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Potential of norethisterone enanthate for male contraception: pharmacokinetics and suppression of pituitary and gonadal function

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Summary

OBJECTIVE Gestagens are known to suppress gonadotrophins in women and are currently also under investigation for the development of hormonal male contraceptives. The aim of the study was to assess the potential of norethisterone enanthate (NETE) for male contraception.

DESIGN AND MEASUREMENTS The suppressive effect of a single injection of 200 mg NETE on serum gonadotrophins, serum testosterone, lipids, spermatogenesis, well-being and sexual function was evaluated in seven healthy men.

RESULTS In this single dose study treatment was well tolerated by all volunteers. NETE led to a rapid, profound and significant suppression of serum LH (day 6 – day 10), FSH (day 2 – day 29), testosterone (day 1 – day 29 and day 35) and SHBG (day 6 – day 35). At study end sperm counts were significantly suppressed. Numbers of spontaneous erections (day 17, 23 and 26), number of sexual fantasies (day 20 and 23) as well as libido (day 20 and 26) were significantly decreased compared to baseline. All other parameters including lipids, augmented glucose, testicular volume and well-being showed no significant alterations.

CONCLUSION Because of its strong, rapid and sustained suppression of serum FSH and testosterone norethisterone enanthate offers great potential for hormonal male contraception if combined with testosterone esters.

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Testosterone is an essential part of all experimental approaches to hormonal male contraception. In about two thirds of Caucasian and almost all Chinese volunteers azoospermia can be achieved by administration of testosterone enanthate alone (World Health Organization, 1990; World Health Organization, 1996). Since gestagens are known to suppress gonadotrophins in women, testosterone preparations were combined with different gestagens to enhance the suppression of spermatogenesis (for review see Nieschlag & Behre, 1998). However, despite better gonadotrophin suppression when gestagens are added to testosterone, in most of these studies suppression of spermatogenesis leaves much to be desired (Bebb *et al*., 1996; Anawalt *et al*., 1999; Büchter *et al*., 1999) or – as in the case of cyproterone acetate – produces an unwanted decline in red blood cell count (Meriggiola *et al*., 1996; Meriggiola *et al*., 1998).

Therefore, the search for more appropriate gestagens continues. Among the candidates for hormonal male contraception norethisterone enanthate (NETE) should be especially considered because of its long lasting gonadotrophin suppressive effect in women (Fotherby *et al*., 1984).

NETE as well as the other norethisterone formulation, norethisterone acetate (NETA), are hydrolysed to release the active compound, i.e. norethisterone (NET) which is a 19-nortestosterone derivate. NET is aromatized to ethinyl estradiol as well as 5α reduced. NET binds to the androgen receptor with an affinity of 45% of that of testosterone, resulting in androgenic activity equal to approximately 10% of testosterone (Couzinet *et al*., 1996) and is capable of restoring male sexual behaviour in castrated rats (Morali *et al*., 1990). As testosterone NET can be 5α-reduced and the 5α-reduction of NET to 5α-NET leads to an enhanced binding affinity for androgen receptors comparable to DHT. However, in contrast to DHT, 5α-NET has strongly diminished androgenic properties due to low intrinsic receptor activity. This results in an antiandrogenic effect of the metabolite (Lemus *et al*., 1997). Although it binds to the androgen receptor in women, the antigonadotrophic activity of NET and its metabolites is not mediated via the androgen receptor and therefore may be mediated through the progesterone (Couzinet *et al*., 1996) or oestrogen receptor (Flores *et al*., 1986). In postmenopausal women NET was converted to ethinyl estradiol, corresponding to an oral dose equivalent of about 4 μg/mg of NET (Kuhnz *et al*., 1997).

In men with prostatic carcinoma, administration of NETA also showed a strong suppression of testosterone (Szendroi,

1984) and was capable of suppressing spermatogenesis to azoospermia (5 or 10 mg NETA/day) when combined with percutaneous testosterone gel (250 mg daily) or oral (10 mg/day) testosterone undecanoate (Guerin & Rollet, 1988). When administered to bonnet monkeys in combination with estradiol benzoate using an Alzet minipump or once a month by intramuscular injection, NETE treatment resulted in azoospermia in all monkeys within 60–150 days (Shetty *et al*., 1997).

For these reasons it appeared desirable to investigate the potential of NETE for male contraception. In this paper we report the pharmacokinetics and the effects of a single injection of NETE on serum hormones and on spermatogenesis.

Subjects and methods

Subjects

The study was approved by the Ethics Committee of the University and the State Medical Board, Münster. Nine Caucasian men aged 18–45 years were recruited by notice board advertisement. After giving informed consent, men entered into the study if they had no clinically significant general medical history (including no current regular medication), an unremarkable physical examination and normal laboratory screening tests including routine clinical chemistry, lipids, haematology, reproductive hormones. Volunteers with clinically relevant abnormalities of these parameters were excluded. Ultimately seven volunteers were included and finished the study.

Study design

After the two screening visits, volunteers received a single intramuscular injection of 200 mg NETE at day 0. Thereafter control examinations were performed on study days 1 and 2 and at every second day up to study day 14 and from then on every third day up to day 44. Further control examinations were performed on study days 48, 51, 55, 63, 70, 77 and 84. Each visit comprised a general examination, evaluation of adverse events, measurements of SHBG, reproductive hormone measurements (FSH, LH, prolactin, testosterone) and completing a questionnaire on general health and sexual behaviour. In addition, at one pre-examination and on study days 41 and 84 evaluation of lipid parameters, basal and augmented glucose (oral challenge with 75 g glucose), semen analysis and sonography of scrotal contents and of the prostate were performed. Normal blood values for routine clinical chemistry and haematology were checked again at study end. As androgen deficiency could be expected under treatment, all volunteers were offered androgen supplementation with oral testosterone undecanoate (Andriol®) upon request.

Measurements

Venous blood samples were taken between 0800 and 1200 hours at every visit. Blood samples for endocrine and NET determinations were separated at 800 g and stored at −20 °C until evaluation. All other blood parameters were measured on the day of investigation.

Assays

For determination of NET a radioimmunoassay was used (Bedolla-Tovar *et al*., 1978), which was performed by Prof. Dr Pär Westlund (Karolinska Institute, Department of Women and Child Health, Stockholm, Sweden). The analytical range was 0·225–14·4 μg/l. Mean intra- and interassay coefficients of variation were 1·3 and 3·5%, respectively. All volunteers had undetectable baseline values. The crossreactivity of the antiserum with testosterone was below 0·3%. Serum levels of LH, FSH, prolactin and SHBG were determined by highly specific time-resolved fluoroimmunoassays (AUTODELFIA, Wallac, Turku, Finland). The lower detection limits for FSH, LH and SHBG were 0·25 IU/l, 0·12 IU/l and 6·3 nmol/l, respectively. The normal range in our laboratory for LH is 2–10 IU/l, for FSH 1–7 U/l, for prolactin < 500 mU/l and for SHBG 11–71 nmol/l. Mean intra- and interassay coefficients of variation during hormone analysis period were 1·6 and 3·5% for LH, 1·7 and 4·7% for FSH, 0·9 and 5·3% for prolactin and 1·3 and 10·0% for SHBG, respectively. Serum testosterone was determined using a commercial fluoroimmunoassay (AUTODELFIA, Wallac, Turku, Finland). The lower detection limit for testosterone was 0·5 nmol/l. Mean intra- and interassay coefficients of variation during hormone analysis period were 2·4 and 6·1% for testosterone. The normal serum level for testosterone in our laboratory is above 12 nmol/l. All samples of one volunteer were analysed within one assay.

Clinical chemistry, clotting factors and haematology parameters were analysed with a Hitachi 947 autoanalyser (Roche Diagnostics, Mannheim, Germany) and a H3 autoanalyser (Bayer Diagnostics, Leverkusen, Germany), respectively. A Hitachi 917 autoanalyser (Roche Diagnostics) was used to quantify serum concentrations of glucose, cholesterol, and triglycerides with enzymatic tests (all from Roche Diagnostics). HDL cholesterol was measured using a homogenous enzymatic assay (Roche Diagnostics), and apoA-I, apoB and Lp(a) were determined with immunoturbidimetric tests (Roche Diagnostics).

Semen analysis

Analysis of semen samples was performed according to the World Health Organization Laboratory Manual (World Health Organization, 1999) and subjected to rigorous internal (Cooper *et al*., 1992) and external quality control (Cooper *et al*., 1999).

In cases of extremely low sperm counts or azoospermia, the ejaculates were centrifuged, and analysis was performed on the sediment. The volunteers were requested to abstain from sexual activity for 48 h to 7 days before investigation.

Evaluation of well-being and sexual function

For evaluation of possible psychosexual effects of the treatment a standardized questionnaire on sexual thoughts and fantasies, sexual interest and desire, satisfaction with sexuality, frequency of erections and ejaculations and number of morning erections was used. This previously described written questionnaire was completed by the volunteers at every visit before any contact with the investigator (Behre *et al*., 1992b). Based on self-ratings, the volunteers are asked to judge sexual thoughts and fantasies, sexual interest and desire and satisfaction with sexuality during the last 7 days on an analogue scale from 0 (low) to 10 cm (high). In addition, frequency of erections and ejaculations and number of morning erections during the last 7 days were also recorded by the volunteers.

Ultrasonography of testicular volume/transrectal ultrasonography of the prostate

Sonographic measurements of testis and prostate volume were performed applying a high frequency 7·5-megahertz sector scanner (Sonoline Versa Pro, Siemens, Erlangen, Germany). All measurements of prostate volume were performed by transrectal ultrasonography applying a mechanical biplanar 7·5 MHz sector scanner (Siemens, Endo-P). Prostate volume was calculated applying the ellipsoid method (Behre *et al*., 1997b).

Statistics

All variables were checked for normal distribution in the Kolmogorov–Smirnov one-sample test for goodness-of-fit and were normally distributed. Variations over time were evaluated by one-way ANOVA for repeated measurements. In case of an overall $P < 0{\cdot}05$ in the ANOVA, differences between baseline values and the following time points were tested by Tukey's post hoc test. Two-sided P-values of 0·05 were considered significant. All analyses were performed using the statistical software GraphPadPrism for Windows version 2·01 (GraphPad Software Inc., San Diego, CA, USA). Results are given as mean ± SEM.

Results

General well-being and sexual function

In general, treatment was well tolerated by all volunteers. Adverse events reported by the volunteers were flu ($n = 8$), gastritis ($n = 2$) and concussion ($n = 1$) and were not related to the medication. No significant changes in physical symptoms, mood ratings and individual well-being were observed at any investigated time point compared to baseline. There was a significant fall in the number of spontaneous erections, libido and sexual fantasies (Fig. 1). In contrast, the number of ejaculations and erections per week as well as satisfaction with sexual life remained unchanged (Fig. 2). Two volunteers requested androgen supplementation during study days 27–32 and they received a total of 12 capsules.

Norethisterone

NET was significantly elevated compared to baseline from study day 1 to study day 14 (Fig. 3). Mean ± SEM AUC, cMax and tMax were $2781 \pm 212\ \mu g/l$*hour, $9{\cdot}7 \pm 2{\cdot}1\ \mu g/l$ and 117 ± 27 h, respectively. The terminal half-life and mean

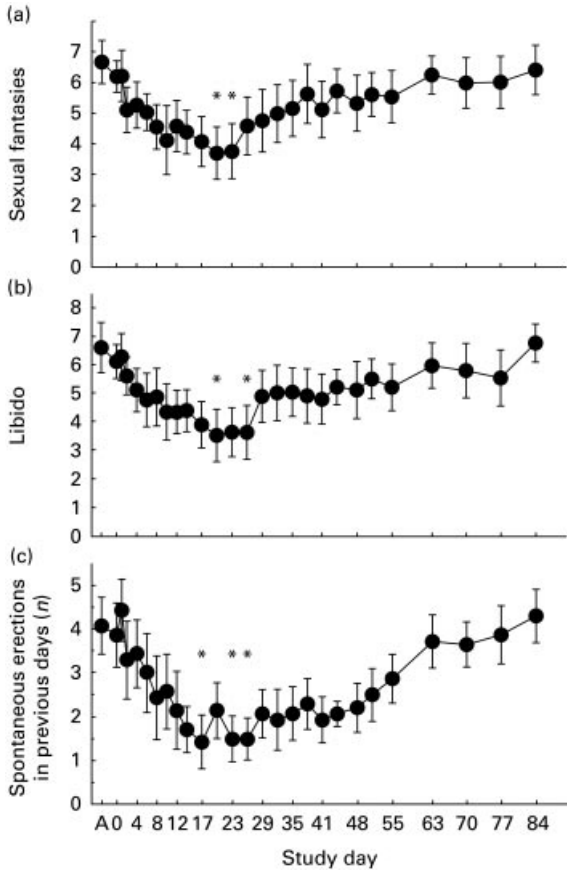

Fig. 1 (a) sexual fantasies, (b) libido and (c) number of spontaneous erections in the previous seven days [mean ± SEM] of seven volunteers after intramuscular injection of 200 mg NETE over time. Libido and sexual fantasies were measured in cm on a free scale line from 0 to 10 cm. *$P < 0{\cdot}05$.

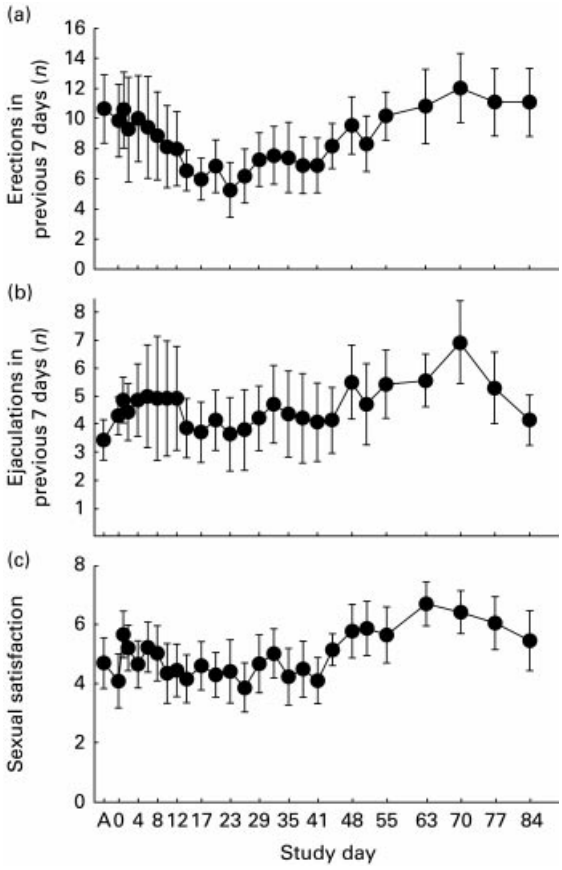

Fig. 2 Numbers of (a) erections per week and (b) ejaculations per week and (c) sexual satisfaction [mean ± SEM] of seven volunteers after intramuscular injection of 200 mg NETE over time. Sexual satisfaction was measured in cm on a free scale line from 0 to 10 cm.

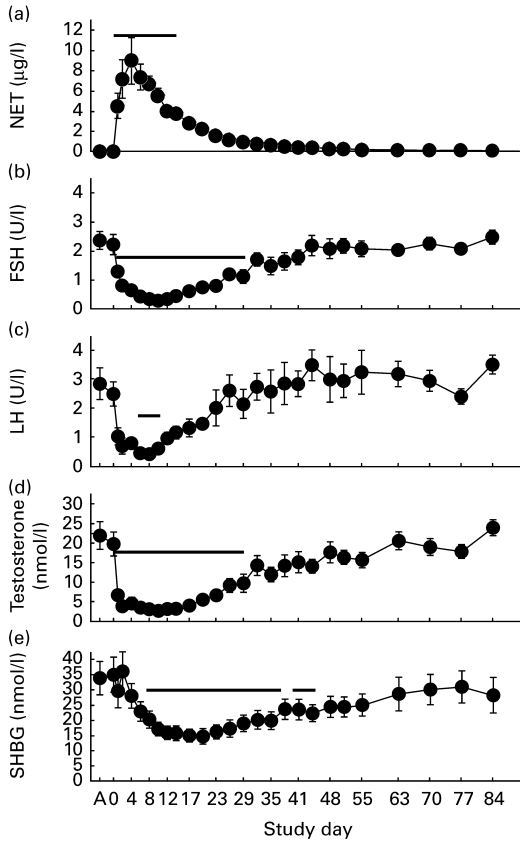

Fig. 3 Serum (a) NET, (b) FSH, (c) LH, (d) testosterone and (e) SHBG levels [mean ± SEM] of seven volunteers after intramuscular injection of 200 mg NETE over time. Significant differences to baseline are indicated by bars ($P < 0{\cdot}05$).

retention time of NET in our male volunteers was 293 ± 40 h and 423 ± 58 h, respectively.

Hormones

NETE lead to significant suppression of serum LH (from day 6 to day 10), FSH (from day 2 to day 29), testosterone (from day 1 to day 29 + day 35) and SHBG (from day 6 to day 35) levels (Fig. 3). No significant changes in serum prolactin levels were observed.

Clinical chemistry and haematology

Clinical chemistry, clotting factors and haematology were not significantly altered at study end. Neither significant differences in lipid parameters nor augmented glucose levels could be detected (Table 1) at any investigated timepoints.

Semen parameters

Compared to baseline (87 ± 21·7 million/ml) a reduction of sperm concentration (Fig. 4) was seen on day 41 (41·3 ± 18·5 million/ml); this became significant on day 84 (26·3 ± 4·3 million/ml). Ejaculate volume (baseline: 3·6 ± 0·6 ml; day 41: 3·3 ± 0·5 ml; day 84: 3·4 ± 0·7 ml), sperm motility, sperm morphology and number of round cells remained unchanged.

Testis and prostate

Compared to baseline (57 ± 4 ml) sonographic total testicular volume and echogenicity remained unchanged on study day 41 (55 ± 3 ml) and study day 84 (63 ± 4 ml). Nor did sonographic prostate volume change significantly on study day 41 (18 ± 1 ml) and study day 84 (18 ± 2 ml) when compared to initial values (18 ± 2 ml).

Table 1 Lipid parameters at baseline and at study day 14, 41 and 84 of seven volunteers after intramuscular injection of 200 mg NET at day 0. Data are given as mean and SEM

	Mean baseline	Day 14	Day 41	Day 84
Cholesterol (mmol/l)	4·66 ± 0·59	4·16 ± 0·41	4·73 ± 0·56	4·78 ± 0·57
HDL-C (mmol/l)	1·50 ± 0·13	1·11 ± 0·10	1·49 ± 0·16	1·53 ± 0·16
LDL-C (mmol/l)	2·74 ± 0·49	2·79 ± 0·44	2·95 ± 0·54	2·84 ± 0·47
Cholesterol/HDL-C	3·2 ± 0·4	3·9 ± 0·5	3·3 ± 0·5	3·2 ± 0·4
Triglycerides (mmol/l)	0·96 ± 0·23	0·56 ± 0·06	0·66 ± 0·14	0·89 ± 0·17
Lp(a) (g/l)	0·28 ± 0·11	0·21 ± 0·08	0·16 ± 0·03	0·29 ± 0·11
ApoA-I (g/l)	1·33 ± 0·09	1·05 ± 0·07	1·43 ± 0·22	1·37 ± 0·12
ApoB (g/l)	0·85 ± 0·14	0·83 ± 0·13	0·87 ± 0·16	0·82 ± 0·13

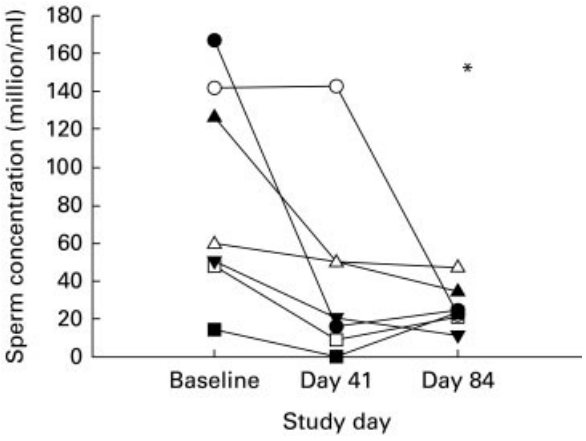

Fig. 4 Individual sperm concentrations (million/ml) at baseline, study day 41 and at study day 84 of seven volunteers after intramuscular injection of 200 mg NETE at day 0. The mean sperm concentration at study day 84 was significantly decreased compared to baseline; $P < 0{\cdot}05$.

Discussion

Probably because NET has strong androgenic properties the short-term treatment was well tolerated by all volunteers and general well-being, clinical chemistry and haematology as well as lipid parameters and ejaculate volume remained unchanged throughout the study. However, as this is a single-dose study, it may well be that effects on the above mentioned parameters would require a prolonged treatment period before they become clinically relevant. On the other hand, the androgenic properties of NET were not strong enough to compensate for specific androgen deficiency symptoms such as number of spontaneous erections, rating of libido and sexual fantasies, while number of ejaculations, number of erections and sexual satisfaction were not significantly altered.

In women after a single intramuscular injection of 200 mg maximum NETE concentrations were 6·06 ± 3·25 μg/l with an AUC of 2318 ± 1498 μg/l* hour (Joshi *et al*., 1989), which is equivalent to our cMax. (9·7 ± 2 mg) and AUC (2781 ± 12 μg/l* hour). In women tMax was reached within 7 days (Sang *et al*., 1981) or 4–8 days (Gerhards *et al*., 1976), comparable to the tMax of NET in our volunteers which was achieved 2–10 days (mean 4·9 ± 1·1 days) after injection. Moreover, the terminal elimination half life (range: 7·3–17·3 days; mean: 12·2 ± 1·7 days) was similar to the terminal elimination half life in women which was reported to be between 7·5 and 22·5 days (Sang *et al*., 1981) or 8·7 ± 4·5 days (Joshi *et al*., 1989). The pharmacokinetic parameters of NETE in male volunteers suggests a body distribution and metabolism similar to that in females, which might also be an indicator that fat storage is negligible for NET.

As seen in men with prostatic carcinoma (Szendroi, 1984), administration of NETA leads to a strong suppression of gonadotrophins. Based on the assumption that similar pharmacokinetics i.e. as in women, would result in similar biological response, we chose a dose of 200 mg NETE for this initial trial. However, gonadotrophin suppression in our volunteers did not persist as long as in females, where serum progesterone levels indicated persistence of anovulation up to 12 weeks after intramuscular injection of 200 mg NETE (Joshi *et al*., 1989). This probably could be explained by the greater vulnerability of gonadotrophin secretion in women to exogenous hormone application (Flores, 1994). Especially FSH was suppressed very rapidly near to the detection limit of the assay and was clearly more strongly suppressed than in a trial for hormonal male contraception with NETA (Guerin & Rollet, 1988). On the other hand, LH suppression was incomplete. However, as shown previously in studies with NETA in women, NET reduces the LH pulse frequency without significantly decreasing the LH pulse amplitude (Couzinet *et al*., 1996). This effect is similar to the inhibitory action of progesterone at the hypothalamic level during the luteal phase in normal cycling women (Knobil & Hotchkiss, 1994). In men, as in women androgens are probably not involved in the gonadotrophin suppressive effect of NET (Couzinet *et al*., 1996). The progestogenic activity of NET in males may also explain the incomplete LH suppression in our study.

However, in postmenopausal women NET was converted to ethinyl oestradiol, corresponding to an oral dose equivalent of

about 4 μg per milligram of NET (Kuhnz *et al*., 1997) and it may well be that at least some of the antigonadotrophic effects of NET observed in our study are mediated through the oestrogen receptor as shown in women previously (Flores *et al*., 1986). This might be also another possible explanation for the gender difference in gonadotrophin suppression which might be due to the different susceptibility of men and women to the consequences of NET oestrogenic metabolites.

Despite the incomplete suppression of LH, serum testosterone levels were reversibly suppressed up to a mean ± SEM nadir of 2·7 ± 0·6 nmol/l at study day 10, followed by a marked suppression and reconstitution of serum SHBG levels. At first view this result is surprising but may be explained by direct local effects of NETE or NET and its metabolites on the Leydig cells. This was shown previously in adult male rats, where placement of NET filled Silastic implants in one epididymal fat pad led to drastic reduction of testicular size and weight and sperm production at the ipsilateral site, while the contralateral testis and weight of both epididymides were not affected by the treatment, suggesting normal serum testosterone levels due to the testosterone production of the contralateral site (Srivastava & Malaviya, 1980).

In humans this profound and rapid suppression of testosterone levels has been seen previously only in trials using GnRH antagonists (Behre *et al*., 1992a; Behre *et al*., 1997a) where the serum testosterone levels of about 2 nmol/l could be attributed mainly to residual adrenal production. In other studies for male contraception gestagens have been added to testosterone preparations to enhance the gonadotrophin suppression (for review see Nieschlag & Behre, 1998). However, neither studies using depot-medroxyprogesterone acetate (Alvarez-Sanchez *et al*., 1977; Frick *et al*., 1977; Faundes *et al*., 1981; Hedman *et al*., 1988; Knuth *et al*., 1989; Wu & Aitken, 1989; Pangkahila, 1991; Handelsman *et al*., 1996) nor studies using other progestins such as levonorgestrel (Anawalt *et al*., 1999; Bebb *et al*., 1996; Büchter *et al*., 1999) have shown such a pronounced and rapid suppression of serum FSH and testosterone comparable to GnRH antagonists or the current study. Contraceptive studies with GnRH-antagonists have shown suppression of spermatogenesis after 12 weeks between 80 and 90% of initial sperm concentration (Pavlou *et al*., 1991; Tom *et al*., 1992; Bagatell *et al*., 1993; Swerdloff *et al*., 1998). The extreme and rapid suppression of serum FSH and testosterone in our study lead to a significant 75% reduction of sperm counts around the end of one spermatogenetic cycle. However, the suppression of sperm counts was not as pronounced as in contraceptive studies with GnRH antagonists or gestagens, as those regimes consist of repeated dosing of the GnRH antagonist or gestagen together with an androgen.

In conclusion, because of its strong, rapid and sustained suppression of serum FSH and testosterone values combined with a favourable long-term injection interval profile and good tolerability, norethisterone enanthate offers great potential for hormonal male contraception if combined with testosterone esters, preferably long acting.

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