

1 **7 α -methyl-19-nortestosterone (MENT) vs. testosterone in combination with**
2 **etonogestrel implants for spermatogenic suppression in normal men.**

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33 **Abstract**

34 Testosterone with a progestogen can suppress spermatogenesis for contraception. The synthetic
35 androgen 7 α -methyl-19-nortestosterone (MENT) may offer advantages as it is resistant to 5 α -
36 reduction and is therefore less active at the prostate. This study aimed to investigate MENT
37 implants in combination with etonogestrel on spermatogenesis, gonadotropins, and androgen-
38 dependent tissues in comparison with a testosterone/etonogestrel regimen.

39 Normal men (n=29) were recruited and randomized to receive 2 etonogestrel implants with either
40 600mg testosterone pellets repeated every 12 wk or 2 MENT implants, for up to 48 wk.

41 Testosterone concentrations in the testosterone group remained in the normal range. Subjects
42 with 2 MENT implants showed peak MENT levels at 4 wk with testosterone concentrations
43 2nmol/L. Sperm concentrations fell rapidly to $<1 \times 10^6$ /ml at 12 wk in 8/10 subjects in the MENT
44 group and 13/16 subjects in the testosterone group with equally suppressed gonadotropins.
45 Thereafter suppression was not maintained in the MENT group and 6 men noted loss of libido.
46 14 men completed 48 wk of testosterone treatment and all became azoospermic. Hemoglobin
47 concentrations rose and HDL-C fell in both groups. The MENT group showed a fall in PSA
48 with no change in bone mass.

49 MENT with a progestogen can achieve rapid suppression of spermatogenesis similar to
50 testosterone but this promising result was not sustained due to a decline in MENT release from
51 the implants. This dose of testosterone, compared to previous studies using a lower dose with a
52 higher dose of etonogestrel, had non-reproductive side effects without any increase in
53 spermatogenic suppression. These data indicate the importance of the doses of progestogen and
54 testosterone for optimum spermatogenic suppression while minimising side effects.

55 **Key Words: male contraception androgen progestogen**

56

57 **Introduction**

58 Hormonal methods of contraception for men are based on suppression of gonadotrophins and
59 hence spermatogenesis. Administration of testosterone alone provides effective reversible
60 contraception (World Health Organisation Task Force on Methods for the Regulation of Male
61 Fertility, 1990; World Health Organisation Task Force on Methods for the Regulation of Male
62 Fertility, 1996) but azoospermia is not universally achieved and the dose of testosterone
63 produces unwanted side effects. Administration of a progestogen with testosterone increases
64 spermatogenic suppression while allowing a reduction in the dose of testosterone towards that of
65 physiological replacement (Anderson and Baird, 2002; Kamischke and Nieschlag, 2004).

66

67 An alternative approach is the use of a synthetic androgen. Testosterone is a pro-hormone in
68 many tissues, converted by 5α -reductase to dihydrotestosterone (DHT) e.g. in the prostate and
69 by aromatase to estradiol e.g. in bone. Tissue selectivity can thus be conferred by altered
70 susceptibility to conversion by these enzymes. This is exemplified by 7α -methyl-19-
71 nortestosterone (MENT), which is resistant to 5α -reduction but a substrate for aromatase
72 (Agarwal and Monder, 1988; LaMorte *et al.*, 1994). Theoretically, this will result in relative
73 sparing of the prostate while maintaining other androgen-dependent functions (Sundaram *et al.*,
74 1993). MENT Ac implants induce suppression of the hypothalamo-pituitary-testicular axis in
75 normal men (Noé *et al.*, 1999; von Eckardstein *et al.*, 2003) and data to support relative sparing
76 of the prostate have been obtained in non-human primates (Cummings *et al.*, 1998) and
77 hypogonadal men (Anderson *et al.*, 2003).

78

79 While many studies have explored a range of preparations of testosterone and progestogens,
80 there are limited data on the interaction between doses (Grimes *et al.*, 2004). We have previously
81 demonstrated that an implant formulation of the progestogen etonogestrel with long-acting
82 testosterone pellets results in dose-dependent suppression of spermatogenesis with minimal non-
83 reproductive effects (Anderson *et al.*, 2002; Brady *et al.*, 2004). The present study was designed
84 to investigate the effectiveness and side effects of MENT as the androgen component of a
85 prototype male contraceptive regimen involving 2 etonogestrel implants in comparison with a
86 testosterone-based regimen. The dose of testosterone was slightly larger than that which we had
87 previously shown to be highly effective at suppressing spermatogenesis when combined with an
88 optimal number of etonogestrel implants (3, (Brady *et al.*, 2004)).

89

90 **Methods**

91 *Subjects*

92 Twenty-nine healthy Caucasian men, mean age 34.1 years (range 23-50) were recruited as
93 previously (Anderson *et al.*, 2002; Kinniburgh *et al.*, 2002; Brady *et al.*, 2004). Inclusion criteria
94 included good mental and physical health, body mass index (BMI) between 18-32kg/m², normal
95 FSH, LH and testosterone concentrations and standard biochemical and hematological variables,
96 and normal physical examination. Blood pressure was measured in the sitting position after 5
97 minutes rest using an automated device, and was required to remain < 140mmHg systolic and <
98 90mmHg diastolic for the duration of the study. Two pre-treatment semen samples were required
99 with sperm concentration >20x10⁶/ml with motility and morphology within local normal limits.
100 All participants provided written informed consent. The study was approved by Lothian
101 Research Ethics Committee.

102

103 *Study design and medication*

104 The study was a randomised, open-label trial investigating MENT implants or testosterone in
105 combination with etonogestrel implants, both groups scheduled for treatment for 48 wks.
106 Following two screening visits subjects were randomised using a computer-generated list and
107 numbered sealed envelopes. Subjects in the MENT group were administered 2 implants each
108 containing 135 mg MENT acetate formulated as in previous studies to release ~400µg of MENT
109 Ac/implant/day (Anderson *et al.*, 2003; von Eckardstein *et al.*, 2003) s.c with local anaesthesia
110 into the medial aspect of one arm, and 2 etonogestrel implants 4cm long, each containing 68mg
111 of etonogestrel (Implanon®, N.V Organon, Oss, The Netherlands) into the other upper arm. The
112 testosterone group were administered 600 mg testosterone pellets (3x200mg, N.V Organon) s.c.
113 into the anterior abdominal wall, repeated at wks 12, 24 and 36, with 2 etonogestrel implants at
114 first administration of testosterone. The etonogestrel and MENT implants were removed at the
115 end of the treatment period at which time the subjects entered the recovery phase.

116

117 Subjects were reviewed at 4 wk intervals. The number of episodes of sexual activity (sexual
118 intercourse and masturbation) over the preceding two weeks were recorded by interview at 12-
119 wk intervals and at 16 wks of the recovery phase at which times physical examination was also
120 performed, and testicular volume was determined using the Prader orchidometer. All subjects
121 were followed up until two semen samples had been submitted with sperm concentrations above
122 $20 \times 10^6/\text{ml}$.

123

124 Transrectal ultrasound using a biplanar probe (Eccoccee, Toshiba, Stirling, UK) was used to
125 measure total prostate volume (3.14/6 x antero-posterior x transverse x longitudinal
126 measurements). The coefficient of variation for repeated measurement was 9.8%. Bone mineral
127 density at the lumbar spine and hip was determined using a QDR-4500A (Hologic Inc, Bedford,
128 MA) with coefficient of variation 1.3%.

129

130 *Assays*

131 Serum was stored at -20°C until assay. Testosterone, FSH, LH and estradiol were measured by
132 time-resolved immunofluorometric assay (DELFLIA, Wallac). Assay sensitivity was 0.3 nmol/L
133 for testosterone, 0.05IU/L for FSH and LH and 50pmol/L for estradiol. Intra-assay coefficients
134 of variation (CVs) were $<7\%$, inter-assay CVs were $<5\%$ for FSH and LH $<10\%$ for testosterone
135 and estradiol. Inhibin B was assayed using a modification of the previously described assay
136 (Groome *et al.*, 1996; Anderson *et al.*, 1998) using an improved primary antibody (46A/F).
137 Results are quantified against the WHO inhibin B standard, and show a very high correlation
138 with the previous method although are generally higher. Assay sensitivity is 7pg/ml with inter-
139 and intra-assay CVs $<10\%$. MENT in serum and in the implants following removal was
140 measured by radioimmunoassay (Kumar *et al.*, 1990; Suvisaari *et al.*, 1997) with intra-assay CV
141 3.8-7.9% and inter-assay CV 8.0-12.3%. Due to cross-reactivity with testosterone and other
142 serum factors a value of 0.39 ± 0.01 nmol/L is seen in samples prior to MENT treatment.
143 Samples were analysed for biochemical and haematological parameters by autoanalyser at 12
144 weekly intervals.

145

146 ***Semen Analysis***

147 Semen samples were submitted following 3-7 days abstinence and sperm concentration
148 determined (WHO Special Program of Research, 1999). Azoospermia was confirmed by
149 thorough examination of the re-suspended pellet following centrifugation at 3660g for 15
150 minutes.

151

152 ***Data Analysis***

153 All results are presented as mean \pm SEM. Serum hormone and biochemical data were log
154 transformed before analysis by ANOVA for repeated measurements and sperm concentrations
155 were cube root transformed before ANOVA, with Tukey's post hoc test. Proportions of men
156 achieving thresholds for spermatogenic suppression were analysed by Fisher's exact test. Sexual
157 activity data were analysed by non-parametric testing. For all comparisons, a P value of <0.05
158 was considered significant.

159

160 **Results**

161 ***Subjects, withdrawals and adverse events***

162 29 subjects were assigned to the treatment groups. Three subjects in the MENT group chose to
163 leave the study after 8 wks of treatment, two because of symptoms of low libido and erectile
164 dysfunction and one for personal reasons. The two men who withdrew from the study because of
165 reduced libido had similar serum MENT concentrations to the rest of the group. Two subjects in
166 the testosterone group withdrew after 24 wks of treatment, one for personal reasons and the other
167 for symptoms of labile mood, sleep disturbance and nocturia. Adverse events included reduced
168 libido and erectile function in 4 additional subjects in the MENT group who completed
169 treatment. In the testosterone group, single testosterone pellets were extruded in 2 subjects, both

170 one wk prior to scheduled re-administration. One subject in the testosterone group reported
171 increased libido and acne.

172

173 Due to the incidence of reports of low libido and early withdrawal in the MENT group it was
174 decided in consultation with the study Data Monitoring and Safety Committee to shorten the
175 MENT treatment period to 24 wks whereas men in the testosterone group completed 48 weeks
176 treatment.

177

178 ***MENT and Testosterone concentrations***

179 Serum MENT concentrations in that group demonstrated an initial peak at four wks of 1.1 ± 0.1
180 nmol/L then declined to 0.54 ± 0.05 nmol/L at wk 24 (figure 1a), similar to assay blank values.

181 Analysis of MENT remaining in the implants after removal demonstrated that the implants had
182 released $31 \pm 2\%$ of content over 24 wks, giving an average release rate of 117 ± 6 $\mu\text{g/day/implant}$.

183 Implants removed after shorter insertion periods (51-60 days, n=3) showed a higher release rate
184 at 329 ± 6 $\mu\text{g/day/implant}$. Testosterone concentrations in this group fell to 2.0 ± 0.4 nmol/L at 4

185 wks ($P < 0.0001$ vs pre-treatment, figure 1b), and remained very low for the duration of treatment.

186 Following removal of MENT and etonogestrel implants, testosterone concentrations rapidly
187 returned to pretreatment values.

188

189 Serum testosterone concentrations in the testosterone group were significantly greater than in the
190 MENT group at all time points during treatment ($P < 0.0001$, figure 1b). There was some

191 fluctuation in keeping with the schedule of testosterone administration at 12 wk intervals.

192 Trough and peak concentrations rose during the study, indicating some accumulation. After an

193 initial fall over the initial 12 wks of treatment ($P < 0.001$) testosterone concentrations were similar
194 to pre-treatment at 24 wks but at 28 and 40 wks were significantly higher than pre-treatment
195 (both $P < 0.001$). However average testosterone concentrations over both the first and second 24
196 wk periods were not significantly different to pre-treatment (22.6 ± 1.9 vs. 20.2 ± 1.4 vs. $26.9 \pm$
197 1.5 nmol/L pre-treatment, 4-24 and 28-48 wks respectively).

198

199 *Sperm concentrations*

200 Both groups showed profound suppression of spermatogenesis initially. At 12 weeks, 8 of 10
201 subjects (80%) in the MENT group and 13 of 16 (81%) in the testosterone group demonstrated
202 suppression to $< 1 \times 10^6$ /ml with 3 and 11 subjects respectively being azoospermic. Thereafter
203 suppression was inconsistent in the MENT group. Only 4 men maintained $< 1 \times 10^6$ /ml until 24
204 weeks whereas the others showed partial recovery. Overall the mean sperm concentration rose to
205 $11.5 \pm 5.2 \times 10^6$ /ml at that time.

206

207 In contrast the testosterone group showed maintained suppression. At 24 weeks 14 of 16 subjects
208 (88%) were azoospermic, and by the end of the treatment period all subjects demonstrated
209 azoospermia ($n=14$). One of the two men not azoospermic at 24 weeks withdrew from the study
210 for personal reasons, and the other became azoospermic at week 44. The range of time to achieve
211 azoospermia was therefore 4 - 44 weeks, median 12 weeks. Once azoospermic, spermatozoa
212 were detected only in the centrifuged pellet of the ejaculate in one individual on one occasion.

213

214 Recovery was rapid following implant removal in the MENT group with 9 out of 10 subjects
215 achieving sperm concentration $> 20 \times 10^6$ /ml within 16 weeks. Slower recovery was seen in the

216 testosterone group, with a median duration of 28 weeks. One subject continued to show sperm
217 concentrations of $10\text{-}15 \times 10^6/\text{ml}$ after over 1 year of follow-up, with normal total sperm number,
218 motility and morphology, and gonadotropin concentrations. A second subject underwent
219 vasectomy before his sperm concentration had recovered to $>20 \times 10^6/\text{ml}$, 64 weeks after removal
220 of etonogestrel implants.

221

222

223 *Other reproductive hormones*

224 Initial suppression of both FSH and LH was rapid and profound in both treatment groups (figure
225 1). In the MENT group mean FSH was 0.18 ± 0.03 IU/L after four weeks. However, progressive
226 partial escape from suppression was evident with a rise to 1.29 ± 0.21 IU/L at 24 weeks ($P=0.04$,
227 ANOVA of treatment values). Concentrations were similar to pre-treatment in the recovery
228 phase. In the testosterone group the initial suppression was well maintained (figure 1) with minor
229 rises at times of trough testosterone concentrations that did not reach statistical significance.
230 Two men showed FSH rises to >1 IU/L at 24 weeks, none at 36 weeks and 1 at 48 weeks
231 treatment. FSH showed an overshoot in the recovery phase ($P<0.01$).

232

233 LH was also profoundly suppressed in both treatment groups initially (figure 1). In the MENT
234 group there was a small rise during continuing treatment but this did not reach statistical
235 significance (figure 1). In the testosterone group the suppression was more complete and
236 consistent for the duration of treatment with only one man showing any detectable recovery of
237 LH to >1 IU/L, at 48 weeks treatment.

238

239 Estradiol concentrations demonstrated a marked fall in the MENT group at 12 and 24 wks
240 treatment ($P<0.001$, figure 1). In the testosterone group the results paralleled the testosterone
241 concentrations with a small fall seen at 12 wks ($P<0.001$), recovering by 24 wks (ns vs pre-
242 treatment, $P<0.01$ vs 12 wks). There was a significant difference in estradiol concentrations
243 between the two groups at 24 wks ($P=0.01$).

244
245 Inhibin B concentrations showed a significant decline during treatment in both groups, but
246 differed between groups ($P=0.01$). In the testosterone group, inhibin B concentrations declined
247 during the first 24 wks of treatment, with little fall thereafter. There was however a significant
248 further fall during the recovery phase ($P<0.0001$ vs wk 48), with a nadir at 12 weeks recovery.
249 There was an inverse relation between inhibin B and FSH during the recovery phase ($P=0.004$ at
250 16 weeks). In the MENT group, inhibin B concentrations were only significantly lower than
251 pretreatment at 8 wks ($P=0.001$).

252
253 ***Hematology and Lipids***

254 Hemoglobin concentrations were significantly increased in the MENT group at 12 weeks but this
255 did not persist at 24 wks (table 2). A similar pattern was also seen in hematocrit but this did not
256 reach statistical significance. In the testosterone group a slower progressive rise in hemoglobin
257 concentration ($P=0.0006$) was observed which only became significant at 48 wks. There was
258 also a significant overall rise in hematocrit ($P=0.009$) in the testosterone group although none of
259 the individual treatment time points were significantly different from pre-treatment (table 2).

260
261 There were significant falls in HDL-C concentrations in both groups during treatment with return
262 to pre-treatment values in the recovery period (table 2). In both groups HDL-C was significantly

263 lower than pre-treatment at all time points during treatment. Cholesterol concentrations in the
264 MENT but not the testosterone group also showed a fall during treatment (table 2). There were
265 no significant changes in triglyceride or LDL-C concentrations in either group. Neither group
266 demonstrated any significant changes in any biochemical variables throughout the study.

267

268 ***Blood pressure***

269 A significant rise in systolic blood pressure was observed in the MENT group throughout the
270 treatment period ($P=0.02$, figure 3) with no significant change in diastolic blood pressure.
271 Systolic blood pressure was not significantly different to pre-treatment during the recovery
272 phase. There were no significant changes observed in the testosterone group.

273

274 ***Testes and Prostate***

275 Both treatment groups showed decreases in testicular volume during the study period. This fall
276 was only transient in the MENT group. In the testosterone group mean testis volume fell to a
277 nadir at 48 weeks ($p<0.001$).

278

279 There was no significant change in prostate volume over the course of the study in the MENT
280 group (table 3). However, in the testosterone group a small but statistically significant increase
281 in prostate volume was seen at the end of the treatment period ($P=0.007$) and it remained slightly
282 elevated after 16 wks in the recovery phase ($P<0.05$ vs pre-treatment, ns vs wk 48). Serum
283 prostate specific antigen (PSA) concentration demonstrated a significant fall in the MENT group
284 (table 3) at both 12 and 24 wks of treatment but was unchanged in the testosterone group.

285

286 ***Body Composition, Sexual Behaviour and Bone Mineral Density.***

287 There was no change in weight in the MENT group, but an increase in the testosterone group
288 (P=0.02, table 3). There were no significant changes in sexual activity or bone mineral density in
289 either group (table 3).

290

291 **Discussion**

292 These data further confirm that administration of sex steroids can suppress spermatogenesis
293 sufficiently and reversibly to allow development as a hormonal contraceptive for men (Anderson
294 and Baird, 2002; Nieschlag *et al.*, 2003; Kamischke and Nieschlag, 2004). What is less clear is
295 how to optimise the rate, extent and inter-individual consistency of spermatogenic suppression
296 while minimising the potentially adverse non-reproductive effects of treatment, which largely
297 reflect administration of supraphysiological doses of testosterone or other androgens.
298 Administration of a progestogen allows considerable dose-sparing of testosterone, and studies
299 using long-duration formulations suggest that this may also allow a reduction in dose while
300 maintaining efficacy (Handelsman *et al.*, 1996; Anderson *et al.*, 2002; Brady *et al.*, 2004).

301

302 The synthetic androgen MENT may offer advantages over testosterone for replacement therapy
303 both in hypogonadal men and as a component of a hormonal male contraceptive (Anderson *et*
304 *al.*, 1999; von Eckardstein *et al.*, 2003). However for the latter, the dose of MENT required was
305 associated with undesirable non-reproductive effects indicating supraphysiological androgen
306 action, i.e. increases in hemoglobin and haematocrit (Noé *et al.*, 1999; von Eckardstein *et al.*,
307 2003), as with testosterone alone. We have previously demonstrated that two MENT implants
308 formulated as in the present study appeared to provide replacement in hypogonadal men

309 (Anderson *et al.*, 2003). Similarly, the dose of etonogestrel used here when combined with
310 replacement doses of testosterone resulted in marked but not maximal suppression of
311 spermatogenesis (Anderson *et al.*, 2002). We therefore investigated this dose of etonogestrel
312 with MENT in comparison with a slightly larger dose of testosterone.

313

314 There was rapid spermatogenic suppression during the initial weeks of treatment, with
315 approximately 80% of both groups achieving $<1 \times 10^6$ /ml at 12 weeks. This is comparable to the
316 most effective regimens previously reported (Kamischke *et al.*, 2002; Turner *et al.*, 2004)
317 indicating the potential value of MENT in male contraception. Thereafter however suppression
318 in the MENT group was inconsistent and some subjects complained of reduced interest in sex.
319 Serum MENT concentrations were similar to those previously reported using this implant
320 formulation (Anderson *et al.*, 2003) but determination of MENT in the implant after removal
321 confirmed that the release rate was low. It therefore appears that a greater sustained release rate
322 than achieved here is necessary for continuing spermatogenic suppression and support of sexual
323 interest in normal men.

324

325 The inconsistent suppression of spermatogenesis in the MENT group was accompanied by an
326 increase in FSH with no significant change in LH or testosterone concentrations during the
327 second half of the treatment period. Measurement of intratesticular testosterone concentrations
328 and of the specific testicular androgen epitestosterone has shown that testicular steroidogenesis is
329 incompletely suppressed during administration of male contraceptive regimens (McLachlan *et*
330 *al.*, 2002; McLachlan *et al.*, 2004; Walton *et al.*, 2006). It appears that the rise in FSH seen here
331 with low but detectable LH concentrations is sufficient to support spermatogenesis in some men,

332 emphasising the need for maximal suppression of both gonadotropins and thus depletion of
333 intratesticular testosterone for optimal spermatogenic suppression.

334
335 The differential effects on spermatogenic suppression were also evident in the divergent patterns
336 of inhibin B which fell only transiently in the MENT group. A more marked fall was
337 demonstrated in the testosterone group, as previously reported (Anderson *et al.*, 1998; Brady *et*
338 *al.*, 2004). However a striking further decline during the early stages of recovery was detected.
339 The basis for this is unclear, although it accounts for the overshoot in FSH during the recovery
340 phase. It is possible that the rapid restoration of LH and thus intratesticular testosterone and of
341 FSH delays reinitiation of spermatogenesis in some men, as demonstrated in rats following
342 radiotherapy (Meistrich *et al.*, 1997; Shetty *et al.*, 2006).

343
344 The men who withdrew from the study because of reduced sexual interest did so after only 8
345 weeks treatment, thus even the peak serum MENT concentrations were insufficient in these
346 normal men. This contrasts with other evidence that the MENT dose was not insufficient, as
347 hemoglobin concentration was increased and HDL-C was reduced at 12 weeks, whereas in the
348 testosterone group both these were unchanged with no evidence of inadequate behavioural
349 support. This discrepancy may indicate that a relatively higher dose of MENT is required for
350 behavioural support in normal men compared to hypogonadal men (Anderson *et al.*, 1999;
351 Anderson *et al.*, 2003) and also compared to trophic effects on the bone marrow and liver, which
352 may reflect its restricted metabolism.

353

354 All men in the testosterone group eventually became azoospermic, with similar suppression to
355 the same dose of etonogestrel with a lower dose of testosterone (Anderson *et al.*, 2002). This
356 contrasts with improved suppression using the lower dose of testosterone but higher dose of
357 etonogestrel (Brady *et al.*, 2004). In these earlier studies there was no evidence of inadequate
358 androgen replacement and there were only minimal non-reproductive side effects. Together these
359 data illustrate dosage effects for both the testosterone and progestogen component and
360 demonstrate the importance of the progestogen dose over the testosterone dose in maximising
361 spermatogenic suppression. Disadvantageous effects of this increased dose of testosterone
362 (600mg/12 weeks) were exemplified by the increase in hemoglobin and decrease in HDL-C
363 concentrations, despite average testosterone concentrations being similar to pre-treatment over
364 the second 24 weeks of treatment, which were not seen in our previous studies using a lower
365 dose of testosterone (400mg/12 weeks). These results are in keeping with the physiological
366 replacement dose of testosterone being nearer 5 than 7 mg/day (Walton *et al.*, 2006) and
367 highlight the importance of measuring the response of androgen-dependent variables such as
368 hemoglobin concentration and hematocrit to determine the optimum replacement dose rather
369 than solely serum testosterone which will be overestimated pre-treatment if only morning
370 sampling is used (Anderson and Baird, 2002).

371
372 The major potential advantage of MENT is that it is resistant to 5 α -reduction and may therefore
373 relatively spare the prostate. The present data also support this, with a fall in serum PSA with a
374 non-significant fall in prostate volume. These results are similar to those in similarly-aged
375 hypogonadal men (Anderson *et al.*, 2003). This interpretation must be tempered however by the
376 above reservations regarding the overall adequacy of this dose of MENT.

377

378 Maintenance of bone mass in men is dependent on serum testosterone and local conversion to
379 estradiol (Vanderschueren *et al.*, 2004). MENT is also a substrate for aromatase, and in an aged
380 orchidectomized rat model, MENT was effective in maintaining bone mass (Venken *et al.*,
381 2005). In hypogonadal men 2 MENT implants did not appear sufficient to maintain lumbar
382 spine bone mass (Anderson *et al.*, 2003). The present data do not show any loss of bone mass in
383 the MENT group over this relatively short duration of treatment although it is possible that with
384 longer duration of treatment bone mass may not be adequately supported. This may reflect
385 differences between normal and hypogonadal men, or the additional administration of the
386 progestogen.

387

388 There was a small but significant elevation of systolic blood pressure in the MENT group with
389 no change in the testosterone group. A similar finding was reported in a previous study
390 investigating MENT alone (von Eckardstein *et al.*, 2003). Systolic blood pressure may reflect
391 arterial stiffness and is increasingly recognised to be a strong cardiovascular risk factor (Oliver
392 and Webb, 2003). Arterial stiffness is inversely related to testosterone concentrations in older
393 men (Hougaku *et al.*, 2006), and was increased by induced hypogonadism in men with prostate
394 cancer (Smith *et al.*, 2001). During MENT administration both testosterone and estradiol
395 concentrations are low. Recent data suggest that endogenous estradiol may be vasculoprotective
396 (Arnlov *et al.*, 2006). Although MENT is aromatised to an active estrogen (LaMorte *et al.*,
397 1994), the low serum concentrations both of MENT and therefore of potential active metabolites
398 may contribute to increased arterial stiffness and a rise in systolic blood pressure which therefore
399 might actually be less affected by administration of a higher, more effective dose.

400

401 In conclusion this study demonstrates that the combination of MENT and etonogestrel results in
402 effective spermatogenic suppression. Formulation of MENT to give consistent release at a dose
403 similar to that in the initial weeks of this study may be a promising approach for hormonal male
404 contraception although non-reproductive effects were detected. The testosterone group also
405 showed rapid spermatogenic suppression, but it is clear that it is of greater value to raise the dose
406 of progestogen than that of the testosterone component to optimise suppression and minimise
407 non-reproductive androgenic effects.

408

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414

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417

418 **References**

- 419 Agarwal AK and Monder C *In vitro* metabolism of 7 α -methyl-19-nortestosterone by rat liver,
420 prostate, and epididymis: comparison with testosterone and 19-nortestosterone. *Endocrinol* 1988;
421 13: 2187-2193.
- 422 Anderson RA and Baird DT Male contraception. *Endocr Rev* 2002; 23: 735-762.
- 423 Anderson RA, Irvine DS, Balfour C, Groome NP and Riley SC Inhibin B in seminal plasma:
424 testicular origin and relationship to spermatogenesis. *Human Reproduction* 1998; 13: 920-926.
- 425 Anderson RA, Kinniburgh D and Baird DT Suppression of spermatogenesis by etonogestrel
426 implants with depot testosterone: potential for long-acting male contraception. *J Clin Endocrinol*
427 *Metab* 2002; 87: 3640-3649.
- 428 Anderson RA, Martin CW, Kung AWC, Everington D, Pun TC, Tan KCB, Bancroft J, Sundaram
429 K, Moo-Young AJ and Baird DT 7 α -Methyl-19-Nortestosterone (MENT) maintains sexual
430 behavior and mood in hypogonadal men. *J Clin Endocrinol Metab* 1999; 84: 3556-3562.
- 431 Anderson RA, Wallace AM, Sattar N, Kumar N and Sundaram K Evidence for tissue selectivity
432 of the synthetic androgen 7 alpha-methyl-19-nortestosterone in hypogonadal men. *J Clin*
433 *Endocrinol Metab* 2003; 88: 2784-2793.
- 434 Arnlov J, Pencina MJ, Amin S, Nam BH, Benjamin EJ, Murabito JM, Wang TJ, Knapp PE,
435 D'Agostino RB, Sr., Bhasin S and Vasan RS Endogenous sex hormones and cardiovascular
436 disease incidence in men. *Ann Intern Med* 2006; 145: 176-184.
- 437 Brady BM, Walton M, Hollow N, Kicman AT, Baird DT and Anderson RA Depot testosterone
438 with etonogestrel implants result in induction of azoospermia in all men for long-term
439 contraception. *Human Reprod* 2004; 19: 2658-2667.

440 Cummings DE, Kumar N, Bardin CW, Sundaram K and Bremner WJ Prostate-sparing effects in
441 primates of the potent androgen 7 α -methyl-19-nortestosterone: a potential alternative to
442 testosterone for androgen replacement and male contraception. *J Clin Endocrinol Metab* 1998;
443 83: 4212-4219.

444 Grimes D, Gallo M, Grigorieva V, Nanda K and Schulz K Steroid hormones for contraception in
445 men. *Cochrane Database Syst Rev* 2004; CD004316.

446 Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather J and McNeilly AS
447 Measurement of dimeric inhibin B throughout the menstrual cycle. *J Clin Endocrinol Metab*
448 1996; 81: 1401-1405.

449 Handelsman DJ, Conway AJ, Howe CJ, Turner L and Mackey MA Establishing the minimum
450 effective dose and additive effects of depot progestin in suppression of human spermatogenesis
451 by a testosterone depot. *J Clin Endocrinol Metab* 1996; 81: 4113-4121.

452 Hougaku H, Fleg JL, Najjar SS, Lakatta EG, Harman SM, Blackman MR and Metter EJ
453 Relationship between androgenic hormones and arterial stiffness, based on longitudinal hormone
454 measurements. *Am J Physiol Endocrinol Metab* 2006; 290: E234-242.

455 Kamischke A, Heuermann T, Kruger K, von Eckardstein S, Schellschmidt I, Rubig A and
456 Nieschlag E An effective hormonal male contraceptive using testosterone undecanoate with oral
457 or injectable norethisterone preparations. *J Clin Endocrinol Metab* 2002; 87: 530-539.

458 Kamischke A and Nieschlag E Progress towards hormonal male contraception. *Trends*
459 *Pharmacol Sci* 2004; 25: 49-57.

460 Kinniburgh D, Zhu H, Cheng L, Kicman AT, Baird DT and Anderson RA Oral desogestrel with
461 testosterone pellets induces consistent suppression of spermatogenesis to azoospermia in both
462 Caucasian and Chinese men. *Human Reprod* 2002; 17: 1490-1501.

463 Kumar N, Didolkar AK, Ladd A, Thau R, Monder C, Bardin CW and Sundaram K
464 Radioimmunoassay of 7 α -methyl-19-nortestosterone and investigation of its pharmacokinetics in
465 animals. *J Steroid Biochem Mol Biol* 1990; 37: 587-591.

466 LaMorte A, Kumar N, Bardin CW and Sundaram K Aromatization of 7 alpha-methyl-19-
467 nortestosterone by human placental microsomes in vitro. *J Steroid Biochem Mol Biol* 1994; 48:
468 297-304.

469 McLachlan RI, O'Donnell L, Stanton PG, Balourdos G, Frydenberg M, de Kretser DM and
470 Robertson DM Effects of testosterone plus medroxyprogesterone acetate on semen quality,
471 reproductive hormones, and germ cell populations in normal young men. *J Clin Endocrinol*
472 *Metab* 2002; 87: 546-556.

473 McLachlan RI, Robertson DM, Pruyers E, Ugoni A, Matsumoto AM, Anawalt BD, Bremner
474 WJ and Meriggiola C Relationship between serum gonadotropins and spermatogenic suppression
475 in men undergoing steroidal contraceptive treatment. *J Clin Endocrinol Metab* 2004; 89: 142-
476 149.

477 Meistrich ML, Wilson G, Zhang Y, Kurdoglu B and Terry NH Protection from procarbazine-
478 induced testicular damage by hormonal pretreatment does not involve arrest of spermatogonial
479 proliferation. *Cancer Res* 1997; 57: 1091-1097.

480 Nieschlag E, Zitzmann M and Kamischke A Use of progestins in male contraception. *Steroids*
481 2003; 68: 965-972.

482 Noé G, Suvisaari J, Martin C, Moo-Young AJ, Sundaram K, Saleh SI, Quintero E, Croxatto HB
483 and Lähteenmäki P Gonadotrophin and testosterone suppression by 7 α -methyl-19-
484 nortestosterone acetate administered by subdermal implant to healthy men. *Human Reprod* 1999;
485 14: 2200-2206.

486 Oliver JJ and Webb DJ Noninvasive assessment of arterial stiffness and risk of atherosclerotic
487 events. *Arterioscler Thromb Vasc Biol* 2003; 23: 554-566.

488 Shetty G, Weng CC, Meachem SJ, Bolden-Tiller OU, Zhang Z, Pakarinen P, Huhtaniemi I and
489 Meistrich ML Both testosterone and follicle-stimulating hormone independently inhibit
490 spermatogonial differentiation in irradiated rats. *Endocrinology* 2006; 147: 472-482.

491 Smith JC, Bennett S, Evans LM, Kynaston HG, Parmar M, Mason MD, Cockcroft JR, Scanlon
492 MF and Davies JS The effects of induced hypogonadism on arterial stiffness, body composition,
493 and metabolic parameters in males with prostate cancer. *J Clin Endocrinol Metab* 2001; 86:
494 4261-4267.

495 Sundaram K, Kumar N and Bardin CW 7 α -methyl-nortestosterone (MENT) : the optimal
496 androgen for male contraception. *Annals of Medicine* 1993; 25: 199-205.

497 Suvisaari J, Sundaram K, Noe G, Kumar N, Aguilleaume C, Tsong YY, Lähteenmaki P and
498 Bardin CW Pharmacokinetics and pharmacodynamics of 7 α -methyl-19-nortestosterone after
499 intramuscular administration in healthy men. *Human Reprod* 1997; 12: 967-973.

500 Turner L, Conway AJ, Jimenez M, Liu PY, Forbes E, McLachlan RI and Handelsman DJ
501 Contraceptive efficacy of a depot progestin and androgen combination in men. *Obstet Gynecol*
502 *Surv* 2004; 59: 270-271.

503 Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R and Ohlsson C
504 Androgens and bone. *Endocr Rev* 2004; 25: 389-425.

505 Venken K, Boonen S, Van Herck E, Vandenput L, Kumar N, Sitruk-Ware R, Sundaram K,
506 Bouillon R and Vanderschueren D Bone and muscle protective potential of the prostate-sparing
507 synthetic androgen 7 α -methyl-19-nortestosterone: evidence from the aged orchidectomized
508 male rat model. *Bone* 2005; 36: 663-670.

509 von Eckardstein S, Noe G, Brache V, Nieschlag E, Croxatto H, Alvarez F, Moo-Young A, Sivin
510 I, Kumar N, Small M and Sundaram K A clinical trial of 7 alpha-methyl-19-nortestosterone
511 implants for possible use as a long-acting contraceptive for men. J Clin Endocrinol Metab 2003;
512 88: 5232-5239.

513 Walton M, Anderson RA, Kicman AT, Elton RA, Ossowska K and Baird DT A diurnal variation
514 in testicular hormone production is maintained following gonadotrophin suppression in normal
515 men. Clin. Endocrinol. 2006; 65:

516 WHO Special Program of Research, Development, and Research Training in Human
517 Reproduction (1999) WHO laboratory manual for the examination of human semen and semen-
518 cervical mucus interaction. Cambridge: Cambridge University Press.

519 World Health Organisation Task Force on Methods for the Regulation of Male Fertility
520 Contraceptive efficacy of testosterone-induced azoospermia in normal men. Lancet 1990; 336:
521 955-959.

522 World Health Organisation Task Force on Methods for the Regulation of Male Fertility
523 Contraceptive efficacy of testosterone-induced azoospermia and oligozoospermia in normal men.
524 Fertil Steril 1996; 65: 821-829.

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Table 1. Pre-treatment characteristics of men in the two groups.

	MENT Group	Testosterone Group
Age (years)	34.9 ± 1.5	32.2 ± 1.2
BMI (kg/m ²)	25.6 ± 0.8	24.7 ± 0.7
LH (IU/L)	3.3 ± 0.4	3.7 ± 0.3
FSH (IU/L)	2.0 ± 0.4	2.3 ± 0.4
Testosterone (nmol/L)	17.6 ± 2.7	21.4 ± 2.5
Sperm conc (x10 ⁶ /ml)	55.1 ± 9.1	58.7 ± 14.0

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Pre-treatment values for all subjects, mean ± SEM. MENT group n=13, testosterone group n=16.

533 Table 2. Serum values of haematology, lipid and PSA results.
534

	Pre-treatment	12 weeks	24 weeks	36 weeks	48 weeks	Recovery
Haemoglobin (g/L)						
T Group	152 ± 2.5	149 ± 2.2	153 ± 2.1	154 ± 2.5	157 ± 2.5**	154 ± 2.5
MENT Group	149 ± 2.9	154 ± 3.3*	148 ± 2.4	-	-	148 ± 2.0
Haematocrit						
T Group	0.45 ± 0.01	0.44 ± 0.01	0.45 ± 0.01	0.46 ± 0.01	0.46 ± 0.01	0.46 ± 0.01
MENT Group	0.44 ± 0.01	0.46 ± 0.01	0.43 ± 0.01	-	-	0.43 ± 0.01
HDL-C (nmol/L)						
T Group	1.4 ± 0.1	1.2 ± 0.1*	1.2 ± 0.1**	1.2 ± 0.01**	1.1 ± 0.1**	1.3 ± 0.1
MENT Group	1.4 ± 0.1	1.2 ± 0.2**	1.2 ± 0.1**	-	-	1.3 ± 0.1
Cholesterol (nmol/L)						
T Group	4.8 ± 0.2	4.4 ± 0.2	4.6 ± 0.2	4.5 ± 0.2	4.7 ± 0.2	5.0 ± 0.2
MENT Group	4.9 ± 0.2	4.6 ± 0.2	4.4 ± 0.2*	-	-	4.9 ± 0.2
LDL-C (nmol/L)						
T Group	2.8 ± 0.2	2.6 ± 0.2	2.9 ± 0.2	2.9 ± 0.2	3.0 ± 0.2	3.1 ± 0.2
MENT Group	2.9 ± 0.2	2.9 ± 0.2	2.5 ± 0.2	-	-	2.9 ± 0.2
Triglycerides (nmol/L)						
T Group	1.3 ± 0.2	1.4 ± 0.2	1.3 ± 0.3	1.1 ± 0.1	1.5 ± 0.2	1.4 ± 0.2
MENT Group	1.5 ± 0.2	1.3 ± 0.2	1.7 ± 0.2	-	-	1.5 ± 0.2

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536 Mean±sem. MENT group n=13, testosterone group n=16. * p<0.05, ** P<0.01 vs pretreatment.
537 There were no significant differences between the MENT and testosterone groups in any of these
538 variables.

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Table 3. Weight, prostate volume and PSA, sexual activity and bone density data in the two treatment groups during MENT/testosterone plus etonogestrel treatment

	Pre-Treatment	12 weeks	24 weeks	36 weeks	48 weeks	Recovery
Weight (kgs)						
T Group	80.8 ± 3.8	80.6 ± 3.4	82.2 ± 3.5	83.6 ± 4.1	84.2 ± 3.9*	84.1 ± 3.8
MENT Group	80.5 ± 3.0	83.0 ± 3.6	81.5 ± 3.7	-	-	81.3 ± 3.3
Prostate vol (ml)						
T Group	14.7 ± 1.3	-	16.2 ± 0.9	-	17.4 ± 0.9**	16.9 ± 1.2
MENT Group	16.3 ± 1.4	-	15.8 ± 2.3	-	-	16.4 ± 1.4
PSA (ng/ml)						
T Group	0.8 ± 0.1	0.87 ± 0.1	0.84 ± 0.1	0.73 ± 0.1	0.72 ± 0.1	0.68 ± 0.1
MENT Group	1.1 ± 0.2	0.77 ± 0.1**	0.74 ± 0.1**	-	-	0.80 ± 0.1
Testis vol (ml)						
T Group	18.6 ± 0.9	13.7 ± 0.9**	12.4 ± 0.6**	12.6 ± 1.2**	11.5 ± 1.1**	19.0 ± 0.9
MENT Group	18.1 ± 0.9	12.9 ± 1.0**	15.4 ± 1.1	-	-	18.6 ± 1.1
Sexual activity						
T Group	7.1 ± 0.9	8.7 ± 1.5	7.8 ± 0.9	7.6 ± 1.8	7.4 ± 1.9	7.1 ± 1.2
MENT Group	6.9 ± 0.9	5.9 ± 1.3	6.4 ± 1.3	-	-	7.9 ± 2.1
Spine BMD (g/m ²)						
T Group	1.03 ± 0.02	-	1.03 ± 0.03	-	1.08 ± 0.02	1.01 ± 0.06
MENT Group	1.05 ± 0.04	-	1.04 ± 0.04	-	-	1.01 ± 0.04
Total Hip BMD						
T Group	1.08 ± 0.02	-	1.08 ± 0.02	-	1.10 ± 0.03	1.08 ± 0.03
MENT Group	1.01 ± 0.05	-	1.01 ± 0.05	-	-	1.01 ± 0.05

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Mean±sem. MENT group n=13, testosterone group n=16. * p<0.05, ** P<0.01 vs pretreatment. There were no significant differences between the MENT and testosterone groups in any of these variables

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576 **Figure legends**

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578 Figure 1: Serum concentrations of (a) MENT, (b) testosterone, (c) FSH, (d) LH, (e) estradiol and
579 (f) inhibin B pretreatment and during treatment and recovery phases. MENT group (open square
580 symbols) n=13, testosterone group (filled round symbols) n=19, mean \pm sem . The MENT group
581 received drug treatment for 24 weeks, the testosterone group for 48 weeks. Testosterone was
582 administered at weeks 0, 12, 24 and 36. In (b) the normal range is indicated by the broken lines.

583

584 Figure 2: Sperm concentrations during treatment and recovery phases. MENT group (open
585 square symbols) n=13, testosterone group (filled round symbols) n=19, mean \pm sem. The MENT
586 group received drug treatment for 24 weeks, the testosterone group for 48 weeks.

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588 Figure 3: Systolic and diastolic blood pressure during treatment and recovery phases. MENT
589 group (open square symbols) n=13, testosterone group (filled round symbols) n=19, mean \pm sem.
590 The MENT group received drug treatment for 24 weeks, the testosterone group for 48 weeks.





