceptive regimens indicates the importance of studying such regimens in different populations. However, we found no marked differences in the degree of suppression of spermatogenesis between Caucasian and Chinese recruits with the present regimen. Indeed, surprisingly, there was a trend towards greater suppression in the Caucasian cohort in the 150 µg group. A testosterone-only group was not included in the design: the same low dose of testosterone pellets administered alone to Caucasian men has been clearly shown to have no suppressive effect on spermatogenesis (Handelsman *et al.*, 2000). However, it should be noted that the additive effect of progestogen has not been clearly demonstrated in Asian men and was not assessed in the present study. Although differences in drug compliance between centres cannot be excluded as the basis for non-suppression in some Chinese men, these data imply that the differences previously obtained with testosterone-only administration (World Health Organization Task Force on Methods for the Regulation of Male Fertility, 1995) may not be a characteristic of all contraceptive regimens. While there appear to be ethnic differences in susceptibility to spermatogenic suppression, some regimens such as that investigated here might override any such differences.

Gonadotrophin secretion was profoundly suppressed during treatment, to consistently undetectable concentrations in the 300 µg group. Suppression was less complete in the 150 µg group, although both LH and FSH were undetectable in most men in most samples. There were, however, significant fluctuations in both LH and FSH in the 150 µg group with a reciprocal relationship to changes in testosterone concentrations. Thus gonadotrophins tended to rise, i.e. escape from suppression, at 12 and 24 weeks at the nadir of testosterone concentrations. These data illustrate the dose-dependency of gonadotrophin suppression by desogestrel, but also clearly show the significant contribution of fluctuations in testosterone concentrations, revealed with the sub-maximal desogestrel dose. These data show that at this threshold dose of testosterone the undetectable decrease in testosterone absorption in the third compared with the first month following insertion (Handelsman, 1998) is of considerable importance to the degree of gonadotrophin suppression achieved. Gonadotrophin concentrations were less completely suppressed in those men who maintained detectable sperm concentrations. It is thus probable that, with a slightly higher testosterone dose, suppression of spermatogenesis in the 150 µg group would have been more complete. Etonogestrel concentrations were comparable with those reported elsewhere with the same doses (Wu *et al.*, 1999; Anawalt *et al.*, 2000) and subjects maintaining spermatogenesis had similar concentrations to azoospermic subjects. In Shanghai, desogestrel was administered by study personnel daily for the first 12 weeks of treatment. Although it is possible that non-compliance thereafter contributed to the lack of suppression to azoospermia in some men, these men showed significant sperm concentrations throughout the treatment period. It is therefore more likely that the incomplete

suppression of spermatogenesis in the group receiving 150 µg desogestrel is due to the dose being insufficient for maximal effect. Testosterone pellet extrusion did not contribute to lack of efficacy, as it did not occur in any men in the 150 µg group.

Measurement of other testicular products may provide insight into the mechanism of suppression of spermatogenesis by this regime. Inhibin B is a product of Sertoli cells and has a role in pituitary feedback on gonadotrophin secretion (Anderson and Sharpe, 2000). Evidence from some contraceptive studies has demonstrated a fall in inhibin B with suppression of spermatogenesis (Anawalt *et al.*, 1996; Anderson *et al.*, 1997b). However, in this study, serum concentrations were maintained despite azoospermia being achieved in all subjects in the 300 µg desogestrel group, consistent with our previous finding in a short-duration study of this regime (Martin *et al.*, 2000b). In contrast, pro-αC concentrations fell rapidly during treatment, indicating the closer relationship between gonadotrophins and pro-αC secretion. Seminal plasma also contains dimeric inhibin B. The significant correlation between seminal inhibin B concentration and sperm concentration suggests that it may reflect the activity of the seminiferous epithelium (Anderson *et al.*, 1998). A marked reduction in the concentration of seminal plasma inhibin B in those subjects who became azoospermic (Martin *et al.*, 2000b) was confirmed with the current study. The disparity between systemic and seminal inhibin B concentrations demonstrates that serum inhibin B concentration is a poor marker of spermatogenesis in this context. The basis for the lack of change in serum inhibin B during gonadotrophin suppression is unclear. It is possible that this reflects the loss of integrity of inter-Sertoli-cell junctions (Kerr, 1991; Muffly *et al.*, 1994), preventing the normal regulation of the bi-directional secretion of inhibin B and allowing the high concentrations within the seminiferous tubule access to the interstitial space.

EpiT is a natural epimer of testosterone secreted predominantly by the testis (Dehennin and Matsumoto, 1993; Kicman *et al.*, 1999). In our previous study in which 24 h urine collections were analysed, EpiT excretion fell to a similar degree to that reported here (Anderson *et al.*, 1997a). 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). EpiT remained detectable in all men during treatment with concentrations severalfold higher than found in hypogonadal men (Kicman *et al.*, 1999). It therefore appears that there remains a low rate of steroidogenesis within the testis during desogestrel/testosterone treatment. The small number of men who did not become azoospermic precludes statistical analysis of EpiT excretion by that variable, but there was no evidence of higher excretion in those men during treatment.

The completeness of suppression of spermatogenesis with the present regimen precludes comparison of responders with non-responders, but comparison may be made with other regimens. A major difference may lie in the characteristics of the testosterone preparation used. In a previous study using oral desogestrel with injectable testosterone enanthate, it appeared that azoospermia was more prevalent in groups receiving lower doses of testosterone (Wu *et al.*, 1999) although group sizes were small. The high prevalence of azoospermia in the present study may therefore

be related to the avoidance of high post-injection testosterone concentrations, as well as the relatively low levels of testosterone overall. The dose of testosterone administered here (400 mg/12 weeks) was calculated to explore the possibility of replacement in the low physiological range, in contrast to the majority of studies in this field which have used higher doses of testosterone. Direct determination of testosterone absorption from these pellets indicates that this dose will release 2.6 mg testosterone per day (Handelsman, 1998), i.e. lower than most estimates of normal production rates (Southren *et al.*, 1968; Vierhapper *et al.*, 1997; Santner *et al.*, 1998). Consistent with this, there was an initial fall in total testosterone concentrations, to ~60% of the pretreatment concentration at 12 weeks. However, as testosterone pellets are fully bioavailable over ~6 months (Handelsman, 1998), repeated administration as in the present protocol involving administration on up to four occasions should mean that the steady-state dose release is ~4.8 mg/day. In fact, testosterone concentrations in those men who received treatment for 48 weeks continued to show very similar fluctuations to that seen in the first 24 weeks. It should, however, be noted that pretreatment samples were taken in the morning, and thus may be higher than the 24 h average in healthy young men (Plymate *et al.*, 1989).

It is important to consider whether maintenance of testosterone concentrations in the low physiological range as here might have adverse or beneficial effects on other reproductive and non-reproductive androgen-sensitive target organs including liver metabolism, haematopoiesis, muscle and bone. Regimens involving administration of testosterone alone or with progestogens to men generally result in potentially adverse changes in lipoproteins, particularly HDL-C (Wallace and Wu, 1990; Bebb *et al.*, 1996; Wu *et al.*, 1996). A decrease in HDL-C and total cholesterol were seen in the Edinburgh but not Shanghai cohorts. Similarly Chinese men did not show changes in lipoprotein concentrations during testosterone-only treatment (Wu *et al.*, 1996). Haematological parameters may be a sensitive index of androgen dosage, and no changes were detected. This contrasts with the increase in haemoglobin and haematocrit observed during supraphysiological testosterone treatment (Wu *et al.*, 1996), and the decrease during testosterone/cyproterone acetate treatment (Meriggiola *et al.*, 1996). Muscle mass and strength were not addressed in this study but possible long-term effects are clearly important. Changes in bone metabolism may also be of value in the investigation of the non-reproductive effects of contraceptive steroid administration as an index of androgenicity. Few studies of male contraceptive steroid regimens have investigated possible effects on bone turnover. One study using testosterone alone showed increases in biochemical markers of bone turnover and also in bone mineral density (Young *et al.*, 1993). Hypogonadism in men results in increased B-ALP activity and urinary excretion of pyridinoline and deoxypyridinoline, indicating increases in both bone formation and resorption, and conversely testosterone treatment of hypogonadal men reduces these parameters and results in increased bone mineral density (Anderson *et al.*, 1997). In the present study bone alkaline phosphatase was reduced, while urinary deoxypyridinoline and pyridinoline were unchanged. A fall in total alkaline

phosphatase has recently been reported in a study in normal men involving administration of a larger dose of testosterone with norethisterone (Kamischke *et al.*, 2001). Changes in these biochemical markers cannot be readily extrapolated to indicate changes in bone mineral density, and the clinical relevance of these findings is uncertain. Biochemical assessment of the adequacy of testosterone replacement other than by testosterone concentrations is uncertain (Bhasin *et al.*, 1998), yet is becoming increasingly important as both contraceptive regimens and hypogonadal replacement therapies may include androgens other than testosterone itself (Sundaram *et al.*, 1993; Anderson *et al.*, 1999; Negro-Vilar, 1999).

In conclusion, oral desogestrel with a long-acting testosterone preparation results in a very high prevalence of azoospermia, which can be maintained for a prolonged period of time. The need for improved testosterone preparations remains, but these data indicate that this is a promising approach to the development of a valid method that will meet the requirements of men and women (Glasier *et al.*, 2000; Martin *et al.*, 2000a).

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