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7a-Methyl-19-Nortestosterone (MENT): Pharmacokinetics and Antigonadotropic Effects in Men

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Academic Dissertation

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Contents

List of Original Publications

This thesis is based on the following original publications which are referred to in the text by the Roman numbers I to IV:

I Janne Suvisaari, Kalyan Sundaram, Gabriela Noé, Narender Kumar, Claude Aguillaume, Yun-Yen Tsong, Pekka Lähteenmäki, C. Wayne Bardin. Pharmacokinetics and pharmacodynamics of 7α-methyl-19-nortestosterone after intramuscular administration in healthy men. Human Reproduction 1997; 12(5):967-973.

II Narender Kumar, Janne Suvisaari, Yun-Yen Tsong, Claude Aguillaume, C. Wayne Bardin, Pekka Lähteenmäki, Kalyan Sundaram. Pharmacokinetics of 7α-Methyl-19-nortestosterone in Men and Cynomolgus Monkeys. Journal of Andrology 1997; 18(4):352-358.

III Gabriela Noé, Janne Suvisaari, Cameron Martin, Alfred Moo-Young, Kalyan Sundaram, Saleh I. Saleh, Eliana Quintero, Horacio B. Croxatto, Pekka Lähteenmäki. Gonadotrophin and testosterone suppression by 7α-methyl-19-nortestosterone acetate administered by subdermal implant to healthy men. Human Reproduction 1999; 14(9):2200-2206.

IV Janne Suvisaari, Alfred Moo-Young, Auni Juhakoski, Kaisa Elomaa, Saleh I. Saleh, Pekka Lähteenmäki. Pharmacokinetics of 7α-Methyl-19-nortestosterone (MENT™) Delivery Using Subdermal Implants in Healthy Men. Contraception 1999; 60(5):299-303.

Abbreviations

BMI	body mass index; weight / height^2 (kg/m^2)
°C	degrees Celsius
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
i.m.	intramuscular
i.v.	intravenous
kg	kilogram
l	liter
LH	luteinizing hormone
m^2	square meter
MENT	7α-methyl-19-nortestosterone
MENT Ac	7α-methyl-19-nortestosterone acetate
mg	milligram
µg	microgram
min	minute
ml	milliliter
mol	mole
nmol	nanomole
SHBG	sex hormone-binding globulin
U	international units for gonadotropin concentrations; same as IU
vs.	versus

Abstract

With the ultimate aim of developing a new hormonal male contraceptive and a new androgen for replacement therapy, we studied the pharmacokinetics and endocrine effects of 7α-methyl-19-nortestosterone (MENT). The results of studies carried out *in vitro* and in animals indicated that MENT is an aromatizable androgen with progestational effects. It does not seem to be toxic, its effect on muscle and its feedback effect on the pituitary are at least 10 times those of testosterone, and it is not activated by 5α-reduction, its effect on the prostate thus being only 4 times that of testosterone. Hence, we assumed that a dose of MENT adequate for androgen substitution and gonadotropin suppression to a level leading to azoospermia would not overstimulate the prostate. Furthermore, subdermal implants releasing MENT acetate (MENT Ac), a pro-drug rapidly converted to MENT *in vivo*, could be made. We studied the concentrations of MENT, gonadotropins, and testosterone in the sera of healthy men who had received single intravenous or intramuscular MENT injections, a series of six daily intramuscular MENT injections, or who had had MENT Ac implants inserted for 4 weeks. We found that MENT has a terminal half-life of about 40 min, a total apparent volume of distribution of about 70 l, and a clearance rate of about 2000 l/day (27 l/kg/day). Intramuscular MENT injections in doses ranging from 1.0 to 4.0 mg as well as 1, 2, or 4 subdermal implants releasing MENT acetate at a rate of approximately 0.3 to 1.2 mg per day caused dose-dependent suppression of gonadotropin concentrations. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations in all men with 4 implants decreased to or below 0.6 U/l. These concentrations are similar to those seen in men rendered azoospermic by testosterone ester injections in male contraception trials, but due to the short duration of our studies we could not investigate the potential of MENT

administration to cause azoospermia. We conclude that MENT is a very promising candidate for hormonal male contraception and long-term androgen replacement therapy.

Introduction

Androgen-based hormonal male contraception may become available and androgen replacement therapy in the elderly may become widespread in the not so distant future. Whether this will happen or not depends largely on the availability of suitable androgen administration methods. The development of transdermal testosterone preparations was an important advance in androgen replacement therapy but even more convenient administration methods are needed. In contrast to current androgen users, most of whom suffer from serious diseases and are thus ready to accept whatever treatment there is, most of the new potential androgen users are only considering hormonal male contraception or androgen replacement therapy as an option and will probably not accept the inconveniences of traditional androgen administration methods. More user-friendly androgen administration methods are clearly needed. Furthermore, only minor adverse effects can be considered acceptable if androgens are to be used in healthy men. (Hayes, 2000)

Hormonal male contraception based on androgen administration has been shown to be feasible ([*Anonymous*], 1990). It is not yet available, and one of the reasons for this situation is the inconvenience of the available methods of androgen administration. More convenient and safer androgen administration methods will have to be developed before hormonal male contraception becomes a viable option (Amory & Bremner, 2000). Hence, much of the development of new androgen administration methods is taking place as part of the development of hormonal male contraception. Other androgen users would, of course, also benefit from the new androgen administration methods developed to satisfy the demands of contraception.

The risks and benefits of long-term androgen administration have not been studied extensively enough (Bardin *et al*, 1991). With widespread androgen use, adverse effects of androgens could become important public health problems. Hence, the development of more convenient androgen administration methods may not be enough; it may also be necessary to find androgens that are safer than testosterone in long-term use, and it would be even better if androgens with non-contraceptive health benefits could be found.

The synthetic androgenic anabolic steroid 7α-methyl-19-nortestosterone (MENT) has properties that could make it a more convenient and safer alternative to all testosterone-based androgen formulations and an exceptionally promising candidate for male hormonal contraception. Previous experiments carried out in vitro and in animals have revealed that MENT does not undergo 5α-reduction and therefore it has a relatively low potency in tissues such as the prostate where the effect of testosterone is amplified by 5α-reductase enzymes. Hence, it is assumed that MENT is less likely than testosterone to over-stimulate the prostate. MENT is also several times more potent than testosterone. This makes it much more suitable for administration via subdermal implants, a convenient long-term administration method. It has also been shown that MENT is aromatized to an estrogenic compound, a metabolic step that is required for many important effects of androgens. (Agarwal & Monder, 1988; Kumar *et al*, 1992; Sundaram *et al*, 1993; LaMorte *et al*, 1994; Kumar *et al*, 1999)

Despite its potential advantages, neither the pharmacokinetic properties nor the effects of MENT in men had been studied previously. Therefore, we investigated the basic pharmacokinetic properties of MENT administered by intravenous and intramuscular injections as well as subdermal implants, and the effects of MENT on gonadotropin and testosterone concentrations.

Review of the Literature

Overview of Androgen Physiology

Synthesis and Secretion of Androgens

Testosterone and its major metabolite dihydrotestosterone (Figure 1) are the most important androgens; weaker and physiologically less significant androgens include androstenedione, dehydroepiandrosterone, and dehydroepiandrosterone sulfate. In men, testosterone is mainly produced by the interstitial Leydig cells of the testis. A small amount results from peripheral metabolism from androstenedione, a weak androgen produced by the adrenal cortex. In normal men, the importance of adrenal androgens is minimal. The daily amount of testosterone produced by the testes is about 3–7 mg (Bagatell & Bremner, 1996; Vierhapper *et al*, 1997).

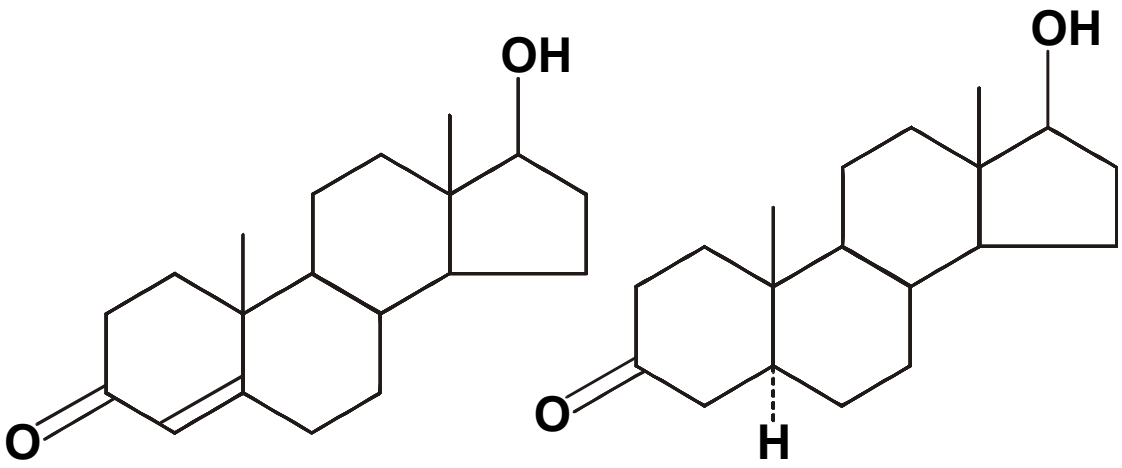

Figure 1. Testosterone (left) and 5α-dihydrotestosterone (right)

In women, testosterone is produced by the theca cells of the ovaries and by peripheral conversion of adrenal androgens. Adrenal androgens are physiologically important in women, since the amount of testosterone produced by the ovaries is much smaller than the amount produced by the testes. The total production rate of testosterone in women is about 0.2–0.4 mg/day (Kirschner & Bardin, 1972; Vierhapper *et al*, 1997).

Testosterone secretion by Leydig cells in men and by theca cells in women is stimulated by LH, which is produced in the anterior part of the pituitary gland. LH secretion is stimulated by the pulsatile secretion of GnRH from the hypothalamus. Androgens, estrogens and progestins exert a negative feedback effect on the secretion of GnRH and LH by their actions on the pituitary and the hypothalamus. Most of the negative feedback effect of androgens is caused by their estrogenic metabolites produced by aromatization. 5α-Reduction does not seem to be necessary for the negative feedback effect of testosterone. (Rittmaster *et al*, 1992; Kumar *et al*, 1995*a*; Hayes et al, 2000)

Testosterone secretion is pulsatile as a result of the pulsatile secretion of LH, which results from the pulsatile secretion of GnRH. There is also a clear circadian variation in testosterone concentrations, which are highest in the morning. There are probably also seasonal, or circannual variations in testosterone concentrations. (Veldhuis *et al*, 1987; Dabbs, 1990; Foresta *et al*, 1997)

Effects of Androgens

Testosterone exerts its actions directly through the activation of androgen receptors, indirectly through its reduction to 5α-dihydrotestosterone which also acts on androgen receptors, or indirectly through its aromatization to estradiol and the activation of estrogen receptors. Androgen and estrogen receptors are ligand-inducible transcription factors. Androgen molecules enter the nucleus of a target cell and associate with unbound androgen receptors. Binding of androgen induces a conformational change in the receptor molecule that leads to its phosphorylation and its dissociation from so-called heat shock proteins. Next, the receptor dimerizes with another androgen receptor that is in the same state. The dimer binds to an androgen response element on an androgen-regulated gene and increases its

transcription rate. Some of the effects of androgens and estrogens, called nongenomic steroid effects, seem to be mediated by different receptors on the cell surface and signal transduction mechanisms similar to those involved in the action of peptide hormones. (Revelli *et al*, 1998; McKenna *et al*, 1999; Prins, 2000)

In tissues such as the prostate, seminal vesicles, epididymis, and certain parts of the skin, testosterone is converted by 5α-reductase enzymes into a more potent androgen, 5α-dihydrotestosterone. The affinity of 5α-dihydro-testosterone for the androgen receptor is about five times that of testosterone (Wilbert *et al*, 1983). (Russell & Wilson, 1994)

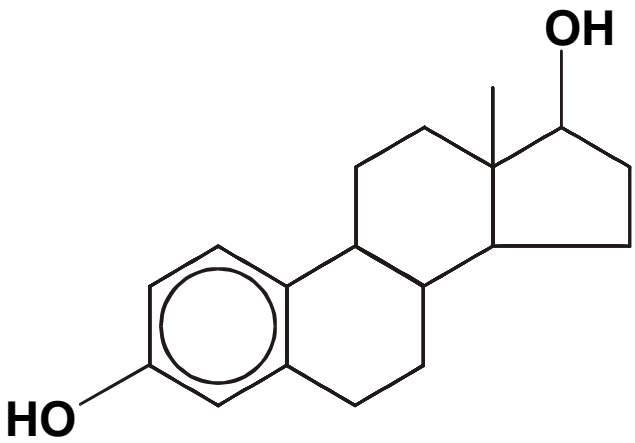

Figure 2. Estradiol

Actions of testosterone that are mediated through its aromatization to estradiol (Figure 2) include most of its negative feedback effect on the secretion of luteinizing hormone (LH) and gonadotropin-releasing hormone (GnRH), some of its behavioral effects, some of its effects on bone, some of its effects on spermatogenesis, and some of its effects on carbohydrate and lipid metabolism. (Finkelstein *et al*, 1991; Bulun, 1996; Grumbach & Auchus, 1999; Hayes *et al*, 2000; Pentikäinen *et al*, 2000; Simpson, 2000)

As far as is currently known, there is only one type of nuclear androgen receptor. It is a ligand-activated transcription factor like other members of the nuclear receptor superfamily, such as the receptors for estrogens,

progestins, glucocorticoids, mineralocorticoids, thyroid hormones, and vitamins A and D_3. The nuclear receptor superfamily also includes a large group of proteins referred to as orphan receptors, for which specific ligands are unknown. There are two types of estrogen receptor, termed alpha and beta, having clearly different structures, tissue distributions, and effects. (Gustafsson, 1999; Enmark & Gustafsson, 1999)

The physiological actions of androgens in males include the development and maintenance of the structure and functions of the reproductive system, including spermatogenesis, and the development and maintenance of secondary sexual characteristics. Male secondary sexual characteristics include a higher bone density and muscle mass, a larger larynx and deeper voice, and a higher hematocrit than in females, as well as male patterns of hair growth, male sexual orientation, patterns of behavior, libido and potency. (Bagatell & Bremner, 1996)

Spermatogenesis is not regulated directly by testosterone or gonadotropins, but by paracrine substances secreted by the Sertoli cells of the seminiferous tubules. The function of the Sertoli cells is hormonally regulated by testosterone and follicle-stimulating hormone. Recent findings suggest that the effect of testosterone on spermatogenesis might also be mediated through its aromatization to estradiol, and estradiol receptors in developing germ cells (Pentikäinen *et al*, 2000). An extremely high local testosterone concentration in the testes, practically achievable only by local testosterone production by the Leydig cells, is necessary for spermatogenesis. Follicle-stimulating hormone (FSH) seems to be necessary for quantitatively and qualitatively normal spermatogenesis and normal fertility, but sperm production and even fertility appears to be possible in the absence of the action of FSH. For example, two out of five men with an inactivating FSH receptor mutation (566C→T, $Ala^{189}Val$) had fathered children, and none of

the five was azoospermic. (Swerdloff *et al*, 1992; Cummings & Bremner, 1994; Tapanainen *et al*, 1997; Themmen & Huhtaniemi, 2000)

Effects of Ageing in Testosterone Concentrations

Serum concentrations of testosterone, free testosterone, and non-SHBG-bound testosterone all decrease with normal ageing in men. Since SHBG (sex hormone-binding globulin) concentrations tend to increase with age, non-SHBG-bound testosterone levels tend to decrease more than those of total testosterone. (Tenover, 1998; Perry, 1999) In women also, testosterone concentrations decline with increasing age. The decline begins in the reproductive years and continues after the menopause. (Davis & Burger, 1998)

Indications for Androgen Administration

The most obvious medical indication for androgen administration is male hypogonadism. Men with hypogonadism are unable to synthesize adequate quantities of androgens and need long-term androgen replacement to maintain sexual behavior, androgen-dependent physiological processes, secondary sexual characteristics, and mental health. The causes of hypogonadism can be divided into two main groups, primary or hypergonadotropic hypogonadism, and secondary or hypogonadotropic hypogonadism. In primary hypogonadism the defect is either in the gonads or post-gonadal, for example in the conversion of testosterone to 5α-dihydrotestosterone or at the level of the androgen receptor. In secondary hypogonadism, the defect is a lack of gonadotropins. The causes of primary hypogonadism include gonadal defects caused by genetic diseases such as Klinefelter's syndrome, anatomical defects, and lesions caused by infections, toxins or radiation, as well as androgen or LH insensitivity

syndromes. The causes of secondary hypogonadism include pituitary and hypothalamic diseases including panhypopituitarism, hyperprolactinemia, isolated gonadotropin deficiencies, and various genetic disorders as well as systemic causes such as chronic disease, starvation, severe obesity, and the adverse effects of certain drugs such as glucocorticoids. Delayed puberty can be considered a constitutional cause of secondary hypogonadism. (Bagatell & Bremner, 1996)

The traditional treatment of choice for male hypogonadism is a testosterone ester such as testosterone enanthate given intramuscularly (100–300 mg every 10 to 21 days). Smaller doses are used in adolescent boys with hypogonadism or boys with a constitutional delay of puberty. Although constitutional delay of puberty is a variant of normal pubertal maturation, low-dose androgen replacement therapy is recommended because it results in psychological and physiological benefits. (Bhasin, 1992; Bagatell & Bremner, 1996; Houchin & Rogol, 1998)

Androgen replacement therapy is potentially useful in a variety of disorders where hypogonadism is a consequence of a severe systemic disease such as chronic renal failure (Handelsman & Liu, 1998) and human immuno-deficiency virus infection. Hypogonadism occurs in approximately 30% of men infected with human immunodeficiency virus. Several clinical trials on the effects of androgen supplementation in men infected with human immunodeficiency virus have been conducted, and the results are encouraging. (Dobs, 1998; Bhasin & Javanbakht, 1999).

Androgens may also be useful in the treatment of eugonadal patients with secondary wasting syndromes. Androgens have been shown to be beneficial in several groups of chronically ill patients who have lost body mass (Dobs, 1999).

The results of several studies indicate that androgen replacement therapy in older men improves muscle strength and bone mass, decreases fat mass, and raises the serum hemoglobin concentration. (Perry, 1999) The increase in hemoglobin and hematocrit may even be excessive (Sih *et al*, 1997). However, available data are still insufficient to permit any major conclusions about the role of androgen replacement in the treatment of age-related physiological changes (Hayes, 2000).

Androgens may be beneficial in the treatment of skeletal muscle dysfunction in chronic obstructive pulmonary disease, although very little experimental evidence exists on this subject. If androgens are administered to patients with chronic obstructive pulmonary disease, the possibilities that androgens may increase hematocrit or worsen sleep apnea are of special concern. (Casaburi, 1998)

Androgens have traditionally been used in the treatment of some forms of anemia and other hematologic disorders, such as aplastic anemia, Fanconi's anemia, anemia of chronic renal failure, paroxysmal nocturnal hemoglobinuria, idiopathic autoimmune hemolytic anemia, agnogenic myeloid metaplasia, and idiopathic thrombocytopenic purpura. Androgens have consistent albeit modest efficacy in the treatment of some of these conditions, such as anemia of chronic renal failure. Androgens are still in limited use in the treatment of hematologic disorders, but they have been largely replaced by newer options such as recombinant erythropoietin and other hematopoietic growth factors, bone-marrow transplantation, anti-thymocyte globulin, and immunosuppressive drugs such as cyclosporine A. (Besa, 1994; Handelsman & Liu, 1998)

Hereditary angioedema, a disease caused by deficiency of C1-inhibitor, can be successfully treated with some synthetic derivatives of testosterone but not with testosterone itself; it seems that a drug's efficacy in preventing

hereditary angioedema attacks is connected to its 17α-alkylation. (Bagatell & Bremner, 1996)

In women, androgen replacement has been used as a component of hormone replacement therapy for the restoration of libido and general well-being in symptomatic testosterone deficiency states following premature ovarian failure, menopause or iatrogenic ovarian failure. Potential indications also include the treatment of premenstrual syndrome, the prevention of glucocorticoid-induced and premenopausal bone loss, loss of libido in premenopausal women, and the management of wasting syndromes secondary to human immunodeficiency virus infection and malignancies. The weak androgen danazol is used in the treatment of endometriosis. As stated previously, women are much more susceptible to the adverse effects of androgens. Hence, high doses of androgens should be avoided altogether in women. Postmenopausal women treated with androgens should usually receive concurrent estrogen replacement therapy to reduce the risk of adverse effects. (Davis & Burger, 1998; Friedrich, 2000)

Non-medical use of androgens is widespread in certain parts of the population in many countries. Athletes have used androgens to increase muscle mass and improve physical performance at least since the 1940s (Bagatell & Bremner, 1996). There is a lack of direct and reliable information on the use of androgens in sports, but, for example, the drastic decline in Olympic results since the introduction in 1989 of out-of-competition doping testing is indirect evidence of the widespread use of androgens and other performance enhancing drugs among top athletes (Franke & Berendonk, 1997). Until recently, in some countries such as the German Democratic Republic, androgen use was customary not only among male athletes but also among female and child athletes (Franke & Berendonk, 1997). The rate of non-medical androgen use to improve physical capacities or appearance is also high in some occidental countries.

For example, in the United States 12% of high school senior boys reported having used androgenic anabolic steroids, although the rate in a survey of the general population was much lower, 0.5% (0.9% for males and 0.1% for females) (Yesalis *et al*, 1993).

A new form of non-medical (or more exactly, non-therapeutic) androgen use would be the use of androgens in male contraception. Hormonal male contraception is discussed below. It has even been proposed that androgens could be useful during space travel (Bhasin *et al*, 1996) — presumably to prevent the effects of the lack of gravitation on bone and muscle.

Beneficial and Adverse Effects of Androgen Administration

Beneficial Effects of Androgen Administration

The obviously beneficial effects of androgen administration related to the indication for androgen administration have been discussed above. Androgens may also have beneficial effects unrelated to the reason for their administration. For example, long-term androgen use for male contraception might have non-contraceptive benefits. It is important to consider such potential benefits in addition to the potential adverse effects of androgens.

A clear increase in muscle size and strength was observed in normal men who received 600 mg of testosterone enanthate each week for 10 weeks (Bhasin *et al*, 1996). This dose is several times higher than those ordinarily used in androgen replacement therapy (about 100 mg per week) or male contraception studies (about 200 mg per week). Although indirect evidence from androgen use in sports (Franke & Berendonk, 1997) also supports the notion that exogenous androgens increase muscle mass and strength, previous investigators could not demonstrate this effect, and it seems that a significant response of muscle to exogenous androgen in healthy men

requires a high dose (Bardin, 1996). Hence, this benefit may not be present or it may be too small to be noticed when androgens are used in androgen replacement therapy or male contraception.

Androgens are undoubtedly beneficial in the treatment of osteoporosis in hypogonadal men, and the results of some studies suggest that androgens may also be beneficial in the treatment of osteoporosis in eugonadal men (Katznelson, 1998). Hence, it is not unreasonable to assume that long-term androgen administration for the purposes of contraception might decrease the risk of osteoporosis later in life.

It has been postulated that a reason for the lower incidence of osteoporosis and Alzheimer's disease in men could be the fact that men have much higher testosterone concentrations than women and hence a better supply of precursors for local estrogen production in estrogen-dependent tissues (Simpson, 2000).

Adverse Effects of Androgen Administration

The adverse effects of androgen replacement therapy in men with hypogonadism are mainly due to the androgen administration methods used; these effects are discussed in the section on androgen administration methods. Raising testosterone concentrations to the levels seen in normal healthy men should not cause other effects than those of endogenous testosterone. There is one exception, however. The effect of exogenously administered testosterone on the testis itself is different from the effect of testosterone produced within the testis. This is because, in contrast to administered testosterone, intratesticular testosterone production leads to the extremely high intratesticular testosterone concentrations (about 2 nmol/g of testis tissue, which is approximately 100-fold compared to circulating testosterone concentrations) (Huhtaniemi *et al*, 1985). A high intratesticular testosterone concentration is necessary for spermatogenesis (Wu, 1988).

Hence, exogenous testosterone administration leads to infertility and testicular atrophy, both of which seem to be reversible if testosterone administration is discontinued (Wu *et al*, 1996; Rolf & Nieschlag, 1998).

Moderately supraphysiological doses of testosterone, such as those used in male contraception trials, have caused acne, gynecomastia, increased or decreased sexual interest, mild behavioral changes, reduction in the circulating concentrations of high-density lipoprotein cholesterol, prostatic enlargement, a decrease in testis size and azoospermia. (Anderson *et al*, 1995; Wu *et al*, 1996; Rolf & Nieschlag, 1998).

Because androgens control the growth and differentiation of the prostate, a major cause of concern is the long-term effect of testosterone administration on the prostate. A small increase in prostate size has been observed in male contraception studies, but only future longer clinical trials will clarify the issue of whether very long-term testosterone administration leads to symptomatic prostatic hyperplasia or an increased risk of cancer of the prostate. (Wallace *et al*, 1993; Rolf & Nieschlag, 1998)

The serious adverse hepatic effects associated with prolonged use of some androgens, such as cholestasis, peliosis hepatis (blood-filled hepatic cysts), hepatocellular hyperplasia, hepatic adenomas, and hepatocellular carcinoma seem to be mostly restricted to users of 17α-alkylated derivatives of testosterone. (Huhtaniemi, 1994; Bagatell & Bremner, 1996; Rolf & Nieschlag, 1998; Winters, 1999)

The results of several observational studies suggest that some men develop marked aggression, or hypomanic or manic symptoms when using high doses of androgenic anabolic steroids (Pope & Katz, 1994). The results of a recent randomized, placebo-controlled, double-blind clinical trial confirmed that doses of 600 mg of testosterone cypionate per week caused a significant increase in measures of manic and aggressive symptoms in a small subset of

men, while there were few changes in most. The symptoms were marked in 2, moderate in 6 and minimal in 42 men; the subgroups were otherwise indistinguishable as regards demographic, psychological, physiological, and laboratory measures. In the same study, 300 mg per week produced only slight effects. (Pope *et al*, 2000) In contrast, no changes in mood or behavior were observed among 43 men who received 600 mg of testosterone enanthate weekly for ten weeks (Bhasin *et al*, 1996). In conclusion, it seems that a small subset of men is prone to develop adverse behavioral effects and mood disturbances during high-dose androgen treatment, but that most men are not vulnerable to such effects.

The question of whether exogenous androgens, at physiological or slightly supraphysiological doses increase or reduce the risk of cardiovascular disease remains controversial. Although changes in lipoprotein levels that are considered atherogenic — mainly a decrease in high-density lipoprotein cholesterol — have been observed (Anderson *et al*, 1995), changes in lipoprotein levels that may be beneficial, such as a decrease in lipoprotein(a) have also been observed. Lipoprotein(a) concentrations are increased in some patients with coronary artery disease and cerebrovascular disease, but the results of prospective studies are conflicting and its role in atherogenesis remains to be established (Jialal, 1998). The consistent finding that low testosterone concentrations in men are associated with common risk factors of coronary artery disease such as a pro-atherogenic lipid profile, systolic and diastolic hypertension, hyperinsulinemia, android obesity, and high fibrinogen levels may indirectly suggest that exogenous testosterone would reduce the risk of cardiovascular disease in some men. (English *et al*, 1997)

Overall, it seems that in men, the risks associated with androgen use have been exaggerated. Really severe side-effects are rare, and many reports on

side-effects are case reports and confounding factors have often not been clarified (Huhtaniemi, 1994; Rolf & Nieschlag, 1998).

The situation is different in women and children. In addition to the adverse effects androgens can cause in men, women are vulnerable to effects that are adverse in women but part of normal androgen action in men, such as hirsutism, temporal balding, and voice deepening, and to adverse effects that are only possible in women, such as clitorolomegaly. (Davis & Burger, 1998) The possibility of an increase in breast cancer risk is another concern about testosterone therapy in women. The results of epidemiological studies have shown both positive and negative associations between androgen levels and breast cancer risk, and this issue remains unclarified (Friedrich, 2000). In pre-pubertal children of both sexes, androgens can cause virilization and premature epiphyseal closure (Rolf & Nieschlag, 1998).

Androgen Administration Methods

The traditional androgen administration methods are intramuscular injections of testosterone 17β-esters and oral administration of testosterone undecanoate and 17α-alkylated derivatives of testosterone. The alkylated derivatives are now used less frequently, since they can have serious hepatic adverse effects. A newer method, transdermal testosterone administration, is now also in routine use. Lately, the shortcomings of traditional androgen administration methods have received more attention and many new androgen administration methods are being developed. (Bhasin, 1992; Bagatell & Bremner, 1996; McClellan & Goa, 1998; Winters, 1999; Hayes, 2000)

Injectable Androgen Preparations

If testosterone is given by intramuscular injection, it is rapidly absorbed and, having a half-life of only approximately 30 minutes, rapidly degraded. Hence, testosterone injections cannot be used as such in androgen replacement. Testosterone esters such as testosterone enanthate (Figure 3) are absorbed slowly when injected intramuscularly in an oily preparation. After absorption, these esters are rapidly deesterified and free testosterone is the active drug. Testosterone enanthate, testosterone cypionate and other testosterone esters as well as mixtures of testosterone esters are routinely used for androgen replacement therapy. The recommended dose for testosterone enanthate or testosterone cypionate is 200 mg every 2 weeks, or 300 mg every 3 weeks. (Bhasin, 1992)

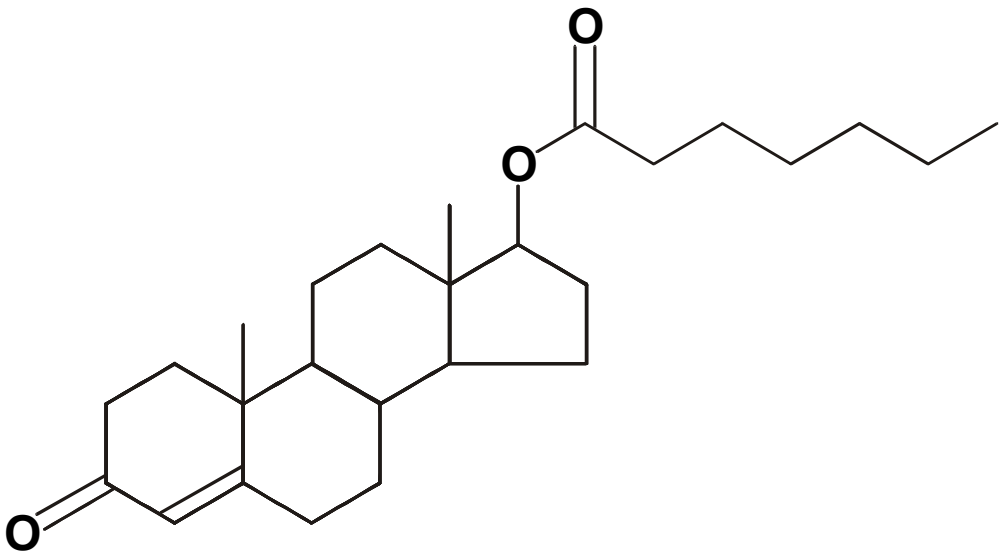

Figure 3. Testosterone enanthate

The drawbacks of conventional testosterone ester regimens include the inconvenient frequency and painfulness of the intramuscular injections as well as the wide fluctuation in testosterone concentrations during each injection interval, concentrations being too high for a few days after the injection and too low shortly thereafter. The unnecessarily high testosterone concentrations early in the injection interval may increase the risk of adverse effects. In addition, the fluctuations in testosterone concentrations

lead to fluctuations in psychological androgen effects such as mood and sexual desire. (Bhasin, 1992; Bagatell & Bremner, 1996; Winters, 1999)

Injectable testosterone undecanoate has a longer duration of action than testosterone enanthate. A single dose has been shown to maintain normal serum testosterone levels in hypogonadal men for at least six weeks (Amory & Bremner, 2000). Injectable testosterone undecanoate was shown to be very effective in suppressing spermatogenesis in Chinese men when administered every four weeks (Zhang *et al*, 1999).

Testosterone buciclate (Figure 4) is a testosterone ester with a benzol ring incorporated into its side-chain. It has a much longer duration of action than testosterone enanthate, presumably because of its more hydrophobic side-chain. It has been developed to solve the problem of short injection intervals. In hypogonadal men, a single injection of 600 mg of testosterone buciclate increased circulating testosterone concentrations to the lower normal range for about 3 months (Behre & Nieschlag, 1992). The potential of testosterone buciclate in hormonal male contraception has also been investigated and the first results have been encouraging (Behre *et al*, 1995).

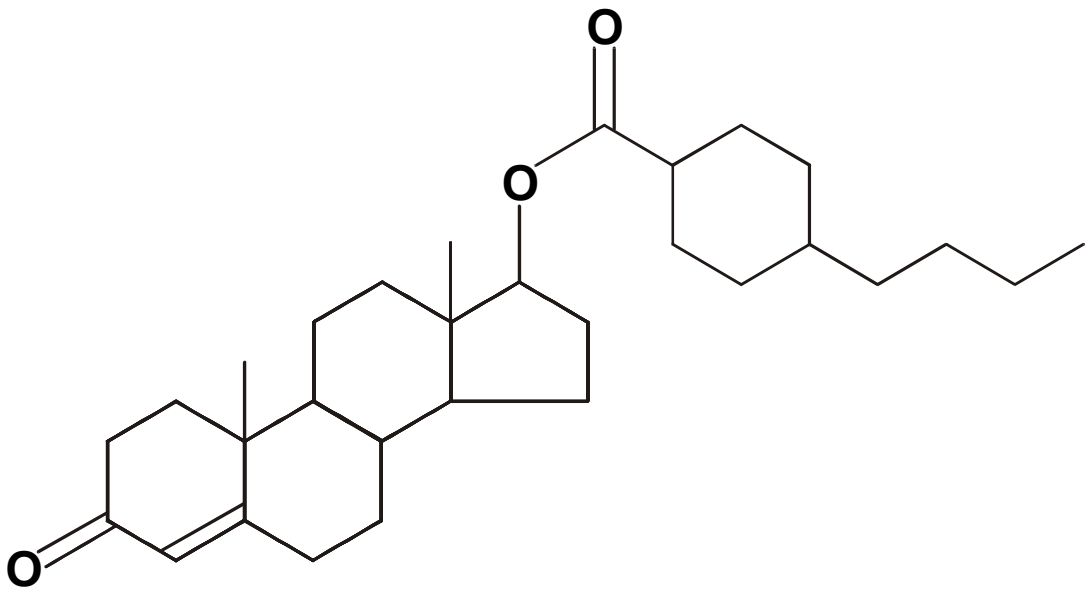

Figure 4. Testosterone buciclate

Another experimental long-acting injectable testosterone formulation is based on the encapsulation of testosterone in biodegradable microcapsules made of a lactide/glycolide copolymer, a substance that is also used in biodegradable sutures. Eugonadal testosterone concentrations could be maintained for 10–11 weeks in hypogonadal men who received 630 mg of testosterone in this depot injection preparation. (Bhasin *et al*, 1992)

Longer-acting injectable testosterone formulations suffer from a common disadvantage: a large amount of drug needs to be administered and consequently the injection volume is also large. For example, in the above-mentioned testosterone microcapsule study, each subject received 5 ml divided into two injection sites (Bhasin *et al*, 1992). The need to administer a large amount of drug is a direct consequence of the potency of testosterone, and can only be remedied by using a more potent androgen.

Transdermal Androgen Preparations

In suitable formulations, testosterone can be absorbed through the skin. Because scrotal skin is at least 5 times more permeable to testosterone than are other skin sites, the first available testosterone transdermal delivery system was designed for scrotal skin. Systems for the delivery of testosterone across nonscrotal skin are now also available. Transdermal testosterone delivery systems provide adequate testosterone replacement for hypogonadal men and, in addition, produce a testosterone concentration pattern similar to the normal circadian fluctuation of testosterone concentrations. (McClellan & Goa, 1998; Winters, 1999)

An obvious inconvenience of both kinds of testosterone patches is the need to remove the old patch and apply a new one each night. The major disadvantage of non-scrotal testosterone patches is that skin irritation at the application site is frequent. Approximately 50% of men who participated in clinical trials reported transient, mild to moderate erythema at the

application site sometime during therapy, and burnlike blister reactions occurred in 12% of men during the clinical trials. (McClellan & Goa, 1998; Winters, 1999)

Skin irritation is less frequent for scrotal patches, since these do not require the enhancers that are required to allow permeation of testosterone through non-genital skin. Disadvantages of the scrotal patches include the inconvenience of having to apply the patch to a shaved scrotum, and an unphysiologically high DHT to testosterone ratio due to the high 5α-reductase activity in scrotal skin. Gel formulations delivering testosterone through the skin have also been tested, and some results are encouraging. A very promising new formulation is a hydroalcoholic gel containing 1% testosterone. In a recent multicenter study this formulation was compared with testosterone patches in the treatment of 227 hypogonadal men and shown to be more or equally effective with a much lower incidence of skin irritation (6% vs 66%) (Wang *et al*, 2000). (Bhasin, 1992; Behre *et al*, 1999; Winters, 1999)

Oral Androgen Preparations

If administered orally, testosterone cannot reach an effective circulating concentration because it is extensively degraded by hepatic first-pass metabolism, as are most testosterone esters. However, there is one long chain fatty acid testosterone ester, testosterone undecanoate, that can be administered orally since it is absorbed into the intestinal lymphatic system whence it passes through the thoracic duct into the systemic circulation, thus avoiding hepatic first-pass metabolism. However, when given orally it has a short duration of action and has to be administered thrice daily, making it somewhat inconvenient for long-term use. It has also been tested as a possible male contraceptive but sufficient suppression of spermatogenesis has not been attained. (Nieschlag *et al*, 1978; Bhasin, 1992)

Testosterone from experimental sublingual and buccal formulations is absorbed through the oral mucosa directly into the systemic circulation thus avoiding hepatic first-pass metabolism. Sublingual and buccal testosterone preparations share with testosterone undecanoate the disadvantage that testosterone concentrations high enough for hormonal male contraception are difficult to achieve. (Kim *et al,* 1995; Salehian *et al,* 1995)

The 17α-alkylated synthetic derivatives of testosterone such as methyl-testosterone, fluoxymesterone, methandrostenolone, oxandrolone, and stanozolol are resistant to hepatic first-pass metabolism and are therefore orally active. However, their use has been associated with serious hepatic adverse effects such as cholestasis, peliosis hepatis, hepatocellular hyperplasia, hepatic adenomas, and hepatocellular carcinoma. (Bhasin, 1992; Bagatell & Bremner, 1996; Walter & Mockel, 1997; Winters, 1999)

Androgen Implants

Subdermal testosterone pellets (implants of crystalline testosterone) have been in clinical use for a long time in some countries. The pellets are inserted into the lower abdominal wall in a minor surgical operation under local anesthesia. Testosterone pellets can provide serum testosterone concentrations in the normal range for several months in hypogonadal men. The main drawback of this method of androgen administration is that extrusion of these pellets occurs relatively frequently. The extrusion rate appears to be approximately once for every 10 implantation procedures. (Handelsman *et al*, 1990; Kelleher *et al*, 1999)

Hormonal Male Contraceptives

Male hormonal contraception has been shown to be both effective and reversible. All methods that have been found to be effective are based on

the same principle: suppression of LH secretion from the pituitary, which leads to the suppression of testosterone production in the testes, and ultimately to suppression of spermatogenesis, since spermatogenesis is dependent on a very high intratesticular testosterone concentration. Recent findings suggest that the effect of testosterone on spermatogenesis might be mediated through its aromatization to estradiol (Pentikäinen *et al*, 2000), but whether testosterone is needed as such or as a precursor for estradiol, a very high intratesticular testosterone concentration is necessary for spermatogenesis. The secretion of LH is stimulated by the pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus. Androgens, estrogens and progestagens exert a negative feedback effect on the secretion of LH by the pituitary and on the secretion of GnRH by the hypothalamus. Thus, LH secretion can be suppressed with exogenous androgens, progestins, estrogens, and substances that block the effect of GnRH. The latter group includes, in addition to GnRH antagonists, GnRH agonists which have an inhibitory effect on the pituitary when administered in a continuous, rather than a pulsatile manner. This paradoxical effect is explained by down-regulation of GnRH receptors in the pituitary. Secretion of LH can also be suppressed by active immunization against GnRH through the use of injections of GnRH combined with tetanus toxoid. (Wu, 1988; Swerdloff *et al*, 1992; Cummings & Bremner, 1994; Amory & Bremner, 2000)

The hormonal contraceptive methods that inhibit LH secretion also inhibit the secretion of the other gonadotropin, FSH. This hormone stimulates spermatogenesis through its effects on Sertoli cells. The suppression of FSH secretion probably contributes to the anti-fertility effects of male hormonal contraceptives, but this effect seems to be of less importance than suppression of LH secretion, since spermatogenesis seems to be possible without the action of FSH. (Matsumoto & Bremner, 1989; Themmen & Huhtaniemi, 2000)

When azoospermia is achieved by using substances other than androgens, androgen replacement is necessary. Because all these methods work by blocking testosterone production in the testes, androgen replacement is necessary to avoid the symptoms of hypogonadism and to maintain normal sexual function and secondary sexual characteristics. When azoospermia is achieved by using androgens alone, two or three times more androgen is needed than the amount used in androgen replacement therapy to sustain androgen-dependent physiological functions when endogenous androgen production is lacking. (Cummings & Bremner, 1997; Amory & Bremner, 1998)

To sum up, hormonal male contraception can be based on the continuous administration of supraphysiological amounts of androgens or on the continuous administration of physiological amounts of androgens combined with either progestins, estrogens, GnRH antagonists or GnRH agonists.

The fact that sperm production can be hormonally suppressed has been known for decades. A lot of small clinical trials having azoospermia as an endpoint have been conducted, and a fairly clear picture of the effectiveness of various methods has emerged (Amory & Bremner, 1998). In addition to the small clinical trials having azoospermia as an endpoint, two larger multicenter studies having pregnancy in the partner as an endpoint have also been conducted. These studies, conducted by the World Health Organization, showed pregnancy rates of 0.8 and 0.0 per 100 person-years among the partners of healthy men rendered azoospermic by weekly testosterone enanthate injections of 200 mg. (In the first study, the partners of men rendered azoospermic were exposed to the risk of pregnancy for 124 person-years and in the second, for 230 person-years during which weekly testosterone enanthate injections were the only form of contraception. In the first study, there was one pregnancy, and in the second, not a single one among the partners of azoospermic men.) The second study also

demonstrated that severe oligozoospermia (sperm counts below 3 million/ml) is associated with a pregnancy rate (8 per 100 person-years) which is low when compared with those associated with currently available reversible male contraceptive methods such as condoms, but higher than the pregnancy rates associated with female hormonal contraceptive methods. Azoospermia or severe oligozoospermia was achieved in 98% of men. ([*Anonymous*], 1990; [*Anonymous*], 1996)

Although only testosterone enanthate injections have been tested with pregnancy as an endpoint, it seems reasonable to assume that azoospermia or severe oligozoospermia produced by other hormonal methods would be equally effective. Combinations of androgens and progestagens can even be marginally more effective than testosterone alone in suppressing spermatogenesis, but these regimens appear to decrease high-density lipoprotein cholesterol concentrations more than testosterone esters alone (Wu *et al*, 1999; Amory & Bremner, 2000). In small trials, the combination of a GnRH antagonist and testosterone enanthate has also been found to be effective, but not in all men (Tom *et al*, 1992; Bagatell *et al*, 1993; Amory & Bremner, 2000). GnRH antagonists are administered via daily subcutaneous injections, which is clearly not a feasible mode of administration for a contraceptive agent. These agents occasionally cause subcutaneous nodules at the injection site and frequently cause injection site irritation, which is probably mediated by histamine release. (Cummings & Bremner, 1997; Amory & Bremner, 2000) Combinations of testosterone and GnRH agonists have been disappointing (Amory & Bremner, 2000).

For unknown reasons, hormonal contraception is not effective in all men. The effectiveness of the best methods is strikingly different in different populations. In non-Asian men, only 40–70% become azoospermic with the optimal hormonal male contraceptive regimens tested thus far. In contrast, more than 95% of Asian men become azoospermic with these regimens, and

the rest become severely oligospermic. The reasons for this difference as well as the reasons for variability in the suppression of spermatogenesis among non-Asian men remain unknown. (Cummings & Bremner, 1994; Amory & Bremner, 2000)

Despite the fact that male hormonal contraception is feasible and there is a need for new alternatives in contraception, male hormonal contraception is not yet available outside experimental settings. There are several reasons — some of them fairly obvious — for the current situation. One is that the most extensively studied male hormonal contraceptive method involves weekly intramuscular injections of testosterone enanthate, a regimen clearly too inconvenient for widespread use. This problem could be solved by new androgen administration methods (Amory & Bremner, 2000). A problem that will not be solved by new androgen administration methods and that is common to all hormonal male contraceptive methods known today is the slow onset of action and the slow recovery of fertility after stopping use. The duration of spermatogenesis and the transit of sperm through the epididymis and vas deferens is about three months (spermatogenesis lasts approximately 74 days and passage through the epididymis 12 days (Ross & Reith, 1985)). (Cummings & Bremner, 1994)

Since hormonal male contraceptive methods seem to exert their effects at the earliest stages of spermatogenesis (Zhengwei *et al*, 1998), viable sperm are present in men using hormonal male contraception for approximately three months after starting use of the method ([*Anonymous*], 1996). For the same reason, it takes three or four months to recover to normal sperm concentrations after the method is stopped ([*Anonymous*], 1996). Sperm counts have to be monitored until spermatogenesis is sufficiently suppressed, and the men in whom spermatogenesis will never be sufficiently suppressed will only know it after three or four months.

The known and unknown adverse effects of male hormonal contraceptives are of great concern. The adverse effects of androgens have already been discussed. If another drug is used to suppress LH in combination with an androgen, a smaller, physiological amount of androgen will be needed and therefore the risk of androgenic side effects will be lower. However, the other drug can be a source of other adverse effects, as discussed previously. Of great concern are the possible effects of male hormonal contraceptives on future fertility. These and other long-term effects will only be elucidated when the results of longer clinical trials are available. The studies conducted thus far show no evidence of irreversible infertility, but spermatogenesis recovers slowly. As mentioned previously, it takes three or four months to recover to low normal sperm concentrations (20 million per ml), and it takes 6–8 months to recover to pretreatment baseline levels ([*Anonymous*], 1996).

7α-Methyl-19-nortestosterone (MENT)

7α-Methyl-19-nortestosterone (MENT) (Figure 5) was synthesized in the 1960s, and studies on animals showed that it has strong androgenic and anabolic effects (Campbell *et al*, 1963). It was also evaluated as a palliative treatment for female breast cancer patients, in whom a virilizing effect was observed (Segaloff *et al*, 1964; O'Bryan & Talley, 1966). In addition, it was administered to three men with carcinoma of the prostate. Although it does not prove anything, it is interesting to note that one of the patients with metastatic cancer of the prostate showed a subjective improvement of 6 months duration. (O'Bryan & Talley, 1966)

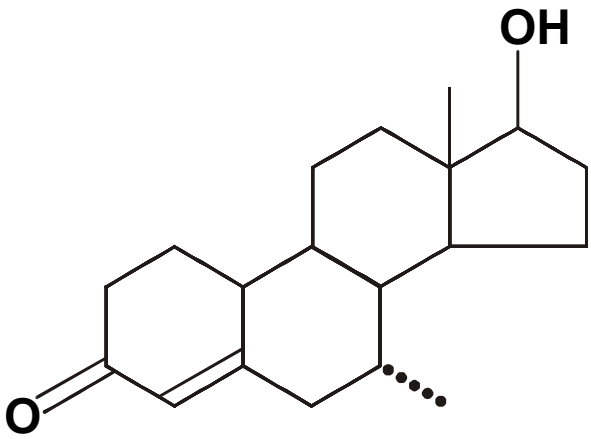

Figure 5. 7α-Methyl-19-nortestosterone (MENT)

The pharmacokinetic properties of MENT were not analyzed in these early studies since serum MENT concentrations could not be measured. The pituitary response to MENT could not be shown directly, since methods for the assay of serum gonadotropin concentrations were unsatisfactory at that time. Urinary excretion of gonadotropins was measured in at least one patient, in whom a 16-fold decrease was observed (O'Bryan & Talley, 1966).

MENT has not been in clinical use. To our knowledge, the pharmacokinetic properties of MENT and its effects on gonadotropin and testosterone concentrations in men had not been studied before the present study.

The development of a radioimmunoassay for the measurement of MENT concentrations made possible the investigation of its pharmacokinetics. The clearance rates and half-lives of MENT in rats and rabbits were found to be comparable to those of testosterone. (Kumar *et al,* 1990)

In *in vitro* studies, the relative binding affinity of MENT to androgen receptors was found to be 3–4 times higher than that of testosterone (Beri *et al*, 1998; Kumar *et al,* 1999). Studies on castrated rats showed that compared with testosterone, the relative potency of MENT is 3–5 times higher in the prostate and seminal vesicles, 10 times higher in muscle, and 12–25 times higher in the suppression of serum gonadotropins (Kumar *et al*,

1992; Kumar *et al*, 1995*b*; Kumar *et al*, 1999). The results of a study on *Macaca fascicularis* monkeys were very similar regarding the relative effects of MENT on the prostate, muscle, and gonadotropins. (Cummings *et al*, 1998) If the potencies of testosterone and MENT are compared by measuring the serum concentrations of these hormones required to achieve complete LH suppression in *Macaca fascicularis*, instead of comparing the doses administered, MENT appears to be more than 60 times more potent. (Cummings *et al*, 1998)

Unlike testosterone, MENT does not undergo 5α-reduction when incubated *in vitro* with homogenized preparations of rat liver, prostate and epididymis (Agarwal & Monder, 1988). This finding can explain the differences in the relative potency of MENT compared with testosterone in tissues that contain or do not contain 5α-reductases. Since MENT is not activated by 5α-reductases, it has a relatively lower potency in tissues that contain 5α-reductase enzymes. Therefore, when administered at doses that maintain normal sexual function and secondary sexual characteristics, it should be less likely to over-stimulate the prostate.

The fact that MENT is not activated by 5α-reductases also raises the question of whether there are any useful physiological effects of 5α-dihydrotestosterone in adults that would not be adequately maintained by MENT. For example, to be an acceptable replacement androgen MENT has to be able to maintain normal sexual function. The results of some studies indirectly suggest that 5α-reduction may amplify the effect of testosterone in maintaining sexual function. In a cross-sectional study in normal men it was found that higher 5α-dihydrotestosterone concentrations were associated with a higher frequency of orgasms, whereas testosterone was unrelated to the frequency of orgasms (Mantzoros *et al*, 1995). Another study revealed a slightly higher incidence of decreased libido, impotence, and ejaculatory disorders among men receiving finasteride, a competitive

5α-reductase inhibitor, compared with men receiving a placebo. (Gormley *et al*, 1992) However, these pieces of evidence are very indirect and there is no known unique beneficial role for 5α-dihydrotestosterone in adults (Cummings *et al*, 1998). All the available evidence concerning MENT suggests that it would maintain sexual behavior and sexual function as well as testosterone. It has been shown that MENT administration can maintain sexual behavior in castrated male rodents (Morali *et al*, 1993; Ogawa *et al*, 1996; Wood *et al*, 1996). In early studies, MENT increased libido in women to the extent that some patients discontinued the treatment because of it (O'Bryan & Talley, 1966). In a recent study in hypogonadal men, MENT improved several measures of sexual function when compared with the situation during a treatment-free period. Furthermore, the effect of MENT, administered by means of subdermal implants releasing MENT acetate, was similar to that of traditional androgen replacement therapy with intramuscular testosterone enanthate injections. (Anderson *et al*, 1999)

As discussed in the Overview of Androgen Physiology, the effects of testosterone on some tissues are, to a greater or lesser extent, carried out by estradiol formed by the aromatization of testosterone. The effects of MENT on these tissues should be physiological, because it has been shown that MENT is also aromatized to an estrogenic compound (LaMorte *et al*, 1994). MENT is also bound to estrogen receptors, but its binding affinity is very low, about 0.03% of that of estradiol (Beri *et al*, 1998).

MENT has been shown to have a progestational effect similar to that of progesterone in rabbits, and the binding affinity of MENT to progesterone receptors is about 60% of that of progesterone (Beri *et al*, 1998).

Toxicological studies in animals have not revealed any important adverse effects. In tolerance studies conducted in the 1960s, oral MENT administration was associated with changes in liver function test results

when doses of 50 or 80 mg per day were administered, but not when the daily dose was 20 mg. (Unpublished data)

The high potency of MENT makes it potentially suitable for long-term sustained release administration preparations. In fact, subdermal implants releasing MENT acetate (MENT Ac) (Figure 6), a pro-drug rapidly converted to MENT *in vivo*, can be made. Such implants have been used in our clinical trials and in another study, in which the effects of MENT Ac implants and testosterone enanthate injections in hypogonadal men were compared (Anderson *et al*, 1999).

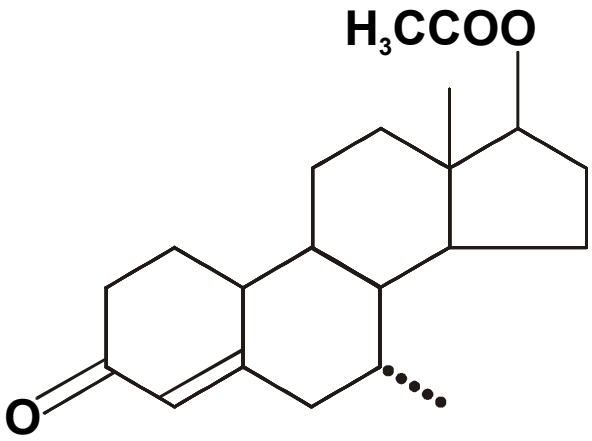

Figure 6. 7α-Methyl-19-nortestosterone acetate (MENT Ac)

Aims of the Study

The main specific aims of the present study were:

To determine the basic pharmacokinetic parameters of MENT by administering MENT by intravenous and intramuscular injections to healthy men and measuring the resulting serum MENT concentrations.

To determine the pharmacokinetics of MENT during sustained release administration by measuring serum MENT concentrations in men who had MENT-releasing implants inserted subdermally.

To determine the effects of MENT administration on the secretion of gonadotropins and testosterone by measuring serum LH, FSH and testosterone concentrations before, during, and after MENT administration in men who received intramuscular MENT injections and in men who had MENT-releasing implants inserted subdermally.

Materials, Methods and Subjects

The present study consists of four phase I clinical trials described in four original publications. The publications are referred to in this text by the Roman numbers I – IV according to the order in which they were published (see the "List of Original Publications"). The trials are discussed in the text in the order in which they were conducted. The single intravenous injection trial was described in publication II, the single intramuscular injection trial, as well as the trial involving six intramuscular injections at daily intervals, were described in publication I, and the MENT implant trial was described in publications III and IV.

Trial Sites

The clinical trials were conducted in the following clinics: the Family Planning Clinic of The Family Federation of Finland (Väestöliitto), Helsinki, Finland, the Consultorio de Planificación Familiar, Instituto Chileno de Medicina Reproductiva, Santiago, Chile, and the Centre for Reproductive Biology, Edinburgh, Great Britain. For brevity, the above-mentioned trial sites are referred to in this text by the names of the cities in which they are located.

The single intravenous injection trial was conducted in Helsinki, the single intramuscular injection trial as well as the trial involving six intramuscular injections at daily intervals in Helsinki and Santiago, and the MENT implant trial in all three clinics. The multicenter trials were conducted according to the same protocol in each clinic.

Design of the Trials

Single Intravenous Injection Trial (Reported in Publication II)

Nine men received a single intravenous injection of 0.5 mg MENT. Two blood samples were collected within 30 minutes before the injection, and the following samples were collected exactly 3, 6, 10, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes after injection.

Single Intramuscular Injection Trial (Reported in Publication I)

A total of 18 men, in two clinics, were randomly allocated to three groups of equal size (three per group in each clinic). Each subject in the first group was given 2 mg of MENT intramuscularly. Subjects in the second and third groups received intramuscular injections of 4 mg and 8 mg of MENT, respectively. The first blood sample was collected just before injection, the following ten samples exactly 15, 30, 60, 90, 120, 180, 240, 300, 360 and 480 minutes after injection and the next samples 1, 2, 3, 4 and 9 days after injection, at the same time in the morning.

Six Intramuscular Injections Trial (Reported in Publication I)

A total of 24 men, in two clinics, were randomly allocated to three groups of equal size (four per group in each clinic). Each subject in the first group was given 1 mg of MENT each morning for six consecutive days. Subjects in the second and third groups received injections of 2 mg and 4 mg according to the same schedule. Blood samples were drawn before treatment and each day just before the MENT injection and thereafter at the same time in the morning, 2, 3, 5, 9, 13 and 24 days after the last injection.

MENT Implant Trial (Reported in Publications III and IV)

A total of 45 men, in three clinics, were randomly allocated into three groups of equal size (five per group in each clinic). Subjects in each group

received either one, two or four MENT acetate-releasing implants. The implants remained in place for 4 weeks. Blood samples were obtained before implant insertion and at one-week intervals for 6 weeks thereafter (1, 2, 3 and 4 weeks after insertion and 1 and 2 weeks after removal).

Subjects

The volunteers who participated in the clinical trials were Finnish, Chilean and Scottish men ranging in age from 19 to 45 years and in weight from 55 to 97 kg. All were judged to be healthy as assessed by medical history, general physical examination and clinical chemistry measures including blood count, liver and kidney function tests, lipid profile and assay of circulating concentrations of prostate-specific antigen. None of the subjects had any personal or family history of prostate cancer.

Nine Finnish men participated in the single intravenous injection trial, which was conducted only in Helsinki (Table 1). A total of 18 men, nine in Helsinki and nine in Santiago, participated in the single-dose intramuscular MENT trial (Table 2). A total of 24 men, 12 in Helsinki and 12 in Santiago, participated in the trial of six consecutive daily intramuscular MENT injections (Table 3). A total of 45 men, 15 in Helsinki, 15 in Santiago and 15 in Edinburgh, participated in the MENT implant trial (Table 4).

Table 1 Median and Range of Anthropometric Characteristics in the 9 Finnish Men who Participated in the Intravenous Injection Trial

Age (years)	34 (22-39)
Weight (kg)	80 (71-95)
Height (cm)	180 (175-187)
BMI (kg/m^2)	25 (21-28)

Table 2 Anthropometric Characteristics of the 18 Men* who Participated in the Single Intramuscular Injection Trial: Median and Range

	2 mg	4 mg	8 mg
Age (years)	29 (21-34)	25 (19-34)	36 (26-39)
Weight (kg)	71 (61-80)	74 (55-86)	72 (68-95)
Height (cm)	175 (169-182)	174 (167-187)	176 (159-185)
BMI (kg/m^2)	23 (21-26)	24 (19-28)	26 (21-28)

* Six men in each dose group (3 Finnish and 3 Chilean)

Table 3 Anthropometric Characteristics of the 24 Men* who Participated in the Six Intramuscular Injections Trial: Median and Range

	1 mg	2 mg	4 mg
Age (years)	27 (20-40)	26 (21-30)	25 (21-32)
Weight (kg)	72 (57-96)	73 (61-76)	71 (63-83)
Height (cm)	175 (169-185)	176 (168-184)	178 (168-188)
BMI (kg/m^2)	23 (20-31)	22 (21-25)	23 (19-28)

* Eight men in each dose group (4 Finnish and 4 Chilean)

Table 4 Anthropometric Characteristics in the Participants of the Implant Trial: Median and Range in Each Dose Group* for all 45 Men and for the 15 Finnish Men

	1 mg	2 mg	4 mg
All Men			
Age (years)	29 (20-42)	32 (22-45)	25 (21-41)
Weight (kg)	73 (65-89)	72 (57-102)	83 (60-95)
Height (cm)	177 (167-190)	178 (164-189)	175 (165-187)
BMI (kg/m^2)	24 (20-31)	23 (20-31)	26 (21-30)
Finnish Men			
Age (years)	34 (24-42)	32 (22-45)	25 (23-41)
Weight (kg)	81 (69-87)	79 (60-97)	85 (71-88)
Height (cm)	184 (178-186)	178 (164-189)	186 (178-187)
BMI (kg/m^2)	24 (22-26)	25 (22-31)	25 (22-26)

* Fifteen men in each dose group (5 Finnish, 5 Chilean, and 5 Scottish)

The anthropometric characteristics of the Finnish men are presented separately, because 7α-methyl-19-nortestosterone (MENT) concentrations are reported only for this subgroup whose samples were analyzed by gas chromatography – mass spectrometry.

Injection Preparations and Implants

The MENT used in these clinical trials was supplied by SRI International, Menlo Park, California, in the form of MENT acetate. To obtain free MENT, MENT acetate was hydrolyzed at The Population Council´s Laboratory in New York with 1 mol/l potassium hydroxide in ethanol. The purity of MENT was verified by reverse-phase high performance liquid chromatography and thin layer chromatography. The identity of MENT was confirmed by infrared spectroscopy and melting point determination (138–141°C).

For intravenous administration, an aqueous solution containing MENT 50 µg/ml, 0.9% sodium chloride and 10% ethanol was used. Ten ml of this solution (containing 0.5 mg of MENT) was injected into a superficial anterior cubital vein. An aqueous suspension of micronized MENT was used for intramuscular administration. The dose of MENT to be administered (1–8

mg) was diluted in 0.5 ml. The injections were given in the deltoid or gluteus muscles. For the implants, MENT acetate was used instead of free MENT since it is released more conveniently and it is rapidly hydrolyzed to MENT *in vivo*. The implants were manufactured at the Center for Biomedical Research of The Population Council in New York. Each implant contained 112 ± 4 mg of MENT acetate in a polyethylene vinyl acetate copolymer, and the release rate of MENT acetate was estimated to be about 300 µg per day, based on studies carried out *in vitro*. The implants had a diameter of 2.7 mm and a length of 45 mm. The implants were inserted subdermally in the medial aspect of the upper arm with the aid of a trocar under local anesthesia and aseptic conditions. The implants were removed through a short incision under local anesthesia and aseptically.

Sample Handling

Blood samples were collected from the upper limb contralateral to the site of the injection or implant. When several blood samples had to be taken at short intervals, an i.v. catheter fitted into a superficial vein on the back of the hand was used for sampling. When sampling was less frequent, blood was drawn by antecubital phlebotomy. The blood samples were allowed to clot and the sera were separated by centrifugation and stored at -20°C in plastic test tubes until assayed.

Assays

Concentrations of MENT were measured by radioimmunoassay as described previously. The detection limit of the assay (defined as 2 standard deviations above the serum blank) was 0.1 nmol/l, its intra-assay coefficient of variation 7.0% and its interassay coefficient of variation 13.8%. The cross-reactivity of testosterone in the MENT radioimmunoassay was 1.1%. (Kumar *et al*, 1990)

Because of this cross-reactivity, low MENT concentrations cannot be measured accurately by radioimmunoassay. Hence, we reanalyzed the serum MENT concentrations of the samples taken in the MENT implant trial in Helsinki by capillary gas chromatography-mass spectrometry. The detection limit of this assay was 0.347 nmol/l, the intra-assay coefficient of variation was 9.8%, and its interassay coefficient of variation was < 10%.

MENT radioimmunoassays of the samples taken in the single intravenous injection trial and in the single intramuscular injection trial were carried out in the laboratories of The Population Council in New York, and MENT radioimmunoassays of the samples taken in the six intramuscular injections trial and the MENT Ac implant trial were carried out in the Steroid Research Laboratory, Institute of Biomedicine, University of Helsinki. MENT concentration measurements by gas chromatography-mass spectrometry were carried out in the laboratories of Leiras Oy, Turku, Finland.

Serum testosterone concentrations were determined by conventional radioimmunoassay according to the standard operating procedure of the World Health Organization (Sufi *et al*, 1990). The limit of detection was 0.5 nmol/l, the intra-assay coefficient of variation 6.8% and the interassay coefficient of variation 13.3%. The cross-reactivity of MENT in the testosterone radioimmunoassay was 1.2%.

Serum DHT concentrations were determined by radioimmunoassay. The limit of detection was 0.09 nmol/l, the intra-assay coefficient of variation 5.0% and the interassay coefficient of variation 15.5%. The concentrations of FSH, LH and SHBG were measured by time-resolved fluoroimmunoassays, using commercially available kits (DELFIA®, Wallac Oy, Turku, Finland). The limit of detection for LH was 0.05 IU/l, the intra-assay coefficient of variation 5.0% and the interassay coefficient of variation 11.7%. The limit of detection of FSH was 0.05 IU/l, the intra-assay coefficient of variation 3.4% and the interassay coefficient of variation 4.9%. For SHBG,

the limit of detection was 0.8 nmol/l, the intra-assay coefficient of variation 8.9% and the interassay coefficient of variation 7.0%. The concentrations of insulin-like growth factor-1 were measured using immunoradiometric kits (Immunotech, Paris, France). The limit of detection in the insulin-like growth factor-1 assay was 1.57 nmol/l, the intra-assay coefficient of variation 7.3% and the interassay coefficient of variation 12.6%.

Concentrations of testosterone, FSH, LH, SHBG, DHT, and insulin-like growth factor-1 were measured in the Steroid Research Laboratory, Institute of Biomedicine, University of Helsinki.

Routine clinical chemistry assays used to evaluate the safety of MENT administration, including blood count, liver and kidney function tests, lipid profile and assay of prostate-specific antigen, were carried out using standard methods in local clinical chemistry laboratories.

Pharmacokinetic Methods

To analyze the MENT concentration data obtained in these trials we used standard pharmacokinetic methods described in standard textbooks of pharmacokinetics (Gibaldi & Perrier, 1982; Rowland & Tozer, 1994).

Pharmacokinetic data were analyzed with the LAGRAN computer program (Rocci & Jusko, 1983) and Microsoft Excel for Windows 95 (Microsoft Corporation, Redmond, Washington; version 7.0 and earlier versions).

Single Intravenous Injection Trial

We determined the pharmacokinetic parameters of MENT by using a two-compartment model and fitting, by the least squares method, a biexponential curve to the serum MENT concentration versus time data. The elimination rate constants and the extrapolated serum MENT concentrations at zero

time were estimated for the initial and terminal phases of the concentration versus time curve. These parameters, as well as the area under the concentration-time curve were used to calculate the other parameters. Half-lives were calculated by dividing the natural logarithm of 2 by the elimination rate constants. The initial apparent volume of distribution and the total apparent volume of distribution were calculated by dividing the dose of MENT administered by the extrapolated serum MENT concentrations at zero time estimated for the initial and terminal phases of the concentration versus time curve. Total clearance was calculated by dividing the MENT dose by the area under the concentration-time curve.

Single Intramuscular Injection Trial

To estimate the pharmacokinetic parameters of MENT after intramuscular administration, an exponential curve was fitted to the combined concentration versus time data by least squares fit. The estimated elimination rate constant and area under the concentration-time curve were used to calculate the other parameters.

The elimination half-life was calculated by dividing the natural logarithm of 2 by the elimination rate constant. Total clearance was calculated by dividing the MENT dose by the area under the concentration-time curve.

Pharmacokinetic parameters were calculated for each dose group in each clinic.

Six Intramuscular Injections Trial

No pharmacokinetic parameters were calculated in this trial, since MENT concentrations were below the detection limit of the MENT radioimmunoassay.

MENT Implant Trial

For the purposes of pharmacokinetic calculations, the implants were considered constant-rate release devices. The release rate of MENT from the implants was estimated by the product of the clearance rate of MENT (determined in the single intravenous injection trial) and the steady state concentration of MENT, obtained by averaging the serum MENT concentrations over the time span from 1 week after insertion to 4 weeks after insertion in each implant group.

Statistical Methods

Analysis of variance was used to test differences between dose groups and between clinics. Variations over time in MENT and hormone concentrations were evaluated by analysis of variance for repeated measures or, for comparisons involving only two time points, by Student's t-test for paired data. In the six intramuscular injections trial, the maximum relative effect of MENT on each hormone was used in statistical tests. The maximum relative effect was the difference between the most extreme hormone concentration and the baseline concentration, divided by the baseline concentration. The purpose of this transformation was to remove the effect of between-subject variation in baseline hormone levels. In the MENT Ac implant trial, logarithmic transformation was used to normalize testosterone, FSH, and SHBG concentrations for analysis of variance. In all statistical tests, probability values of wrongly rejecting a true null hypothesis (*P*-values) were reported two-tailed, and values below 0.05 were considered statistically significant.

Data were analyzed with Microsoft Excel for Windows 95 (Microsoft Corporation, Redmond, Washington; version 7.0 and earlier versions) and

SPSS for Windows (SPSS Inc., Chicago, Illinois; version 9.0.1. and earlier versions).

Ethical Issues

The clinical trials were conducted in accordance with good clinical practice guidelines and the Declaration of Helsinki ([*Anonymous*], 1997). The Ethics Committees of each clinic and the Institutional Review Board of The Population Council approved the trials. All subjects received detailed information about the trials and gave written informed consent before enrollment.

Results

Pharmacokinetics of MENT

To study the pharmacokinetics of MENT, we used serum samples taken after single intravenous and intramuscular injections as well as after the insertion of MENT-releasing implants. The samples taken in the trial involving six intramuscular injections at daily intervals could not be used to study the pharmacokinetics of MENT, since blood samples were taken 24 hours or several days after the injections and no MENT could be detected in these samples.

Single Intravenous Injection Trial

Peak serum MENT concentrations were measured in the first samples, taken 3 minutes following the intravenous injection of 0.5 mg MENT (Table 5). Thereafter, MENT concentrations decreased rapidly, reaching the detection limit of the MENT radioimmunoassay 3–4 hours after injection.

Table 5 Median and Range of Serum 7α-Methyl-19-Nortestosterone (MENT) Concentrations (nmol/l) in 9 Finnish Men Following the Intravenous Injection of 0.5 mg of MENT

Minutes after injection	MENT (nmol/l)
3	48 (28-88)
6	29 (15-54)
10	21 (9.0-35)
15	16 (7.6-26)
30	12 (4.9-15)
45	8.0 (2.8-9.7)
60	4.9 (2.1-7.6)
90	2.4 (1.4-5.2)
120	1.7 (0.0-2.8)
150	1.4 (0.0-2.4)
180	0.0 (0.0-1.7)
210	0.0 (0.0-1.4)
240	0.0 (0.0-1.0)

Serum MENT concentrations were used to calculate the pharmacokinetic parameters of MENT (Table 6). The observed serum MENT concentrations in 7 subjects could be adequately described by a biexponential equation and the pharmacokinetic parameters of MENT for these subjects were calculated according to the two-compartment model described in the pharmacokinetic methods section. The concentrations in two subjects could not be described by a biexponential equation and the pharmacokinetic parameters for these subjects were calculated using a non-compartmental model.

Table 6 Median and Range of Pharmacokinetic Parameters of 7α-Methyl-19-Nortestosterone (MENT) Calculated Using the Serum MENT Concentrations in 8* Finnish Men Following the Intravenous Injection of 0.5 mg of MENT

Volume of distribution	68 (47-94)
Terminal half-life	42 (22-53)
Clearance rate l/day	2000 (1400-2700)
Clearance rate l/kg/day	27 (20-31)

*The obviously erroneous data from one man were omitted from these summary statistics. (A total of nine men participated in this study.)

Single Intramuscular Injection Trial

Serum MENT concentrations after single intramuscular injections of 2, 4, or 8 mg of MENT were dose-dependent (Table 7). The differences between the three dose groups in peak MENT concentrations were statistically significant ($P = 0.028$). Over the four-fold dose range tested, each doubling of the dose of MENT resulted in an increase of approximately 60% in the peak MENT concentration. The time to reach peak concentrations appeared to be longer at the higher doses, but for this variable, the differences between dose groups were not statistically significant. The median and range of the clearance rates for all subjects were 1600 (720–3200) l/day and 22 (10–49) l/kg. If the probably erroneous clearance rate of one subject, 724 l/day and 10 l/kg, is omitted, the clearance rates for all subjects were 1600 (1100–3200) l/day and 23 (15–49) l/kg. The differences in clearance rates between dose groups were small and not statistically significant.

Table 7 Serum 7α-Methyl-19-Nortestosterone (MENT) Concentrations (nmol/l) in 18 Men* Following the Intramuscular Injection of 2, 4, or 8 mg of MENT: Median (Range) of Each Dose Group

Time after injection	2 mg	4 mg	8 mg
15 min	20 (6.6-38)	30 (5.5-65)	37 (25-74)
30 min	25 (9.0-38)	34 (8.4-61)	45 (30-120)
60 min	21 (9.4-32)	30 (14-61)	48 (27-97)
90 min	19 (10-29)	28 (12-42)	40 (26-99)
2 h	17 (8.0-21)	31 (18-35)	39 (19-123)
3 h	14 (7.6-21)	30 (19-36)	40 (18-112)
4 h	5.7 (4.9-12)	19 (12-24)	36 (15-108)
5 h	4.3 (2.0-5.0)	10 (7.7-15)	30 (11-72)
6 h	2.8 (1.6-4.9)	10 (5.9-16)	22 (10-58)
8 h	1.5 (0.0-3.5)	6.0 (2.8-15)	17 (9.0-32)
24 h	0.0 (0.0-0.0)	0.0 (0.0-2.4)	0.0 (0.0-2.4)

* Six men in each dose group (3 Finnish and 3 Chilean).
Concentrations more than 24 h after injection were all below the detection limit of the MENT radioimmunoassay (0.1 nmol/l).

To obtain an estimate of the (apparent) elimination half-life of MENT after a single i.m. injection, we combined the concentration vs. time data of all subjects. The estimated elimination half-life was 224 min.

MENT Implant Trial

Serum MENT concentrations measured by gas chromatography-mass spectrometry are more accurate than the concentrations measured by radioimmunoassay. Hence, the MENT concentrations reported here are based on the subset of 15 Finnish men whose samples were analyzed by gas chromatography – mass spectrometry.

Serum MENT concentrations during implant use were clearly dose-dependent (Table 8). The overall difference between the groups with 1, 2 and 4 implants, as well as the differences between each pair of two groups were all statistically significant ($P < 0.001$ for the between-subject effect of implants, $P = 0.003$ for comparison of the 1-implant group and the 2-

implant group, $P < 0.001$ for comparison of the 1-implant group and the 4-implant group, and $P = 0.003$ for comparison of the 2-implant group and the 4-implant group).

Table 8 Serum 7α-Methyl-19-Nortestosterone (MENT) Concentrations (nmol/l) in 15 Men* Before, During, and After the Use of 1, 2 or 4 Subdermal Implants Releasing 7α-Methyl-19-Nortestosterone Acetate (MENT Ac): Median (Range) of Each Dose Group

Weeks after insertion	1 Implant	2 Implants	4 Implants
1	0.5 (0.4-0.8)	1.2 (1.0-1.9)	2.3 (1.6-3.7)
2	0.6 (0.5-1.0)	1.2 (0.9-1.9)	2.3 (1.3-2.8)
3	0.5 (0.4-0.6)	1.4 (1.0-1.7)	2.1 (2.0-3.5)
4	0.6 (0.4-0.7)	1.8 (0.7-1.8)	2.2 (1.3-2.5)

* Five Finnish men in each dose group.

No MENT could be detected in the samples taken 1 and 2 weeks after removal of the implants.

In all dose groups, MENT could only be detected in the samples taken 1, 2, 3 or 4 weeks after insertion of the implants. MENT concentrations in these samples were relatively constant and there were no statistically significant differences in MENT concentrations over time within dose groups. Among the subjects who had one implant inserted, serum MENT concentrations 1–4 weeks after insertion ranged from 0.4 to 1.0 nmol/l. For the subjects who had two implants inserted, MENT concentrations ranged from 0.7 to 1.9 nmol/l, and for those who had four implants, from 1.3 to 3.7 nmol/l. Since MENT concentrations did not change significantly over the time span from 1 week after insertion to 4 weeks after insertion, the implants could be assumed to be constant-rate release devices for the purposes of pharmacokinetic calculations. The release rate of MENT from the implants was estimated to be approximately 0.3 mg/day in the 1-implant group, 0.8 mg/day in the 2-implant group, and 1.3 mg/day in the 4-implant group. The

corresponding values for MENT acetate were 0.4 mg/day, 0.9 mg/day, and 1.5 mg/day.

Release Rate of MENT Ac Implants

The release rate of 6 unused MENT Ac implants *in vitro*, measured over a 33-day period was 0.40 mg per day. The release rate of 25 used MENT Ac implants *in vitro*, recovered intact after the MENT Ac implant trial, and measured over a 19-day period, was 0.36 mg per day. Thus, the release rate of MENT Ac implants used for 4 weeks was 91% of the release rate of new implants.

The release rate *in vivo* of 100 MENT Ac implants recovered intact after the MENT Ac implant trial was measured and found to be 0.48 mg/day in the 1-implant group, 1.03 mg/day in the 2-implant group, and 1.94 mg/day in the 4-implant group.

The release rates of MENT from the implants calculated from the product of the clearance rate of MENT, about 2000 l/day, and the mean serum MENT concentrations in each subject in the samples taken 1, 2, 3, and 4 weeks after insertion were 0.32 mg/day in the 1-implant group, 0.81 mg/day in the 2-implant group and 1.33 mg/day in the 4-implant group. The corresponding values for MENT acetate were 0.36 mg/day, 0.93 mg/day and 1.53 mg/day.

Effects of MENT on Gonadotropin and Testosterone Concentrations

To study the effects of MENT on gonadotropin and testosterone concentrations, we used the serum samples taken during and after the series of six daily intramuscular injections as well as after the insertion of MENT-releasing implants. The samples taken in the single intravenous injection

trial and the single intramuscular injection trial could not be used to assess the effect of MENT on hormone concentrations, since blood samples were taken at different times of the day and the effects of the circadian variation in hormone concentrations could not be differentiated from the possible effects of MENT. In contrast, in the trial involving six intramuscular injections and in the MENT Ac implant trial all samples from each subject were taken at approximately the same time in the morning.

Six Intramuscular Injections Trial

There were no significant differences in baseline LH concentrations between clinics. The concentrations of LH decreased in all dose groups after the i.m. injections (Table 9), and the decreases were significant ($P < 0.001$). The mean of the maximal relative decrease in LH concentrations was dose-dependent: 52 ± 3%, 62 ± 6% and 70 ± 5% in the groups who received 1, 2 or 4 mg doses, respectively. The differences between dose groups were statistically significant ($P = 0.048$).

Table 9 Serum Luteinizing Hormone Concentrations (U/l) in 24 Men* Before, During and After a Series of Six Intramuscular Injections of 1, 2 or 4 mg of 7α-Methyl-19-Nortestosterone (MENT) Given at Daily Intervals: Median (Range) of Each Dose Group

	1 mg	2 mg	4 mg
Before 1st injection	3.5 (2.5-4.7)	4.7 (2.9-9.6)	4.0 (2.5-7.0)
Before 2nd injection	3.8 (1.9-4.8)	3.2 (1.9-6.3)	2.9 (1.5-6.2)
Before 3rd injection	2.3 (1.9-2.8)	2.4 (1.0-8.8)	2.5 (1.0-6.5)
Before 4th injection	2.4 (1.7-3.6)	2.9 (1.2-5.8)	2.0 (0.3-5.5)
Before 5th injection	2.2 (1.3-2.9)	3.1 (1.4-6.4)	1.1 (0.5-3.7)
Before 6th injection	2.6 (1.9-4.5)	2.1 (0.7-3.6)	1.6 (0.5-3.5)
2 Days after last injection	4.2 (1.7-6.2)	3.6 (2.7-5.9)	2.9 (1.8-7.3)
3 Days after last injection	3.4 (2.3-6.0)	5.1 (3.3-8.4)	4.9 (3.8-8.1)
5 Days after last injection	3.4 (1.7-5.3)	4.7 (2.6-7.3)	5.1 (2.9-8.2)
9 Days after last injection	2.9 (2.6-4.4)	4.3 (2.6-5.4)	4.4 (2.9-6.2)
13 Days after last injection	2.7 (2.3-5.2)	4.0 (2.4-7.1)	3.5 (1.3-6.1)
24 Days after last injection	2.8 (2.1-4.2)	4.3 (2.3-5.8)	3.2 (3.0-7.0)

* Eight men in each dose group (4 Finnish and 4 Chilean)

The baseline FSH concentrations in the Finnish subjects were significantly higher than those of the Chilean subjects (3.9 ± 0.4 vs. 2.4 ± 0.2 U/l, P = 0.009). After the i.m. injections, FSH concentrations decreased in all dose groups (Table 10), and the decreases were significant ($P < 0.001$). The mean of the maximal relative decrease in FSH concentrations was dose-dependent: 25 ± 5%, 41 ± 5% and 57 ± 4% in the groups who received 1, 2 or 4 mg doses, respectively. The differences between dose groups were statistically significant ($P = 0.001$).

Table 10 Serum Follicle-stimulating Hormone Concentrations (U/l) in 24 Men* Before, During and After a Series of Six Intramuscular Injections of 1, 2 or 4 mg of 7α-Methyl-19-Nortestosterone (MENT) Given at Daily Intervals: Median (Range) of Each Dose Group

	1 mg	2 mg	4 mg
Before 1st injection	3.0 (1.7-5.7)	3.1 (0.9-3.6)	2.6 (0.8-5.8)
Before 2nd injection	2.7 (1.4-6.4)	2.6 (0.8-3.9)	2.2 (0.6-4.2)
Before 3rd injection	2.8 (1.3-6.5)	2.0 (0.7-2.9)	2.0 (0.5-4.0)
Before 4th injection	2.6 (1.4-5.3)	1.8 (0.6-2.8)	1.8 (0.4-3.8)
Before 5th injection	2.2 (1.5-5.2)	1.8 (0.5-3.0)	1.4 (0.4-3.5)
Before 6th injection	2.6 (1.3-5.9)	1.5 (0.4-2.6)	1.4 (0.3-2.8)
2 Days after last injection	2.9 (1.7-7.0)	2.5 (0.6-3.4)	1.9 (0.7-4.4)
3 Days after last injection	3.0 (1.7-7.8)	3.3 (0.7-4.3)	2.6 (0.9-5.9)
5 Days after last injection	3.1 (1.6-9.0)	3.0 (0.8-4.0)	3.6 (1.0-6.0)
9 Days after last injection	3.1 (1.9-7.4)	2.9 (0.8-3.7)	3.1 (0.9-5.4)
13 Days after last injection	2.9 (1.8-6.1)	2.6 (0.7-3.1)	2.5 (0.7-5.4)
24 Days after last injection	2.8 (1.5-6.1)	2.8 (0.8-3.5)	2.5 (0.7-4.7)

* Eight men in each dose group (4 Finnish and 4 Chilean)

Baseline testosterone concentrations in the Finnish subjects were significantly lower than those in the Chilean subjects (15.5 ± 1.2 vs. 20.6 ± 1.7 nmol/l, $P = 0.029$). Testosterone concentrations decreased in all dose groups after the i.m. injections (Table 11), and the decreases were significant ($P < 0.001$). The mean of the maximal relative decrease in testosterone concentrations was dose-dependent: 41 ± 4%, 59 ± 3% and 74 ± 5% in the groups who received 1, 2 or 4 mg doses, respectively. The differences between dose groups were statistically significant ($P < 0.001$).

Table 11 Serum Testosterone Concentrations (nmol/l) in 24 Men* Before, During and After a Series of Six Intramuscular Injections of 1, 2 or 4 mg of 7α-Methyl-19-Nortestosterone (MENT) Given at Daily Intervals: Median (Range) of Each Dose Group

	1 mg	2 mg	4 mg
Before 1st injection	16 (9.1-29)	18 (9.8-29)	17 (13-23)
Before 2nd injection	17 (9.7-21)	19 (9.9-30)	14 (8.6-24)
Before 3rd injection	16 (9.4-19)	15 (4.6-28)	9.2 (5.8-16)
Before 4th injection	13 (8.2-20)	15 (5.3-26)	6.4 (2.4-14)
Before 5th injection	12 (5.3-17)	12 (5.4-21)	5.1 (2.3-14)
Before 6th injection	10 (4.5-19)	12 (2.9-16)	4.9 (2.2-14)
2 Days after last injection	13 (6.2-23)	12 (6.0-19)	6.6 (4.0-11)
3 Days after last injection	13 (7.2-21)	14 (6.9-21)	6.9 (4.5-15)
5 Days after last injection	16 (11-23)	13 (9.2-22)	15 (8.9-24)
9 Days after last injection	19 (13-25)	16 (11-29)	19 (12-37)
13 Days after last injection	18 (10-29)	15 (10-28)	17 (11-26)
24 Days after last injection	19 (14-31)	18 (13-22)	19 (13-34)

* Eight men in each dose group (4 Finnish and 4 Chilean)

Nine days after the last injections, LH, FSH, and testosterone levels had returned to near their baseline values and the concentrations in the last two samples were similar.

MENT Implant Trial

There were no significant differences in baseline LH, FSH, T or SHBG concentrations between clinics. The results of each dose group in the three clinics were combined, resulting in 15 subjects per dose group. After insertion of MENT Ac-releasing subdermal implants, serum LH (Table 12), FSH (Table 13), and testosterone (Table 14) concentrations decreased in all dose groups. During implant use the concentrations of gonadotropins and testosterone were significantly lower than baseline in all dose groups, and in the two- and four-implant groups, significantly lower than in the one-

implant group. Serum LH, FSH, and testosterone concentrations in the two- and four-implant groups were not significantly different. The concentrations of gonadotropins and testosterone remained at approximately the same level in all samples taken during implant use.

Table 12 Serum Luteinizing Hormone Concentrations (U/l) in 45 Men* Before, During and After the Use of 1, 2 or 4 Subdermal Implants Releasing 7α-Methyl-19-Nortestosterone Acetate (MENT Ac): Median (Range) of Each Dose Group

	1 Implant	2 Implants	4 Implants
Before Insertion	3.2 (1.4-5.2)	3.2 (1.9-8.1)	3.4 (1.0-5.7)
1 Week after insertion	2.1 (0.5-4.4)	0.3 (0.2-2.0)	0.1 (0.0-2.5)
2 Weeks after insertion	1.9 (0.6-4.4)	0.2 (0.0-2.9)	0.1 (0.0-0.3)
3 Weeks after insertion	1.5 (0.7-4.7)	0.1 (0.0-2.0)	0.1 (0.0-1.2)
4 Weeks after insertion	1.7 (0.7-6.8)	0.1 (0.0-2.4)	0.1 (0.0-0.6)
1 Week after removal	3.7 (2.1-6.6)	4.3 (0.1-9.4)	5.4 (2.5-11.8)
2 Weeks after removal	3.7 (1.7-5.9)	4.5 (1.2-10.9)	3.3 (1.4-16.2)

* Fifteen men in each dose group (5 Finnish, 5 Chilean and 5 Scottish)

Table 13 Serum Follicle-stimulating Hormone Concentrations (U/l) in 45 Men* Before, During and After the Use of 1, 2 or 4 Subdermal Implants Releasing 7α-Methyl-19-Nortestosterone Acetate (MENT Ac): Median (Range) of Each Dose Group

	1 Implant	2 Implants	4 Implants
Before Insertion	2.6 (1.4-7.4)	3.1 (1.1-5.7)	2.8 (1.2-8.6)
1 Week after insertion	1.7 (0.9-6.0)	0.9 (0.3-2.1)	0.5 (0.2-1.4)
2 Weeks after insertion	1.8 (0.8-4.6)	0.4 (0.2-1.1)	0.2 (0.1-1.0)
3 Weeks after insertion	2.1 (0.8-5.3)	0.3 (0.1-1.1)	0.1 (0.1-0.6)
4 Weeks after insertion	2.4 (0.8-6.1)	0.3 (0.1-1.4)	0.1 (0.0-0.5)
1 Week after removal	3.0 (1.3-9.2)	2.8 (1.5-9.2)	3.9 (1.3-15.8)
2 Weeks after removal	2.7 (1.2-7.2)	2.3 (0.9-7.4)	2.2 (0.8-10.6)

* Fifteen men in each dose group (5 Finnish, 5 Chilean and 5 Scottish)

Table 14 Serum Testosterone Concentrations (nmol/l) in 45 Men* Before, During and After the Use of 1, 2, or 4 Subdermal Implants Releasing 7α-Methyl-19-Nortestosterone Acetate (MENT Ac): Median (Range) of Each Dose Group

	1 Implant	2 Implants	4 Implants
Before Insertion	21 (14-28)	17 (7.9-22)	16 (11-49)
1 Week after insertion	8.2 (1.4-19)	1.2 (0.7-5.8)	1.1 (0.7-3.8)
2 Weeks after insertion	6.5 (1.7-20)	1.0 (0.5-2.8)	0.8 (0.6-3.7)
3 Weeks after insertion	7.6 (1.7-17)	0.9 (0.5-3.1)	0.9 (0.4-5.9)
4 Weeks after insertion	7.5 (2.6-23)	1.1 (0.6-3.2)	1.0 (0.5-4.3)
1 Week after removal	17 (12-32)	13 (9.6-18)	11 (7.4-19)
2 Weeks after removal	17 (7.7-30)	19 (8.2-29)	17 (11-45)

* Fifteen men in each dose group (5 Finnish, 5 Chilean and 5 Scottish)

Effects of MENT on Other Analytes

SHBG

In the six intramuscular injections trial, serum SHBG concentrations decreased in all dose groups. There were no statistically significant differences either in baseline SHBG concentrations or in the decreases in SHBG concentrations between clinics or between dose groups, and therefore the results of all 24 subjects are reported together. The median and range of the baseline serum SHBG concentrations in all 24 subjects was 30 (15–55) nmol/l. Two and 24 days after the last injection, the respective values were 24 (13–56) nmol/l and 28(14–56) nmol/l. The difference in SHBG concentrations between baseline and two days after the last injection was statistically significant ($P < 0.001$).

In the MENT implant trial, SHBG concentrations decreased in all dose groups, but the decrease was statistically significant only in the four-implant group ($P = 0.011$). In the 1-implant group, the median (and range) serum SHBG concentration before implant insertion was 27 (12–49) nmol/l. Four

weeks after insertion the respective value was 22 (14–55) nmol/l, and two weeks after removal, 25 (14–56) nmol/l. In the 2-implant group, the respective concentrations were: before insertion 28 (18–59) nmol/l, four weeks after insertion 27 (17–45) nmol/l, and two weeks after removal 25 (19–57) nmol/l. In the 4-implant group, the respective concentrations were: before insertion 28 (16–86) nmol/l, four weeks after insertion 22 (13–56) nmol/l, and two weeks after removal 28 (15–71) nmol/l.

Dihydrotestosterone and Insulin-Like Growth Factor-1

Dihydrotestosterone concentrations, which were measured only in the MENT implant trial and only in a subgroup of 23 subjects, decreased by 60% in the 1-implant group, 76% in the 2-implant group, and by 77% in the 4-implant group. Serum insulin-like growth factor-1 concentrations, measured in the MENT implant trial and only in a subgroup of 10 subjects, remained constant throughout the trial.

Prostate-Specific Antigen

In all trials, serum concentrations of prostate-specific antigen remained well below the upper limit of the reference range (4 μg/l).

Other Clinical Chemistry Variables

Although we did not detect any consistent changes in clinical chemistry variables, small individual increases in total serum cholesterol, low density lipoprotein cholesterol, triglycerides, aspartate amino transferase, alanine amino transferase, lactate dehydrogenase and creatinine kinase levels were observed in some subjects in both intramuscular injection studies as well as in the MENT implant trial.

Other Adverse Effects

In the intravenous and intramuscular injection studies, the subjects did not report any adverse effects. In the MENT implant trial, 7 subjects experienced pain or other sensations at the site of implant insertion, 7 subjects had androgenic side-effects such as acne and increased libido, 2 subjects experienced sweating and insomnia and 4 subjects reported mood changes. One of the cases of mood change was major depression that developed some days after removal of the implants. The subject required psychiatric treatment. One subject had local infection at the site of insertion of four implants. The infection responded to antibiotic treatment and the implants were allowed to remain in place. In another subject, four implants remained in place for 6 weeks due to problems in removal.

Discussion

The Androgenicity of MENT in Men

This study shows for the first time that MENT is a potent suppressor of LH, FSH and testosterone secretion in men. Gonadotropin secretion can be suppressed by androgens, estrogens and progestins, which all have a negative feedback effect on the pituitary and the hypothalamus, and therefore gonadotropin suppression as such does not prove the androgenicity of MENT. However, if we take into account what is previously known about MENT — its virilizing effects on women and its androgenic actions found in animal studies — it seems almost certain that MENT is also a potent androgen in men. Further support for this claim is given by the fact that intramuscular MENT injections and MENT Ac implants also caused a decrease in SHBG concentrations, a known effect of androgens, and by the

fact that some typical androgenic side-effects such as acne and increased libido were observed in the men who took part in the MENT Ac implant trial.

It is important to note that even though we did not have placebo groups, the changes in serum concentrations of LH, FSH, and testosterone described in this study cannot plausibly be explained by any other reason than MENT administration. Since the samples in the trial involving six intramuscular injections and in the MENT Ac implant trial were all taken at the same time in the morning, the natural circadian variation in the concentrations of these substances could not influence these results. (We did not report the hormone concentrations measured in the single intravenous and single intramuscular injection trials, because in these trials we could not control the effects of circadian variation.)

Pharmacokinetics of MENT

The terminal half-life of MENT, about 40 min, is approximately two times that of testosterone (10–20 minutes). The clearance rate of MENT, about 2000 l/day, is more than 50% higher than the clearance rate of testosterone, which is about 1200 l/day (Bhasin, 1992). These differences in pharmacokinetics are probably due to differences in metabolism of these two androgens, and to the fact that SHBG binds MENT very weakly, their relative binding affinity being about 6% of that of SHBG and testosterone (Kumar *et al*, 1999). One of the main metabolic pathways of testosterone is 5α-reduction, and this pathway is not available to MENT. The metabolic fate of MENT is not exactly known, but it seems that its metabolism is slightly slower than that of testosterone. This would explain the slightly longer half-life of MENT. On the other hand, the fact that MENT is very weakly bound by SHBG should reduce the effect of slower metabolism because a higher fraction of MENT is free and available for metabolic reactions.

The clearance rate after i.m. administration (median 1600, range 720–3200 l/day) was not very different from the clearance rate determined following i.v. administration, (median 2000, range 1400–2700 l/day) — in fact, the ranges overlap. In contrast, the half-life estimated from the data of the single intramuscular injection trial (224 min) was more than five times longer than the 42 min elimination half-life determined using a single i.v. dose of 0.5 mg MENT. This difference is almost certainly explained by the slow absorption of MENT from the i.m. injection site. If absorption is slow enough, it is rate-limiting in the decay phase of MENT concentrations, and the estimated half-life corresponds to the absorption half-life (Rowland & Tozer, 1994). Since MENT is poorly soluble in water, its absorption from muscle could be relatively slow. Slow absorption of MENT might help to explain the fact that in spite of its short half-life, MENT injections given at 24-hour intervals in the trial involving six intramuscular injections had a cumulative suppressive effect on serum LH, FSH and testosterone concentrations.

In the trial involving six intramuscular injections, MENT could not be detected in samples taken 24 hours after the injections, and in the implant trial, MENT could not be detected in the samples taken 1 and 2 weeks after removal of the implants. This is consistent with the short half-life of MENT. Thus, it can be concluded that there is no detectable accumulation of MENT in the body with either intramuscular MENT injections or MENT Ac implants.

MENT Implants

The results of this study also show that MENT acetate implants function properly (for at least four weeks) and can be considered constant-rate release devices. In the 4 samples taken when the implants had been in place from 1 to 4 weeks, MENT levels were similar, showing some fluctuations,

but rising or decreasing trends were not detected and there were no statistically significant changes over time.

The differences between the release rates calculated from the amount of MENT Ac actually lost from the implants and the release rates calculated using MENT concentrations and MENT clearance obtained from the results of the intravenous injection trial were most likely due to a higher release rate during the first few days after insertion. As the samples used in the calculation of release rates were obtained from 1 to 4 weeks after insertion, and each sample reflects the release rate at the time the sample was drawn, the calculated release rate does not include information on the release rate during the first few days. Furthermore, subdermal implants usually show a relatively high release rate for a few days after insertion. The fact that the clearance rate used in the calculations was obtained from the intravenous injection trial and was not the actual clearance rate in the men who took part in the implant trial, could also account for part of the differences. In spite of the small differences, the clearance rates calculated from the amount of MENT Ac lost and the release rates calculated using MENT concentrations are similar. This indicates that the bioavailability of MENT from the implants is good.

The type of implant used in this study contains about 112 mg of MENT Ac. Hence, it can be roughly estimated, using the release rate calculated from the amount of MENT Ac lost, that the implants would last about 112 mg/0.48 mg/day = 233 days. Of course, this is an oversimplification, since the release rate will fall as the amount of MENT Ac in the implants decreases, and after some time the release rate will no longer be sufficient. Based on all the information available, it seems reasonable to propose that the effective life of this type of implant would be about 6 months.

Lack of 5a-Reduction

MENT is not activated by 5α-reduction in animals, and probably not in men either, although this has not been shown. The consequences of this property can only be guessed. It is hoped that MENT would be a safer androgen because of this property, since it implies that it should not over-stimulate the prostate. This property should also reduce the incidence of some other adverse effects of androgens, such as acne and male pattern baldness. If used in women, MENT could be less prone to cause hirsutism.

It is also possible that not being activated by 5α-reductases could also be a handicap. We do not yet know whether MENT can support all androgen-dependent bodily functions and properties. Probably it will, because even though 5α-reduction is essential for male sexual development during fetal life, there are currently no known beneficial effects of 5α-reduction in adults. Furthermore, though MENT does not undergo 5α-reduction it is not without effect in 5α-dihydrotestosterone-dependent tissues. The effects of 5α-dihydrotestosterone are mediated through androgen receptors, and MENT binds more tightly to these receptors than 5α-dihydrotestosterone itself. The differences in the actions of MENT in different androgen-sensitive tissues are quantitative rather than qualitative.

Hormonal Male Contraception

Our ultimate aim is the development of a hormonal male contraceptive based on MENT. The contraceptive would consist of MENT Ac-releasing implants, either alone or in combination with another inhibitor of gonadotropin secretion such as a GnRH antagonist or a progestin. Preferably, if another inhibitor of gonadotropin secretion needs to be used, it should be a

substance that can also be administered by subdermal implants, so that it would not make the method less convenient.

We do not yet know whether MENT will suppress spermatogenesis sufficiently to achieve reliable contraception. Semen analysis has not been included in our trials because they have all been of short duration. The MENT Ac implants were in place for only four weeks, and it is known from a multitude of previous male contraception studies that about four months are required for the complete suppression of spermatogenesis by androgens (Cummings & Bremner, 1994). However, we could have detected a smaller decrease in sperm concentrations. In the first large testosterone enanthate contraception study of the World Health Organization, sperm concentrations were already suppressed to 65% of baseline at 1 month of treatment in those 157 men who later became azoospermic, and to 84% in those 68 who did not (Handelsman *et al*, 1995).

Assuming that the mechanism of contraceptive action of MENT is the same as that of testosterone, namely, suppression of gonadotropin secretion, the results of our study make it reasonable to predict that MENT will be no less efficient than testosterone enanthate in the suppression of sperm counts. Two or four MENT Ac implants suppressed LH concentrations to levels similar to those seen in subjects rendered azoospermic by testosterone enanthate injections.

A longer trial is about to begin, in which healthy men will use 1, 2 or 4 MENT Ac implants for 6 months. In this trial, sperm counts will be measured. The results will be very interesting, not only because of the information obtained on the effect of MENT on male fertility but also because of the information obtained about the safety of MENT. Since MENT is not activated by 5α-reductase enzymes, the trial may also reveal something interesting about the physiological significance, if any, of 5α-reduction in healthy adult men.

Limitations of This Study

Our clinical trials were neither blinded nor placebo-controlled. The number of subjects enrolled in these trials was small, and even the longest treatment lasted for only four weeks. Hence, no reliable conclusions can be drawn about the safety of MENT or the possible significance of the few adverse events that were reported.

The possible effects of single intravenous and single intramuscular MENT injections on hormone concentrations could not be determined, because in the absence of placebo groups, we could not control the confounding effect of circadian variation in testosterone and gonadotropin concentrations.

The short duration of our studies precluded investigation of the effects of MENT on sperm counts and therefore the conclusion we have drawn about the possible contraceptive efficacy of MENT is an extrapolation derived from the effects of MENT on LH and FSH concentrations.

Conclusions

The main conclusions that can be drawn from the results of this study are as follows:

MENT has pharmacokinetic properties that make it suitable for long-term androgen administration via subdermal implants releasing MENT acetate, a MENT ester that is rapidly converted to MENT *in vivo*.

MENT has strong suppressive effects on serum LH, FSH, and testosterone secretion.

The results of this study are consistent with the results of previous *in vitro* and animal studies. All available data on MENT suggest that it is a potent androgen and a very promising candidate for hormonal male contraception and long-term androgen replacement therapy.

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