PSYCHO-ENDOCRINOLOGICAL ASPECTS IN AGING MALES AND TRANSSEXUAL PERSONS

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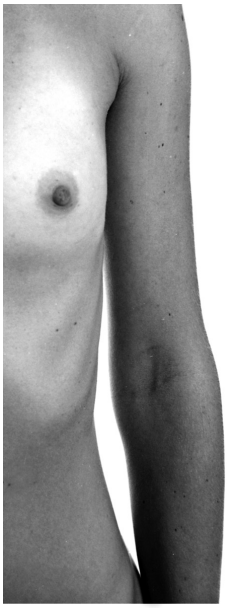
Voor mijn ouders

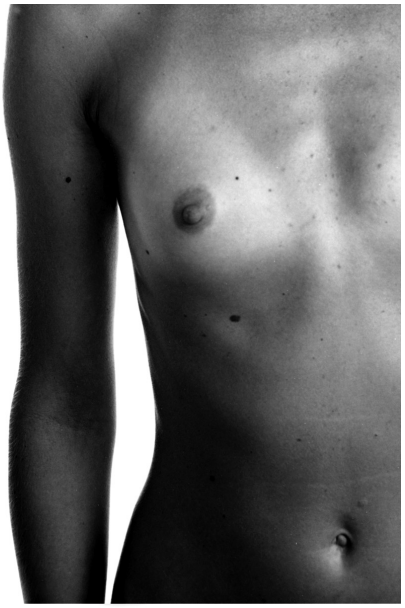
"Una es más auténtica cuando más se parece a lo que ha soñado de sí misma..."

Agrado in Pedro Almodóvar's 'Todo sobre mi Madre'.

CONTENTS

ADAM	Androgen Deficiency in the Aging Male
ADL	Activities of Daily Living
AMS	Aging Males' Symptoms
AR	Androgen receptor
BDI	Beck Depression Inventory
BioE2	Bioavailable Oestradiol
BioT	Bioavailable Testosterone
BMD	Bone Mineral Density
BMI	Body Mass Index
CAD	Coronary Artery Disease
CES-D	Center for Epidemiologic Studies Depression Scale
CV	Coefficient of Variation
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
DHT	Dihydrotestosterone
E2	Oestradiol
Fe	Iron
FE2	Free Oestradiol
FSH	Follicle Stimulating Hormone
FT	Free Testosterone
GDS	Geriatric Depression Scale
GH	Growth Hormone
GnRH	Gonadotropin Releasing Hormone
HDS	Hamilton Depression Scale
IGF-1	Insulin-like Growth Factor
LH	Luteinizing Hormone
MCR	Metabolic Clearance Rate
MMAS	Massachusetts Male Aging Study (MMAS)
PADAM	Partial Androgen Deficiency in the Aging Male
RDRS	Rapid Disability Rating Scale
SD	Standard Deviation
SF-36	Medical Outcome Survey Short–form 36
SHBG	Sex Hormone Binding Globulin
sTfR	Soluble Transferrin Receptor
T	Testosterone





CHAPTER 1

AIMS OF THE THESIS AND GENERAL INTRODUCTION

AIMS OF THE THESIS

It is clear that oestrogens and androgens have profound effects, not only on primary and secondary sex characteristics, but also on the biological and psychological systems in both sexes. However, the description of a clear relation between the sex steroid serum concentration and its effects is not that straightforward, considering that hormonal action is the resultant of many determining processes and interactions. Especially studies on the relationship between behaviour and sex steroids do not always provide consistent results.

The aim of this thesis is to contribute to the clarification of certain endocrinological and psychoendocrinological aspects in two different clinical contexts. More subtle changes in androgen levels in aging males and profound cross-sex hormone administration treatment in transsexual individuals have been considered.

Whereas it has been well documented that gonadal steroid levels decrease with age in men, the mechanism underlying this decrease has yet not been fully elucidated. In fact, unless testosterone levels are severely depressed or other hormonal abnormalities coexist, the aetiology of the hypogonadism is rarely related to overt gonadal or pituitary pathology. There is evidence supporting the idea that relative androgen deficiency may contribute to the clinical changes in aging men. This ill-defined male climacterium syndrome is often referred to as 'andropause', with the underlying implication that it is at least in part related to (relative) androgen deficiency. It remains controversial whether a constellation of clinical symptoms in aging, in particular those related to psychological well-being, may indicate low androgen levels.

The group of transsexual individuals and the profound cross-sex hormonal treatment deserve attention, as there is relatively limited information available on long-term health implications and sexual function. Also, the hormonal treatment in transsexual individuals creates the possibility to study the effects of changing sex steroid status in different organ systems.

Chapter 2 focused on the effects of (inhibiting) oestradiol feedback on gonadotropin and testosterone secretion in young versus elderly men. The hypothesis is tested that decreased T in aging men might result from increased E2 negative feedback. To this end, we compared in young and elderly men the effect on gonadotropin and testosterone secretion of aromatase inhibition by administration of letrozole, a specific and potent fourth-generation aromatase inhibitor. Letrozole, currently indicated as a treatment for breast cancer, reduces systemic E2 concentration in males by 30-50 %. The premise was that if increased E2 negative feedback were instrumental in the age-related decline of testosterone levels, aromatase inhibition would result in a greater gonadotropin response in elderly men compared to young men.

The aim of the study in **Chapter 3** was to correlate clinical symptoms tentatively described as 'andropause' with biochemical measures of androgen status; the usefulness of questionnaires to detect hypoandrogenism in the elderly being dependent on their ability to predict (subnormal) androgen levels. Recently the 'Aging Males' Symptoms' (AMS) rating scale was developed aimed at a more systematic description of severity of symptoms related to a clinically defined 'male climacteric'. AMS is a more general, composite measure of well-being or health status specifically designed for aging men. In this context we examined the relation between (free and bioavailable) testosterone levels and dehydroepiandrosterone sulphate (DHEA-S) with

the results of AMS and other questionnaires assessing the perception of health in a community-based population of ambulatory older men participating in an observational study on the relationship between androgen status and bone mineral density.

The literature on the relation of depression, depressive symptoms and/or depressive mood with testosterone levels in elderly men has not been unequivocal. In the same cohort of elderly men we wished to determine the prevalence of depression as assessed by a 30- item Geriatric Depression Scale (GDS) score and to describe the association between this score and sex steroids, an androgen receptor polymorphism and general health related quality of life and functionality **(Chapter 4)**.

In Chapter 5 and 6, we no longer evaluated relative sex hormone deficiencies, but described the effects of drastic induction of overt hypogonadism followed by cross-sex hormonal substitution in transsexual persons.

In the first study (**Chapter 5**) the regulatory role of oestrogens and androgens on haemoglobin and the haematocrit in transsexual persons undergoing cross hormone administration was monitored. To this aim, we assessed a quantitative assay of bone marrow erythropoietic activity, the soluble transferrin receptor.

Since the start of treating transsexual persons in the multidisciplinary Ghent Gender Team it has been decided to advise a dual-phase hormonal schedule, with a first reversible part where sex specific features are suppressed together with starting the real life test. In the second phase cross-sex hormones are given resulting in –to a great extent- irreversible feminisation and masculinization. We aimed to describe the Ghent hormonal treatment regimen for the first time, to explain the rationale behind it and to evaluate its long-term safety (**Chapter 6**). Also, while most studies on transsexual people focus on long term psychological, surgical and physical health a surprisingly small number of studies have focused on the sexual life of post-operative transsexuals, although adequate sexual functioning is universally acknowledged as an important component of mental health. Little attention has been given to this subject and, indeed, the fast majority of follow-up studies investigated sexual functioning only as part of the psychological or surgical outcome. Our centre is presently among those with extensive experience in phalloplasty. This gave us the opportunity to focus on the sexual consequences of this particular surgical intervention. We were able to compare male-to-female and female-to-male transsexuals for different topics of sexual health.

1.1. SEX STEROID HORMONES AND AGE- ASSOCIATED DECLINE OF ANDROGENS

While genetic males and females both have oestrogens and androgens, the quantitative sex hormone production is different and genetically predetermined. In men, androgens are the predominant present hormones. Androgens are substances that determine the differentiation of male internal and external genitalia as well as the development and maintenance of male secondary sex characteristics and male reproductive function. Also, they have important metabolic effects on protein, carbohydrate and fat metabolism, and as such they contribute to the determination of muscle mass and strength and to that of bone and fat mass, while they indirectly also influence insulin sensitivity. **Testosterone, dihydrotestosterone (DHT), androstenedione, dehydroepiandrosterone** (DHEA) and its **sulfate** (DHEAS) are the major androgens in the systemic circulation.

1.1.1. Testosterone.

In males, almost exclusively the testes secrete testosterone. Only about 20% of circulating DHT originates from direct testicular secretion, the remaining being derived from 5a-reduction of testosterone in peripheral tissues (Hammond *et al.*, 1977). Androstenedione originates for 15 % from peripheral conversion of DHEA and testosterone, the remainder resulting from direct secretion by the testes and the adrenals in approximately equal parts (Horton & Tait, 1966-67). DHEA and DHEAS originate almost exclusively from the adrenals. These adrenal androgens possess weak intrinsic androgenic activity and are an important source of androgens in the female. The biosynthesis of distinct sex steroids is highly interrelated and most compounds can be readily converted into others, making it difficult to determine the effects of single compounds.

Biologically, the most important plasma androgen is **testosterone**. It is largely bound to plasma proteins, only 1 to 2 % being free, 40 to 50 % being loosely bound to albumin, and 50 to 60% being specifically and strongly bound to the sex hormone binding globulin (SHBG) (Vermeulen & Verdonck, 1968; Dunn *et al.*, 1981). Unbound testosterone diffuses passively through the cell membranes into the target cell where it binds to the specific androgen receptor (AR) (Giorgi & Stein, 1981). The serum **free testosterone** (FT) and the albumin-bound testosterone represent the fractions readily available for biological action. Albumin-bound testosterone dissociates during tissue transit, whereas the strong binding of testosterone to SHBG will usually not allow dissociation during the tissue transit time (Pardridge, 1986). The non-SHBG-bound testosterone, i.e. the combined free and albumin-bound testosterone, is often referred to as the "*bio-available testosterone*" (bioT). An alternative to the free hormone concept was considered, which proposed a hypothetical mechanism that -at least in some target cells- androgens bound to SHBG are the biologically relevant molecules (Siiteri & Simberg, 1986)

The clinical significance of plasma DHT is very limited as most DHT formed in peripheral tissue acts locally (Toorians *et al.*, 2003), only a limited fraction escaping to the circulation where DHT is strongly bound to SHBG, only 0.8% being free. Androstenedione as well as DHEA are loosely bound to albumin, the binding to SHBG being negligible; DHEAS on the other hand is relatively strongly bound to albumin (Plager, 1965).

Androgenic actions of testosterone are mediated via binding to the **androgen receptor** (AR), either directly or after 5a-reduction to DHT (Mooradian *et al.*, 1987). Part of the physiologic actions of testosterone results from its aromatisation to **oestradiol** that binds to **oestro-**

gen receptors. The androgen receptor does not bind androstenedione, DHEA or DHEAS and it is assumed that the androgenic effects of these steroids are attributable to their transformation to testosterone in the tissues. Testosterone can also exert rapid, non-genomic effects, in part via binding to a G protein-coupled membrane receptor for the SHBG-testosterone complex that initiates a C-AMP mediated, transcription-independent signalling pathway affecting calcium channels (Rosner *et al*., 1992).

In healthy, adult males *morning* levels of serum testosterone vary between around 315 and 1000 ng/dL (11 and 35 nMol/L) (Vermeulen, 2001), the blood production rate (concentration multiplied by metabolic clearance rate (MCR)) ranging from 4 to 10 mg/day (14 to 35 μMol/day) (Vermeulen, 2003). Plasma levels show circadian variations with amplitude of approximately 35%, highest levels in the morning and lowest levels in the late afternoon (Resko & Eik-Nes, 1966).

1.1.2. Age-Associated Decline of Serum Testosterone.

In the early seventies several authors reported an age-associated decline of serum testosterone levels from the fourth or fifth decade. Although this has long been controversial, this decline has now been confirmed both by a large series of cross sectional studies (Vermeulen, 1991 for review) and by several longitudinal studies (Feldman *et al*. 2002; Harman *et al*., 2001; Morley *et al.*, 1997; Zmuda *et al.*, 1997). There is an age-associated increase of SHBG levels by about 1.2% per year (Feldman *et al.*, 2002), so that the decrease of FT and bioT serum levels is larger than that of total serum testosterone (Deslypere & Vermeulen, 1984; Ferrini & Barrett-Connor, 1998; Vermeulen *et al*. 1996). Moreover, in the elderly the amplitude of the circadian variation of serum testosterone, FT and bio T is reduced (De Slypere & Vermeulen, 1984; Diver *et al.*, 2003). Most recent studies (Feldman *et al*. 2002; Harman *et al.*, 2001; Vermeulen *et al*. 1996; Zmuda *et al.*, 1997) in ambulatory men in whom sampling was performed in the morning show that at age 75 yr mean total serum testosterone level is about two thirds of the levels at age 25, whereas the mean FT and bioT serum levels are only about half of those in young men. Nevertheless, there is at all ages an important between-subject variability of serum (F or bio) T levels. In the age group above 60 yr, about 20% have serum testosterone levels in the upper normal range for young men, while over 20% have testosterone levels below the range for young males and an even larger proportion have levels below this limit when considering FT or bioT (Kaufman & Vermeulen, 1997). In the Baltimore Longitudinal Study of Aging 19, 28 and 49 % of men over 60, 70 and 81 yr, respectively, had total testosterone levels below the young reference range (Harman *et al.*, 2001).

1.1.3. Intra-Subject Variability and Random Effects.

There have been reports of circannual variations in plasma testosterone with amplitude of up to 30% with maximum concentrations around October to December for studies performed in the Northern hemisphere (Dabbs, 1990; Svartberg *et al*. 2003). But the reports are not univocal with some studies finding no significant variation (Tancredi *et al*., 2005) or maximum levels rather in spring or summer. At present it is also not possible to differentiate between potential contributory factors such as climate and/or diet. The circadian variation should not contribute substantially to the inter-individual variability of serum testosterone levels as long as serum testos-

terone is being consistently evaluated in the first part of the *morning* (around 7 to 10 a.m.). Moreover, although persisting to some extent, the circadian variation of serum testosterone is markedly blunted in the elderly (Diver *et al*., 2003).
In middle-aged men single point measurements of testosterone have been found to reflect rather well the long-term hormonal levels (Vermeulen & Verdonck, 1992) and it was found that in 248 healthy community-dwelling men over age 70 yr two single point measurements of total testosterone at one year interval were strongly correlated (r=0.82) (Kaufman & Vermeulen, 1998). Therefore, to apply single point measurements is an acceptable approach for the purpose of epidemiological studies.

1.1.4. Inter-Subject Variability

Part of the inter-individual variability in serum testosterone levels is explained by heredity, physiological factors and lifestyle-related factors. The genetic basis underlying the heredity of testosterone and FT is presently unknown. Adiposity as assessed by the body mass index (BMI; i.e. body weight in kg over square of body height in m) is an important negative determinant of total serum testosterone levels, mainly via its effects on SHBG levels (Demoor & Goosens, 1970). The latter are in turn positively associated with insulin sensitivity, as indicated by the consistent finding of a negative correlation of SHBG with insulin serum levels (Zumoff *et al*., 1990). As far as the influence of diet is concerned, reports in the literature are rather divergent. At all ages in adult men, serum testosterone and FT levels are 5 to 15% higher in (actual) smokers as compared to non-smokers (Dai *et al*., 1988; Field *et al*., 1994). Moderate alcohol consumption has no marked effect on serum testosterone (Longcope *et al*., 2000). A controversial issue is whether and how sexual activity influences mean serum testosterone levels: the data available are inconsistent and do not allow for a conclusion (anonymous, 1970; Christiansen, 1998; Exton *et al*., 2001; Jannini *et al*., 1999). At all ages serum testosterone levels may be transiently or more permanently affected by diseases or their treatment (Handelsman, 1994; Kaufman *et al*., 2004; Vandenberghe *et al*., 1994). In the elderly there is a rather high prevalence of use of medication and in particular frequent concomitant use of multiple drugs. Age-related decline of Leydig cell function, which may already be worsened by intercurrent disease, may thus also be accentuated by use of drugs. A typical example of the latter situation is systemic administration of glucocorticoids in older men with chronic obstructive pulmonary disease (Kamische *et al*., 1998). Adverse drug actions on adult Leydig cell function and their underlying mechanisms have not been extensively studied.

Recently, there has been considerable interest in a possible role of an AR gene polymorphism. The AR gene contains in exon 1 a polymorphic trinucleotide CAG-repeat, which encodes a functionally relevant polyglutamine tract of variable length. A CAG-repeat length exceeding the normal range of 15-31 results in a diminished AR transactivation capacity (Chamberlain *et al*., 1994). In X-linked spinal and bulbar muscular atrophy (Kennedy's disease) the CAG-repeats in the AR exceed 40 and the clinical picture includes signs of mild androgen resistance (La Spada *et al*., 1991; Lumbroso *et* al., 1997; Sobue *et al*., 1994). Clinical studies have associated shorter repeat lengths with higher prevalence of several androgen-sensitive diseases, including prostate cancer (Giovannucci *et al*., 1997), although this is not confirmed in all studies; a polymorphic GGC-repeat encoding a polyglycine tract in the AR has also been associated with prostate cancer (Nelson *et al*., 2003). An association of shorter AR CAG-repeat length with

greater longitudinal decline of serum testosterone and bioT levels has been reported for a subgroup of middle-aged men in the Massachusetts Male Aging Study, although there was no association found between CAG-repeat length and baseline serum levels of either testosterone or FT, nor was there a consistent association with follow-up hormone levels (Krithivas *et al*., 1999). No association was found between the polymorphic AR CAG-repeat and sex steroid serum levels in a cohort of community-dwelling healthy men over age 70 years in Belgium (Van Pottelbergh *et al*., 2001), in accordance with the lack of association between this polymorphism and serum testosterone levels in a study in Chinese and Australian men (Jin *et al*., 2000), in studies in German men (Zitzmann *et al*., 2001-03) and in a study in Finish men (Härkönen *et al*., 2003). As to the LH levels, which might be expected to vary according to differences in androgen sensitivity at the hypothalamic-pituitary level, there are no consistent findings with lack of association between AR CAG-repeat length and LH levels in some studies (Krithivas *et al*., 1999; Van Pottelbergh *et al*., 2001), weak positive associations between repeat length and LH reported by others (Zitzmann *et al*., 2001) or even observations of a negative association in a Finnish cohort, although no longer significant after adjustment for age (Härkönen *et al*., 2003). From the whole of these studies it can be concluded that the AR CAG-repeat polymorphism does not appear to contribute substantially to the determination of androgen levels in elderly men. On the other hand, there have been reports of associations of the AR CAG-repeat polymorphism with clinical parameters modulated by androgen action. The concentration of AR is influenced by androgens (increase), oestrogens (increase of AR in prostate) and by aging, which has been reported to be accompanied by a decrease of AR concentration in different tissues (Roth & Hess, 1982).

1.1.5. DHT, DHEA(S) and E2.

The concentration of **DHT** in plasma varies between 23 and 73 ng/dL (0.8 and 2.5 nMol/L). Only 15 to 20 % is secreted by the testes, 80% originates from conversion of testosterone by 5a-reductase type 2 in the peripheral target tissues, whereas DHT that is formed in the liver under influence of 5a-reductase type 1 is not released into the general circulation but is probably immediately glucuronoconjugated (Ishimaru, 1978).

Plasma **DHEA and DHEAS** are secreted almost exclusively by the adrenals. Only about 10% of DHEA is derived from the gonads, while about 50 to 70% derives from desulfatation of DHEAS in peripheral tissues (Longcope, 1986). DHEA metabolism is very rapid with a MCR around 2000 L/day. Serum levels are highly age-dependent, with mean levels of about 430 ng/dL (15 nMol/L) at age 20 yr, decreasing to 140 ng/dL (5 nMol/L) at age 75 yr (Orentreich *et al*. 1992; Vermeulen, 1980; Vermeulen *et al*., 1996; Zumoff *et al*., 1980). The age-associated decline of serum DHEA contrasts with maintained or even increased serum cortisol concentrations during aging. The serum levels are subject to a circadian rhythm with highest values in the morning; the daily production rate amounts to 2 to 7 mg. The blood conversion rate to testosterone is about 0.6% and hence, its contribution to plasma testosterone levels is negligible in adult men. DHEAS is by far the most abundant androgen in plasma. Its mean concentration in young males is about 220 μg/dL (6μMol/L), i.e. 10 to 20 times the concentration of cortisol. Its metabolism is slow (MCR around 15 L/day) and the blood production rate in young males lies as high as 25 to 30 mg/day (Wang *et al*., 1967). Due to its slow metabolism, plasma DHEAS levels do not show circadian variations. Its hormonal and metabolic effects are probably essentially attributable to its transformation to testosterone and oestrogens in the tissues (Labrie *et al*., 2003).

There is a rapidly growing body of evidence that a number of physiologic actions of testosterone in men are mediated by the oestrogen receptors after its biotransformation by the aromatase cytochrome P450 enzyme in the tissues (Gooren & Toorians, 2003). Documented oestrogen-mediated actions of testosterone in men include a role in the feedback regulation of LH (Finkelstein *et al.*, 1991; Hayes *et al.*, 2000), a role in the regulation of skeletal homeostasis (Khosla *et al.*, 2002; Riggs *et al.*, 2002), as well as a role in lipid metabolism and cardiovascular physiology (Sudhir & Komesaroff, 1999); amongst other possible oestrogen actions in men there are indications for a role in the brain (McEwen & Alves, 1999) and in spermatogenesis (Lindzey & Korach, 2003). The conversion rate of testosterone to **oestradiol** is around 0.2%. Plasma E2 originates for 80 % from aromatisation of testosterone and androstenedione, mainly in (subcutaneous) fat and striated muscle although aromatase activity is present in many other tissues, including in bone and in the brain; no more than 20% E2 in the circulation is secreted by the testes. Oestradiol serum concentration in the adult male is 2 to 3 ng/dL (70 to 110 pmol/L), with a production rate of around 45 μg/day. Plasma E2 is also bound to SHBG but with only half the affinity of testosterone. Total plasma E2 levels in adult men do not vary with age: indeed the decrease in precursor levels (i.e. testosterone and androstenedione) is compensated by an increase of fat mass and tissue aromatase activity with age (Hemsell *et al.* , 1974; Vermeulen *et al.*, 2003). As a consequence of the age-associated increase in SHBG binding capacity, the serum concentrations of free oestradiol (FE2) and non SHBG-bound or "bioavailable" oestradiol (bioE2) do show a moderate age-associated decrease (Ferrini & Barrett-Connor, 1988; van den Beld *et al.*, 2000). There is a decrease with age of the serum testosterone over oestradiol ratio.

1.1.6. The Hypothalamo-Pituitary-Gonadal Axis

In mammals, reproductive function is controlled by the hypothalamo-pituitary-gonadal neuroendocrine axis (fig. 1.1.). The function of the gonads is dependent on stimulation by the pituitary gonadotropic hormones, luteinzing hormone (LH) and follicle-stimulating hormone (FSH). The main action of FSH is to regulate Sertoli cell function in the male, whereas LH plays a predominant role in the control of gonadal steroidogenesis (Blake, 1999; Kaufman 1996).

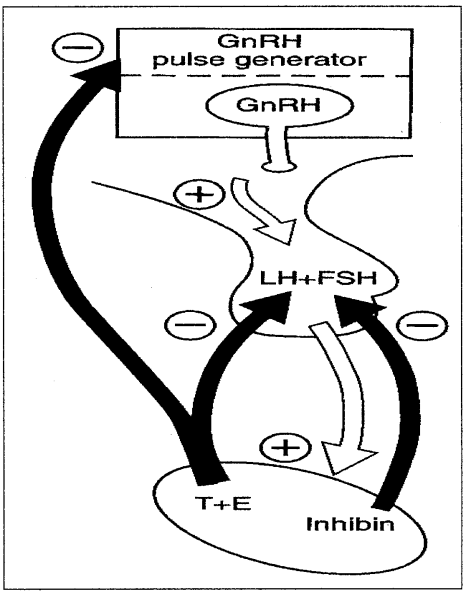


Fig. 1.1. Components of the hypothalamo-pituitary-testicular axis and some important interactions (reproduced from Kaufman, 1996).

Secretion of the pituitary gonadotropins is, in turn, controlled by the hypothalamus, predominantly through the release of a decapeptide, gonadotropin-releasing hormone (GnRH) into the hypophyseal portal circulation (Crowley *et al.*, 1999). The release of GnRH is pulsatile and it has been shown that this intermittent pattern of secretion is obligatory for maintenance of its stimulatory action on the gonadotropin synthesis and release (Belchetz *et al.*, 1978). Consequently, the pituitary gonadotropins are also secreted in a pulsatile manner, dictated by the intermittent GnRH stimulation. The GnRH responsible for tonic stimulation of gonadotropin secretion is being released by a network of GnRH-neurons, in primates mainly located in the medial basal hypothalamus (Marshall & Kelch, 1986; Plant, 1982). The neural oscillator responsible for the rhythmic pattern of GnRH secretion has not been definitely identified.

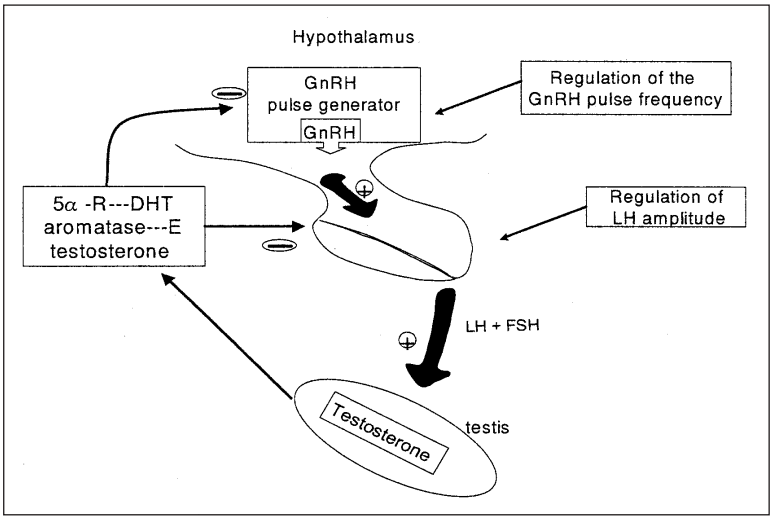


Fig 1.2. The feedback effect of T and E_2 in the regulation of LH secretion. T may act directly and indirectly following its metabolization: oestrogens produced by aromatization, and its 5α-reduced metabolite DHT, can act at the pituitary to influence the LH pulse amplitude and at the hypothalamus to influence the GnRH/LH pulse frequency (and amplitude).

The secretion of gonadotropins is subjected to a tonic inhibition by gonadal steroids, exerted at both the hypothalamic and the pituitary levels (fig. 1.2.). Whereas gonadal steroids are the main feedback regulators of LH secretion, gonadal peptides, in particular inhibin B, plays a major role in feedback regulation of FSH secretion (Hayes *et al.*, 1998, Hayes *et al.*, 2001 a + b).

Human subjects with primary testicular hypogonadism have a markedly increase LH pulse frequency (Matsumoto & Bremner, 1984; Winters *et al*. 1992), which can be progressively decelerated by T replacement (Veldhuis *et al.*, 1984). Administration of T to normal men reduces LH pulse frequency (Finkelstein *et al.*, 1991). Together, these observations indicate that T reduces at the levels of the hypothalamus the frequency of intermittent GnRH release, which is responsible for the pulsatile LH secretion.

T may also act directly at the pituitary level. Indeed, in GnRH-deficient men in whom the frequency of GnRH was fixed, a discernible effect of T was seen at the level of the pituitary with decreases in mean LH levels and LH pulse amplitudes following its administration (Crowley *et al.*, 1991). This indicates that T has a dual site of action. Besides slowing down the GnRH pulse frequency, and possibly reducing the GnRH amount released intermittently by the hypothalamus, T also decreases the responsiveness of pituitary gonadotrophs to GnRH stimulation. Quantitating these effects in men would indicate that approximately half of the effect of T is due to its anterior pituitary site of action, whereas half seems due to its hypothalamic effect on GnRH frequency as judged by suppression of mean LH levels (Crowley *et al.*, 1991).

Whereas the negative feedback action of T on LH secretion is well established, the mechanisms underlying it have still not been fully elucidated. It seems that the inhibitory effect of T is exerted mainly through an androgen receptor-dependent mechanism that does not require a prior conversion of T to E2 (Canovatchel *et al.*, 1994; Veldhuis *et al.*, 1984, Winters *et al.*, 1992), although another mechanism mediated through T aromatization may contribute (Crowley *et al.*, 1991). The biotransformation of T to DHT seems also to play a role in the regulation of LH secretion. Indeed, it has been shown that in subjects with 5a-reductase deficiency, serum LH levels are elevated above normal values, despite the presence of normal or elevated serum levels of T and E2, while serum DHT levels are usually decreased in these patients (Canovatchel *et al.*, 1994; Martini *et al*., 1979).

Oestrogens contribute substantially to the negative feedback regulation of gonadotropin secretion (Bagatell *et al.*, 1994; Hayes *et al.*, 2000). See also (**Chapter 2)** Anti-oestrogen treatment in human males induced larger amplitude LH pulses (Boyar *et al.*, 1973; Tenover *et al.*, 1987; Winters & Troen, 1985). Infusion of E2 at a dose twice the endogenous normal male production resulted in a similar degree of inhibition of gonadotropin secretion in the normal male and in GnRH-deficient men with fixed frequency of exogenously administrated GnRH, suggesting that the predominant inhibiting action of E_2 is exerted at the pituitary levels in the male (Crowley *et al.*, 1991). However, using anastrozole, a selective aromatase inhibitor, for depleting the endogeneous oestrogens in healthy men or in GnRH-treated GnRH-deficient men as well, Hayes *et al*. (2000) demonstrated that in the human male oestrogens also have a dual site of negative feedback action, i.e. acting at the hypothalamus to decrease GnRH pulse frequency and at the pituitary to decrease the responsiveness to GnRH.

1.1.7. Mechanisms of the Age-Associated Decline in Blood Androgen Levels.

There are three different aspects to the changes in serum testosterone levels in aging men: first, there are primary testicular changes with a diminished testicular secretory capacity; second, there is an independent increase of SHBG binding capacity; third, there is an altered neuroendocrine regulation of the Leydig cells with apparent failure of the feedback mechanisms to be fully operational (Kaufman & Vermeulen, 1997-98).

The decrease in testicular secretory reserve appears to involve a reduction of the number of Leydig cells (Neaves *et al.*, 1985; Rubens *et al.*, 1974). The substantial age-related increase of SHBG (about 1.2%/yr) is remarkable as it occurs in the face of an increase of fat mass and insulin levels, which are strong negative determinants of SHBG levels (Giagulli *et al.*, 1994; Vermeulen *et al.*, 2003). Presently, the mechanisms responsible for the age-associated increase

of serum SHBG are yet to be uncovered.

Consistent with a primary testicular cause of decreased testosterone production, mean serum LH levels in the male population tend to increase with age (Tsitouras & Bulat, 1995; Vermeulen, 1991), but this increase is of modest amplitude and inconsistent (Morley *et al.*, 1997). Many elderly men with a serum testosterone concentration below the range for young men do not have elevated LH levels. Moreover, the modest increases in basal serum LH in elderly men appear to be in part underlied by a slower plasma clearance rather than by increased pituitary secretion (Bergendahl *et al.*, 1998; Kaufman *et al.*, 1991). Aging in men is thus also accompanied by manifest alterations in the regulation of LH secretion, the regulation of FSH secretion being better maintained (Mahmoud *et al.*, 2000-03). Assessment of the secretory capacity of the pituitary gonadotropes by in vivo challenge with small 'near physiological' doses of synthetic GnRH has revealed a maintained (Mulligan *et al.*, 1999) or, in accordance with a state of relative hypoandrogenism, even a slightly increased LH response in the elderly as compared to young men (Kaufman *et al.*, 1991). Taken that the pituitary secretory capacity is preserved in the elderly, the apparent failure of the feedback regulatory mechanisms to produce an adequate rise of serum LH must result from changes at the hypothalamic level. The pulsatile secretion of LH, governed by episodic release of hypothalamic GnRH into the pituitary portal circulation, has in the elderly an increased irregularity as compared to young men (Pincus *et al.*, 1997) with essentially unchanged (Deslypere *et al.*, 1987; Tenover *et al.*, 1987; Urban *et al.*, 1988; Winters *et al.*, 1984) or slightly increased (Veldhuis *et al.*, 1992) LH pulse frequency, but with a diminished frequency of large amplitude LH pulses and a reduced mean LH pulse amplitude, which is a parameter of the stimulating effect on the Leydig cells. It can be assumed that the diminished amplitude of LH pulses in the elderly reflects a reduced size of the GnRH bolus intermittently released into the pituitary portal circulation, which might in turn be the consequence of a reduced number of functional hypothalamic GnRH neurones, of a less efficient intermittent recruitment and/or synchronisation of these neurones and/or of a down regulation of their activity by local or systemic factors. As to the latter, an important observation is that elderly men have an increased sensitivity to the negative feedback effects of androgens (De Slypere *et al.*, 1987; Mulligan *et al.*, 1997; Winters & Atkinson 1997).

1.1.8. The Aging Male.

1.1.8.1. Introduction

In distinction to women, for whom the menopause signs the irreversible end of reproductive life as well as the end of cyclic ovarian activity, with as a consequence low sex hormone levels in all postmenopausal women, in men fertility persists until very old age. Also, the age-associated decrease in testosterone levels is slowly progressive with in a majority of men until the 8th decade still (F and bio) T levels within the normal range for young men. Subnormal testosterone levels are thus not a generalised feature of aging and as a rule androgen deficiency is only partial (Gooren, 1996-98). Therefore, the terms **partial androgen deficiency of the aging male** (PADAM) or late onset hypogonadism have been proposed as more appropriate than the terms andropause or male climacteric, which have the connotation of a generalized phenomenon and of permanent infertility.

Frequent clinical manifestations of aging in males are decreased libido and sexual activity or impotence (Davidson *et al.*, 1983), decreased virility with decreased sexual body hair and beard growth, decreased energy, work capacity and cognitive function. Objective signs are decreased muscle mass and strength (Rantanen *et al.,* 1998), decreased bone mineral density (BMD) with increased fracture risk (Nguyen *et al.*, 1996; Santavirta *et al.*, 1992), (abdominal) obesity (Fink *et al.*, 1983; Hughes *et al.*, 2002), and slightly decreased haematocrit (Basaria & Dobs, 1999). Signs of neurovegetative dystonia, nervousness, insomnia, and sometimes hot flushes often accompany these changes (Urban, 1992). The analogy with symptoms of hypogonadism in young males is striking: impaired virilisation with poor development of sexual body hair and beard growth, decreased bone and muscle mass with decreased physical strength, weakness, decreased libido and often erectile dysfunction, abdominal obesity and/or difficulty to concentrate (Comhaire, 2000). This symptomatology in the elderly develops, however, only slowly and progressively. The symptoms are subtle, variable and not specific (Liu *et al.*, 2003). Hence, the clinical symptomatology does at best only suggest the possibility of a hypoandrogenic state in the elderly. A major limitation is the scarcity of controlled data available as to date. No more than 576 elderly men in total have been included in controlled trials and have received active androgenic treatment of 3 weeks to 36 months duration under controlled conditions (Kaufman & Vermeulen, 2005).

1.1.8.2. Sexual function

Decline in sexual activity, interest and desire has been reported by different investigators. About 50% of older adults express sexual desire in the ninth decade and about 15% are sexually active (Mulligan & Katz, 1988). It is presently not established that age-associated decline of testosterone in healthy men contributes substantially to decreased libido and sexual activity in the elderly male population (Gruenewald & Matsumoto, 2003). The correlation of libido with plasma testosterone levels is rather poor (Schiavi, 1996; Davidson *et al.*, 1983). Nevertheless, Tsitouras *et al.* (1982) reported that elderly subjects with higher sexual activity had higher testosterone levels than the men in a low activity group, whereas Schiavi *et al.* (1988) reported that men with hypoactive sexual desire had lower testosterone levels than controls. Schiavi *et al.* (1990) reported that, in married couples or those in stable relationships between the ages of 45 and 75, sexual desire, arousal, coital frequency and prevalence of erectile problems correlate with nocturnal penile tumesence measures.

Testosterone treatment improved or tended to improve libido in studies in which it was assessed (Holmang *et al.*, 1993; Nankin *et al.*, 1986; Schiavi *et al.*, 1997; Tenover, 1992). As to erectile function it may be concluded that, except for the few cases of erectile dysfunction due to pituitary or testicular pathology, erectile dysfunction in elderly men is largely non-hormonal in origin. Indeed, besides hypoandrogenism and hyperprolactinemia many non-hormonal factors such as diabetes mellitus, atheromatosis, alcoholism, polyneuropathy or renal insufficiency, may be a cause of erectile dysfunction, whereas transient dysfunction may be caused by stressful situations, loss of attractiveness of the (same) sexual partner or monotony of sexual life, socio-economic problems, acute infections and a variety of drugs, in particular antihypertensive, psychotropic, and opioid medications (Kaufman & T'Sjoen, 2002; T'Sjoen *et al.*, 2003).

Frequency of erectile dysfunction increases dramatically with age. Psychological conditions such as depression and psychosocial stresses (such as divorce, death of spouse, loss of

social status, loss of job, health-related family problems) are prevalent in older adults and contribute to sexual problems (Cole, 1993). Sexual dissatisfaction is also related to marital relationship problems, which vary from interpersonal problems and inadequate communication of sexual needs to poor sexual techniques. Other issues such as problems commitment, power struggle, and lack of trust may all reflect dissatisfaction on the partner's behalf and may relate to sexual problems. Androgens, which acts both centrally as well as peripherally (Mills *et al.*, 1996) - where testosterone stimulates nitric oxide synthase in the corpora cavernosa (Lugg *et al.*, 1995)- are essential for normal penile erection. Possibly in relation to the stimulatory effect of testosterone on nitric oxide synthase activity, a synergistic effect between androgens and inhibitors of 5-phosphodiesterase type 5 has been observed (Aversa *et al.*, 2003). Nevertheless, testosterone deficiency is rarely a major cause of impotence in elderly males, although it might play a role in 6 to 45% of cases (Morley, 1986), the most prevalent cause of erectile dysfunction being atherosclerotic pelvic arterial insufficiency (Sullivan *et al.*, 2001). In men with marked hypogonadism, frequency, amplitude and rigidity of the erections are significantly reduced, but they remain normal at moderately decreased androgen levels (Carani *et al.*, 1995) and a threshold level of 200 ng/dL has been suggested for sleep related erections (Buena *et al.*,1993; Gooren, 1987). This explains probably why in most studies in elderly men no correlation was observed between erectile dysfunction and serum testosterone levels (Feldman *et al.*, 1994; Korenman *et al.*, 1990; Rhoden *et al.*, 2002). There is no adequate clinical documentation to support the use of androgen treatment with the specific aim to improve sexual function in elderly men (Liu *et al.*, 2002; Snyder *et al.*, 1999). Many of the studies have used rather crude instruments to evaluate (the perception of) sexual function so that further studies are needed to allow for conclusions on the effects of androgen administration on sexual function in elderly men. Nevertheless, whereas the design of the more numerous trials that have included hypogonadal men in a broader age range does not allow for confirmation of the efficacy of androgen treatment on sexual function specifically in the subgroup older study subjects; it can be noted that they provided also no manifest indication that frankly hypogonadal elderly cannot respond to the treatment (Steidle *et al.*, 2003).

1.1.8.3. Cognitive function

Cognition is defined as the various thinking processes through which knowledge is gained, stored, manipulated and expressed. Although many cognitive skills decline with age, the extent and pattern of decline vary both at individual level and according to the type of function. Some individuals age successfully and maintain a similar cognitive function to that of the young, and some functions may even improve with aging. Other individuals may have some intact cognition functions (e.g. long-term memory, complex motor skills) while there is a decline in other areas of cognition (e.g. learning new information). Cognitive function is affected by a number of variables such as demographic factors (age, education), work and leisure activities and individual differences. Psychiatric problems such as depression and substance abuse also affect cognition (Tariq & Morley, 2002).

Older age is associated with functional deterioration throughout the body, including some aspects of cognitive performance. Although only some elderly individuals develop dementia, a decrease in cognitive functioning and minor cognitive impairment impacts on daily living for many. Recently, hormonal effects in the central nervous system have become a focus of interest, with emphasis on potential anti-aging effects of hormonal replacement therapy. Studies in

humans concerning the relationship between endogenous androgen levels and cognitive performance have produced inconsistent results, although there do exist striking sex differences in spatial abilities, broadly defined as those tasks that include visual perception, spatial attention, object identification and visual memory processes (Kimura, 1996; Silverman *et al.*, 1999). In healthy young men, positive relationships have been observed between endogenous plasma testosterone levels and visual-spatial orientation (Gordon & Lee, 1986; McKeever & Deyo, 1990), but other studies have failed to find such an association (Hier & Crowley, 1982; Kampen & Sherwin, 1996; McKeever *et al.* 1987). Patients with isolated hypogonadotropic hypogonadism show an impairment of spatial abilities (Buchsbaum & Henkin, 1980), which is improved by androgen treatment (Cherrier *et al.*, 1988; Morley *et al.*, 1997).

As to the effects of endogenous testosterone levels on cognitive functions in elderly males, Morley *et al.* (1997) reported a significant correlation of the endogenous testosterone levels with visual and verbal memory and a variety of cognitive performance tests, whereas in the Rancho Bernardo study (Barrett-Connor *et al.*, 1999), a higher bioT was significantly associated with better long-term verbal memory and score for a cognitive screening test (Selective Reminding Test). Yaffe *et al.* (2002) found in a cross-sectional study in 310 community-dwelling men with a mean age of 73 yr that a higher bioT was associated with significantly better scores on 3 cognitive tests, i.e. the Mini-Mental State Examination, the Trail Making B and the Digit Symbol tests. In the same study cognitive function was also found to be associated with the CAG-repeat polymorphism of the AR gene (Yaffe *et al.*, 2003). In volunteers of the Baltimore Longitudinal Study of Aging, a higher free testosterone index (FAI = ratio serum testosterone over SHBG) was associated with better scores on visual and verbal memory, visuospatial functioning and visuomotor scanning, and with a reduced rate of longitudinal decline in visual memory (Moffat *et al.*, 2002). Recently, it has been reported from the same study that lower values for FAI were associated with an increased incidence of Alzheimer disease in 574 men aged 32 to 87 yr at baseline and followed for a mean duration of 19.1 years (Moffat *et al.*, 2004). A recent study (Muller *et al.*, 2005) confirmed higher testosterone levels to be associated with better cognitive performance (Mini-Mental State Examination) in the elderly. It may therefore be concluded that the age-associated decrease in (F and bio) T levels appears to contribute to the impaired cognitive functions of elderly men.

There are limited observations of beneficial effects of testosterone treatment on cognitive function in elderly men, which warrant further investigation (Kenny *et al.*, 2002; Muller *et al.*, 2003; Sih *et al.*, 1997). Several intervention studies have shown an improvement in spatial cognition and working memory and a decrease in verbal fluency after treatment with testosterone (Cherrier *et al.*, 2001; Janowsky *et al.* 2000; Ly *et al.*, 2001; Wolf *et al.*, 2000) whereas other studies found no such associations (Cherrier *et al.*, 2002 ; Kenny *et al.*, 2002; O' Connor 2001). However, presently the limited information available with essentially negative findings for the few longer duration studies does not allow to claim clinical benefits on cognition of testosterone administration to elderly men.

1.1.8.4. Depression

Older men are less likely than older women to be diagnosed with depression. This had lead to the hypothesis that sex hormones are involved in the aetiology of at least some types of depression.

Psychological and behavioural changes accompanying aging in males are lack of energy,

decreased cognition, fatigue, memory impairment and sleep disturbances. The literature on the relation of depression, depressive symptoms and/or depressive mood with testosterone levels in elderly men has not been unequivocal. Schweiger *et al.* (1999) reported depressive subjects-scored with the Hamilton Depression Scale (HDS) - to have lower testosterone levels than controls. Based on analysis of subgroups of subjects from the MMAS study, Seidman *et al.* (2001) found lower serum testosterone levels in elderly men with dysthymic disorder but not in those with major depressive disorder and they also reported that the relationship between testosterone levels and depression may be modulated by the CAG repeat length polymorphism of the AR gene. From a review of the literature on testosterone and depression in aging men it was concluded that the available data suggest, but do not demonstrate that testosterone secretion may be reduced in some men with major depression (Seidman & Walsh, 1999).

However, mild depressive symptoms are common among elderly men as is hypogonadism or relative testosterone deficiency. Moreover, the psychiatric symptoms of hypogonadism overlap with symptoms of depression and the relatively high prevalence of depression in the institutionalised setting may be a reflection of the significant medical co-morbidity associated with depression in the older adult (Ganzini *et al.*, 1992). The pathofysiology of depression has not been fully elucidated. Even less well understood are the observed age and gender differences in the manifestations of depression.

Recently, in a historical cohort study in a health care setting for US veterans hypogonadism was associated with increased incidence of depressive illness and a shorter time to diagnosis of depression, even after adjustment for age and medical morbidity (Shores *et al.*, 2004-05). In 856 men aged 50 to 89 years participating in the Rancho Bernardo Study (Barrett-Connor *et al.*, 1999), bioT was significantly inversely associated with a depressive mood score as assessed with the Beck Depression Inventory (BDI), independently of age, weight or physical activity. In addition, in the 25 men with true depression, bioT was 17% lower than that in the rest of the group. There was no assocation between total or bioavailable oestradiol and dysphoria. In a follow-up study in 40 men, mean age 72.4 yr, treated for prostate cancer, chemical castration was associated with an increased BDI score (Almeida *et al.*, 2004). Booth *et al.* (1999) examined the interaction of social behaviour and dysphoria in 4393 men. In males with a below average level of testosterone there was an inverse relationship between T and dysphoria, while in those with above average T levels T was directly related to dysphoria. This supports a parabolic model for the relationship of testosterone to dysphoria. The MMAS study, which included 1709 male subjects 39-70 yr old, showed that an androgen deficiency pattern was accompanied by low dominance, moreover, in 25 men with true depression bioT was 17 % lower than in the rest of the group (Gray *et al.*, 1991). In men 50 to 70 years, who participated in a screening program on prostate cancer and "andropause", FT was inversely correlated with depressive symptoms score according to the Caroll Rating Scale, but was not related to threshold scores considered significant for depression (Delhez *et al.*, 2003). Several other studies (Kaneda & Fujii 2002, Levitt & Joffe, 1988; Moffat *et al.*, 2002; Rubin *et al.*, 1989) failed to find an association between (F or bio)T and depressive scores, whereas in contrast Perry *et al.* (2001) even reported that declining bioT levels were associated with lower levels of depressive symptoms on the HDS in men 55-75 years old. In summary, available evidence does not indicate that the age-related decline in testosterone is a risk factor for clinically significant depression, although a role in depressive mood cannot be excluded. (see also **Chapter 4**).

Several trials with androgen treatment included questionnaires on mood and or depres-

sion. Gray *et al.* (2005) could not find effects of graded testosterone doses in older men (60-75 years) on HDS scores. Other studies failed to demonstrate a treatment effect on either mood (Janowsky *et al.*, 1994-2000; Kunelius *et al.*, 2002; Liu *et al.*, 2003;) or scores for geriatric depression scales (Kenny *et al.*, 2004; Sih *et al.*, 1997) with the single exception of the observation in the small study by Bakhshi *et al.* (2000) of a larger improvement of the score for a geriatric depression scale in the active treatment group (n=9) than in the placebo group (n=6) in male frail geriatric patients admitted for revalidation. In younger HIV-infected men, Grinspoon *et al.* (2000) found a higher BDI score in the hypogonadal compared to the eugonadal males. Testosterone therapy resulted in a significant decrease in the BDI score.

1.1.8.5. Quality of life

Health perceptions related to a decrease in androgen levels during aging have not been investigated extensively in men. Untreated young men with hypogonadism reported higher scores for anger, fatigue and confusion than did eugonadal controls (Kenny *et al.*, 2002). Similarly, older hypogonadal men reported decreased irritability, tiredness, anxiety and sadness, and increased energy levels, cheerfulness, and sense of well being on T replacement (Ly *et al.*, 2001). It is evident that aging is often associated with a decrease of quality of life. However, so far the rare available studies failed to show a relationship between (F or bio) T and quality of life in elderly men as assessed with the SF-36 questionnaire (Dunbar *et al.*, 2001). Neurovegetative symptoms such as "hot flushes" may be more common in elderly men than generally suspected (Spetz *et al.*, 2003), but an association with the endogenous sex steroid levels has not been established.

In a small study, elderly men with moderately low serum androgens treated for 1 month with testosterone failed to accurately guess treatment assignment (Janowsky *et al.*, 2000), while in another study 6 months treatment with DHT gel failed to improve the score of a questionnaire on general well-being (Kunelius *et al.*, 2002). Several studies assessed health-related perception of quality of life using the 'Medical Outcome Survey Short–form 36' (SF-36) questionnaire (English *et al.*, 2000; Kenny *et al.*, 2002; Liu *et al.*, 2003; Ly *et al.*, 2001; Reddy *et al.*, 2000; Snyder *et al.*, 1999). The findings in the latter studies were mostly negative (Liu *et al.*, 2003; Ly *et al.*, 2001; Kenny *et al.*, 2002; Reddy *et al.*, 2000). The instruments used in the negative studies to assess quality of life may not have been sensitive to change. One exception was the report of significantly less worsening of the perception of physical function in elderly men treated for 3 year by testosterone scrotal patches compared to placebo-treated subjects in the study by Snyder *et al.* (1999). Interestingly, improvement of the score for perception of physical functioning during active treatment was observed in men with the lowest pre-treatment serum testosterone levels, but it should also be noted that physical health perception is only 1 out of 8 dimensions assessed by the SF-36 questionnaire. In the latter study, there was no treatment effect for any other sub-score of the SF-36 questionnaire. The only other exception was the finding by English *et al.* (2000) of improved scores on pain perception and role limitation resulting from physical problems during testosterone administration to elderly men with CAD. In men requiring long-term systemic glucocorticoid treatment, Crawford *et al.* (2003) found that treatment with testosterone, but not with nandrolone, improved overall quality of life as assessed with the 'Qualeffo 41', a dedicated questionnaire for patients with osteoporosis.

The findings for treatment effects on mood, depression and quality of life in elderly men are mostly negative. In how far the failure to demonstrate beneficial effects may have resulted

from the use of instruments that are insufficiently sensitive to detect changes in study populations consisting of men with rather good general health status remains to be established. We have no data indicating that treatment may have substantial longer-term effect on quality of life. Nevertheless it can be noted that the only few positive results were all obtained in men with impaired health (Bakhshi *et al.*, 2000; Crawford *et al.*, 2003, English *et al.*, 2000) or in a subset of men with manifestly low serum testosterone (Snyder *et al.*, 1999). Some healthy older men reported increased feelings of well-being and energy during testosterone treatment (Holmang *et al.*, 1993; Marin *et al.*, 1992; Tenover, 1992), but energy (Snyder *et al.*, 1999) and mood (Benkert *et al.*, 1979; Janowsky *et al.*, 1994; Janowsky *et al.*, 2000; Schiavi *et al.*, 1997; Sih *et al.*, 1997) were unchanged in other studies of healthy men. Administration of moderately high doses of testosterone for contraception has failed to reveal adverse effects on male aggressive behaviour (Bahrke *et al.*, 1996).

1.1.8.6. Activities of daily living

Because androgens are associated with muscle mass and strength and with cognitive functioning, it is reasonable to expect that androgens are related to activities of daily living (ADL). Research has suggested that the age-related decline in testosterone is associated with a decline in functional status; that is, the ability to perform simple tasks necessary for the maintenance of ADL (Morley, 2001). Several studies on physiological androgen levels and ADL have been reported, which were confined to men aged 65 years and over. In general, higher DHEA-S levels appear to be associated with higher functioning levels, even in men over 90 years of age (Berr *et al.*, 1996; Ravaglia *et al.*, 1996). In male nursing home residents, higher sex hormone levels (T and DHEA) were associated with better ADL performance (Breuer *et al.*, 2001). However, DHEA-S supplementation in healthy elderly men during a cross-over trial did not result in significant changes in self-reported ADL (Flynn *et al.*, 1999)

1.1.8.7. Erythropoiesis

Adult men have higher hemoglobin and red cell mass than adult women (Hawkins *et al.*, 1954), differences that become only apparent after puberty (Vahlquist, 1950) and are independent of menstruation as they persist when considering young hysterectomized women. Haemoglobin levels increase by 15 to 20 % in boys at puberty. Elderly men tend to have a similar or slightly lower haematocrit than young men (Garry *et al.*, 1983). An association between endogenous serum androgen levels and indices of erythropoiesis in elderly men has not been documented.

Androgens stimulate erythropoiesis and in most studies haematocrit increased by 2 to 5% over baseline values during testosterone treatment, 6 to 25% of subjects developing erythrocytosis with haematocrit over 50% (Amory *et al.*, 2002, Amory *et al.*, 2004, Clague *et al.*, 1999, Drinka *et al.*, 1995, Hajjar *et al.*, 1997, Kenny *et al.*, 2001, Ly *et al.*, 2001, Sih *et al.*, 1997, Snyder et al. 1999, Tenover, 1992, Wittert *et al.*, 2003). Erythrocytosis might increase the risk of stroke and requires corrective measures, i.e. temporary interruption of treatment, dose adaptation and/or phlebotomy. Testosterone treatment by IM injection might be associated more frequently with erythrocytosis than the transdermal route of administration, most likely because traditionally applied treatment regimens with IM injection of testosterone esters at bi-weekly to

monthly interval result in serum testosterone levels that are transiently, but markedly supraphysiological following each injection (Dobs *et al*., 1999; Sih *et al*., 1997). Dobs *et al*. (1999) compared a transdermal nonscrotal testosterone patch with intramuscular injections of testosterone enanthate and observed that 15.4 percent and 43.8 percent of patients, respectively, had at least one documented elevated haematocrit value (defined as over 52%) during the course of the study. Erythrocytosis was associated with supraphysiological levels of bio T and E2, and it occurred more frequently in the group that received intramuscular injections of T. However, significant increases of haematocrit and haemoglobin levels are also seen during transdermal administration of either testosterone (Snyder *et al*., 1999, Steidle *et al*., 2003) or DHT (Kunelius *et al*., 2002; Ly *et al*., 2001). Wang *et al*. (2000) demonstrated a direct relation between T dosage and the incidence of erythrocytosis. Erythrocytosis occurred in 2.8 percent of men receiving 5 mg per day by nonscrotal patches and in 11.3 percent and 17.9 percent of men treated with gel preparations of 50 mg per day and 100 mg per day, respectively.

It is recommended that haematocrit or haemoglobin blood concentration are determined at initiation of treatment and at follow-up visits (Rhoden & Morgentaler, 2004), with close monitoring if the patient also has a condition that may itself be associated with an increase in haematocrit, such as chronic obstructive pulmonary disease (Viallard *et al*., 2000).

1.1.8.8. Other symptoms and problems concerning the aging male

Other associations between clinical manifestations of aging and sex steroid status have been described. As these variables were not assessed in the experimental work of this thesis, we have not repeated the data here. For further information I would like to name important reviews on some of these subjects. Recent reviews on arteriosclerosis and androgens (Wu & Von Eckardstein, 2003), on osteoporosis (Vanderschueren *et al*., 2004) and on body composition (Vermeulen *et al*., 2003) are recommended papers. A detailed review paper on sex steroids in elderly men, clinical significance of the decrease in androgen levels, diagnosis of ADAM and androgen treatment was recently published (Kaufman & Vermeulen, 2005).

1.1.9. Diagnosis of androgen deficiency in the aging male.

1.1.9.1. Introduction

In 1889, Brown-Séquard, aged 72, reported dramatic rejuvenating effects after self-administering testicular extracts of dogs and guinea-pigs. His report resulted in widespread use of testicular extracts throughout Europe and North America for several decades (Brown-Séquard, 1889).

Given that the signs and symptoms suggestive of androgen deficiency in aging males are not specific, diagnosis of androgen deficiency should evidently not be based solely on the clinical picture and additional biochemical evidence is required for its ascertainment (Gooren, 2003). However, in view of the still unresolved issue of what should be considered "physiological" or "optimal" androgen levels in the elderly, in many cases a margin of uncertainty will persist. Therefore, diagnosis should preferably be based on an appropriately conservative evaluation of the convergence between clinical and biochemical findings (Kaufman & Vermeulen, 2005). Androgen replacement therapy with testosterone still remains controversial (Comhaire, 2002; Snyder, 2004)

1.1.9.2. Clinical evaluation

A number of questionnaires are being used in clinical or epidemiological settings to help describe a variety of symptom clusters that are clinically relevant to elderly men, such as questionnaires on self-perceived health status, on depressive mood or depression, on cognitive function, on urinary symptoms, on erectile function or on coping with activities of daily living. There are also several questionnaires that are explicitly or implicitly proposed by their proponents as tools to summarize or evaluate symptoms of androgen deficiency in aging men (table 1).

The **Androgen Deficiency in Aging Males (ADAM)** questionnaire has been developed by Morley *et al*. (2000) as a screening tool for identifying middle-aged and older males with testosterone deficiency. This simple self-administrated questionnaire consists of a set of 10 questions that are to be answered dichotomously by 'yes' or 'no' and relate to present or absent perception of decreased libido, lack of energy, decrease in strength, loss of height, decrease in enjoyment of life, feeling sad and/or grumpy, less strong erections, deterioration in the ability to play sports, falling asleep after dinner and deterioration in work performance. In a group of 316 volunteering Canadian physicians with mean age of only 52 yr (range 40 to 82 yr) the questionnaire had a sensitivity of 88% and a specificity of 60% to identify subjects with low serum bioT defined in this study as a level below <70 ng/dL as seen in 25% of the study group. Legros & Delhez (2002) using a French translation of this questionnaire in 754 men aged 50-70 yr (mean 59.5 yr) who were taking part in a prostate cancer screening and volunteered for an additional "andropause" evaluation observed a sensitivity of 80%, but a specificity of only 32 % to identify FT below the range for young men (i.e. 7 ng/dl). This questionnaire evidently lacks specificity, with part of the false positive tests being associated with symptoms of depression (Delhez *et al*., 2003; Gladh *et al*., 2004). In the study by Tancredi *et al*. (2004) in a group op 5028 men, aged 50-70 years, spontaneously consulting for the assessment of their gonadal function, the sensitivity and specificity of the ADAM score were 81% and 21.6% respectively. The results of ADAM resulted in an appropriate classification of the population in eu- or hypogonadal subjects in 44.5% of the cases.

The **Aging Male Symptoms scale** (AMS), based on subjective evaluation of symptomatology, was developed by Heinemann *et al*. (1999), to help describe and quantify the clinical syndrome of andropause and was not intended as a screening tool for androgen deficiency and not validated against androgen levels by the authors. However, it has been proposed as outcome measure for treatment of androgen deficiency (Daig *et al*., 2003; Moore *et al*., 2004). Basar *et al*. (2005), Dunbar *et al*. (2001) and Tsujimura *et al*. (2005) could not find an association with testosterone levels (see also **Chapter 3**), whereas other studies, published in abstractform, found close associations (Itoh *et al*., 2004; Jankowska *et al*., 2004; Soh *et al*., 2004). Selected items from AMS such as decreased beard growth, depressed mood and wish to be dead correlated significantly with the CAG repeat number in Härkönen's study (2003). The comparison with the generic quality-of-life scale SF-36 showed sufficiently good correlations (Daig *et al*., 2003).

Whereas the latter scale is based on subjective symptomatology, Smith *et al*. (2000) constructed a **self-administered 8 item screener** for testosterone deficiency in aging men that is based on age, BMI, occurrence of diabetes, of asthma and of headache, on sleep pattern, on dominance preference and on smoking. This questionnaire, which takes into account aspects of co-morbidity, has a similarly low specificity of only 49% for a sensitivity of 75% (positive predictive value between 28 and 52%).

The two screening scales for androgen deficiency (ADAM and Smith's 8item screener), measure a similar phenomenon compared to the AMS, even if AMS was not developed as a screening instrument (Heinemann *et al*., 2004).

Although clinical examination may point towards specific causes of hypogonadism, there are no specific findings for age-related partial androgen deficiency. In a study in healthy elderly men over age 70 yr combined testicular volume was decreased by about 20% with a combined testicular volume of 14.3 ml as best cut-off having a sensitivity of 46% and a specificity of 79% to predict a bioT below the range for young men (Mahmoud *et al*., 2003).

Table 1. Attempts to characterize an Andropauze Syndrome, which includes behavioural factors.

Author	Heinemann *et al*. 1999	Morley 2000	Smith 2000
Source	Aging Male	Metabolism	Clin Endocrinol
Behavioural factors	Decreased general well-being Joint pain Muscular aches Excessive sweating Sleep problems Tiredness Irritability Nervousness Anxiety Physical exhaustion Decreased muscular strength Depressive mood Feeling past your peak Feeling burnt out Decrease in beard growth Decrease in ability to perform sexually Decreased libido Decreased morning erections	Decreased libido Lack of energy Decreased strength/endurance Loss of height Decreased enjoyment of life Sad or grumpy Decreased work performance Decreased ability to play sports Decreased strength of erections Falling asleep after dinner	Low dominance Headaches Sleeplessness

1.1.9.3. Biochemical evaluation

Based on data obtained in large groups of healthy, non-obese men the lower limit of the normal range is generally considered to be around **315 ng/dL** (11 nMol/l) **for total** testosterone and around **6.5 ng/dL** (0.225 nMol/L) **for FT** (Vermeulen, 2001), corresponding to a **bioT** of around **140 ng/dL** (5 nmol/L) (Mahmoud *et al*., 2003). Most authors use similar val-

ues (Harman *et al*., 2001; Rudman *et al*., 1994, Tenover, 1992; Wang *et al*., 2000). Using these limits, less than 1% of healthy aged 20 to 40 years but more than 20% of men over 60 years old have subnormal total testosterone levels, a percentage which is slightly higher when using FT.

As to biochemical parameters of androgen deficiency in elderly men, there is no clinically useful biochemical marker of tissular androgen action, so that one has to rely upon measurement of testosterone concentrations in the systemic circulation. Limits of normality are somewhat arbitrary as the sensitivity threshold for androgen action may vary from tissue to tissue and according to age. It is not known whether androgen requirements in the elderly are similar, lower or higher compared to those in young men. There are indications of a greater sensitivity of some tissues, with in particular greater sensitivity of the elderly to negative feedback suppression of LH secretion by androgens (Deslypere *et al*., 1987; Mulligan *et al*., 1997; Winters *et al*., 1984; Winters & Atkinson, 1997). (see also Chapter 2).

Diagnosis should be based on the convergence of clinical symptoms and subnormal testosterone levels. The latter should be frankly low as measured in blood sampled in the morning (before 10 a.m.) and confirmed on a second occasion, preferably separated by a few weeks such as to allow for recuperation following eventual transient causes of hypogonadism. Whereas total testosterone will provide adequate information in many cases, assessment of FT or bioT should be preferred in situations characterized by substantial changes in SHBG levels.

1.1.9.4. Conclusion

In the present state of the art, androgen supplementation should only be considered in the presence of androgen serum levels clearly below the lower normal limit for younger men, together with unequivocal signs and symptoms of androgen deficiency, in the absence of other reversible causes of decreased androgen levels and after screening for contraindications. The longer term risk-benefit ratio for androgen administration to elderly men is unknown, yet, there is no a priori medical or moral justification for withholding the benefits of substitutive treatment from symptomatic hypogonadal elderly men. Conversely, there are at the moment no compelling arguments for the substitutive treatment of the asymptomatic hypogonadal elderly man. Furthermore as an age-connected decrease in testosterone levels has been accepted, the definition of the hypogonadal man strictu sensu can only be based upon reference values according to age. A prudent approach is advisable in view of the limited data and clinical experience for this population and of the potential for a greater susceptibility for adverse treatment effects in the elderly, such as prostatic carcinoma, benign prostatic hyperplasia, polycythemia, sleep apnoea, gyneacomastia and breast carcinoma, fluid retention, hypertension, lipid alterations, and atherosclerosis (Rhoden & Morgentaler, 2004). So far, limited data on safety of testosterone replacement therapy in the elderly has been rather reassuring, however, larger scale studies of longer duration are still needed.

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1.2. TRANSSEXUALISM

1.2.1. Definition

Transsexualism is a condition in which a person experiences a discongruency between their assigned sex and what they feel their genderidentity is. For example, a person who was identified as "female" at birth, who experienced normal somatic sexual differentiation, who was raised as a girl, and has lived being perceived by others as a woman, may feel that the core sense of who they are is a closer fit with "male" or "man." If this feeling is strong and persistent, this person may decide to take steps, hormonally and surgically, to ensure that others perceive them as a man. In other words, they may decide to the transition to living as the sex that more closely matches their internal gender (De Cuypere, 2001; Gooren, 2004). A person with gender dysphoria experiences anxiety, uncertainty or persistently uncomfortable feelings about their birth gender.

Self-diagnoses are confirmed by psychological assessment, which includes a trial period, 'the real life test'. This period when hormonal treatment starts and subjects are required to live socially the live of the desired sex is necessary before irreversible surgical reassignment is considered. Cross sex hormonal treatment is desired by transsexual persons to help them successfully live as a member of their identified gender.

Gender Identity Disorder (GID) has three criteria according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV)(American Psychiatric Association, 1994):

- the desire to live and be accepted as a member of the opposite sex, usually accompanied by the wish to make his or her body as congruent as possible with the preferred sex through surgery and hormonal treatment,
- the presence of persistent discomfort with his or her sex or sense of inappropriateness in the gender role of that sex
- the disorder is not a symptom of another mental disorder or a chromosomal abnormality.

The persistent cross gender identification that results transcends a desire for any cultural advantages of being the other sex (Levy *et al*., 2003). All strata of society are affected by gender identity disorders (Hoenig & Duggan, 1974). Transsexualism must be distinguished from sexual orientation, and transsexuals like non-transsexuals may be heterosexual, homosexual, bisexual or asexual.

The most recent prevalence information from the Netherlands is that transsexualism occurs in one of 11.900 males and in one of 30.400 females (Bakker *et al*., 2002; van Kesteren *et al*., 1996). Unpublished data for Belgium show similar figures: one of 15.185 males and one of 38.665 females (Carael, 2004). The similar prevalence across Western and Eastern Europe, Singapore and The Indian subcontinent suggests that the influence of culture on the underlying condition is relatively small. Higher prevalence figures have been given by Tsoi (1988), possibly as a result of the high quality of surgical services available in Singapore, the absence of repression against transsexuals, and the subsequent lessened fear of role change. The aetiology of transsexualism remains uncertain (Gooren, 1990, Zhou *et al*., 1995). Most biological investigations of transsexuals have found that there are no abnormalities in chromosomal pattern, in the gonads or genitals, or in circulating peripheral sex steroid levels that could account for the condition (Gooren, 1984). Limited case reports have described the association between transsexual-

ism and various chromosomal abnormalities such as 47, XXX (Turan *et al*., 2000), 47, XYY (Buhrich *et al*., 1978; Snaith *et al*., 1991), Klinefelter's syndrome (Davidson, 1966; Prior *et al*., 1989; Seifert & Windgassen, 1995) or Kallmann's syndrome (Meyenburg & Sigusch, 2001). On the base of data reported in the literature and of our own research, we believe that the neuroendocrine regulation of LH-secretion is not different between transsexuals and non-transsexuals (T'Sjoen *et al*., 2005).

It is unknown whether the mechanism controlling sexual differentiation of the human brain is hormonally determined. The volume of the bed nucleus of the stria terminalis (BSTc), a brain area that is essential for sexual behaviour, shows all characteristics of a female differentiation in a sample of male to female transsexuals. A male differentiation was found in the brain of a single female-to-male transsexual (Kruijver *et al*., 2000; Zhou *et al*., 1995). The results of these studies may have been confounded to some extent by the small numbers available for study and may be subjected to various other forms of bias.

However, in the final analysis, the aetiology of transsexualism and gender dysphoria remains unknown and is most probably multifactorial.

1.2.2. Treatment Strategies

1.2.2.1. Diagnosis

Gender dysphoria is a self- diagnosis often supported by friends and family with no supporting tests other than persistence of dysphoria for at least two years alleviated by cross gender identification. It is an incurable condition associated in a significant proportion of cases with social isolation; however it is amendable to hormonal and surgical palliation (Levy *et al*., 2003). The effects of treatment are often irreversible, and therefore the process of psychological evaluation to determine whether the treatment facilitates the patients' comfort and integration into society is far reaching. This decision involves input from different sources such as psychiatrists, various medical and surgical specialities, nurses, social workers, speech therapists, … (Monstrey *et al*., 2001). A multi-disciplinary clinical structure with continual professional development of staff should be available to optimise integration of these specialist areas.

Psychiatric assessment

The international organisation involved with professional help to transsexuals, the Harry Benjamin International Gender Dysphoria Association has drafted Standards of Care (Meyer *et al*., 2001). The major purpose of the Standards of Care (SOC) is to articulate this organisations' professional consensus about the psychological, medical and surgical management of gender identity disorders. Persons with gender identity disorders, their families and social institutions may use the SOC as a mean to understand the current thinking of professionals. During the diagnostic phase there are two objectives:

- to assess the authenticity and amplitude of gender dysphoria including the degree of transsexual conviction
- to give information concerning the treatment procedure, including both possibilities and limitations of hormonal therapy and surgery.

Even though well-defined criteria are available to the psychologist and psychiatrist the diagnosis

of transsexualism remains difficult and assessment by at least two senior specialists is recommended, often involving a number of in-depth interviews over several months during which psychiatric and psychological state, personality profile, physical and emotional development, school and employment background, adult relationships, physical health and current of level of social functioning are addressed (De Cuypere, 1995; Levine, 1980). Not all who enter the process do proceed to full gender reassignment surgery (Landen *et al*., 2001). The diagnostic phase may last for at least three to six months but can be extended to over two years, before making the precise diagnosis. Families of transsexual persons may also benefit from receiving counselling, as they too face difficult changes.

The real life test

When hormone treatment starts or maybe even earlier, the real life test should begin. It is an extended period of full time living as a member of the desired sex. The transsexual person should dress in accordance with his or her new gender, maybe choose a new first name, and inform his or her different social partners of the future hormone and surgical reassignment. Every day confrontation with reactions from the social milieu can represent one of the major difficulties in sex reassignment. Embarking on the real life test may be done in a stepwise fashion, for instance first in a trusted environment and later in public. The subject should have lived at least one full year full time in the new sex before irreversible surgical reassignment is considered. In our current procedure, we ask that the subject stays in contact with a mental health professional to allow assessment of the success of the test and to discuss how to overcome problems.

Clinical assessment

Physical assessment is to be approached with care and should include a full examination of secondary sexual characteristics. Laboratory examination, like measurement of sex hormone levels and karyotyping, baseline cholesterol, urea and electrolytes, glucose and liver function test are unlikely to yield anything more than confirmation of biological sex, aside from potentially disclosing evidence of self treatment (Cohen-Kettenis *et al*., 2004). Currently in our gender team basic medical monitoring includes a serial physical examination relevant to treatment effects and side effects, vital measurements before and during treatment, weight measurements and laboratory assessment. Before any physical intervention is considered, a clear explanation of the irreversible effects of hormonal therapy on body habitus is necessary. The physician should counsel the patient about realistic expectations from treatment and discuss the treatment options hormonal and surgical.

Biologic males, specially those who have not already reproduced, should be informed about sperm preservation options, and they can consider banking sperm prior to hormone therapy (De Sutter, 2001). Biologic females do not presently have readily available options for gamete preservation.

1.2.2.2. Hormonal treatment

Hormonal reassignment has two aims:
- suppression of the hypothalamic-pituitary-gonadal axis leading to a reduction of endogenous

oestradiol or testosterone secretion, and hence, reduction of secondary sex characteristics of the original sex as much as possible. Complete elimination, however, is rare. For example, in male-to-female transsexuals, the previous effects of androgens on the skeleton, such as the greater height of man than women, the size and shape of hands, feet, jaws and pelvis cannot be reversed. Universally, the relatively lower height and the broader hip configuration of female-to-male transsexuals compared to men will not change under androgen-treatment.
- induction of secondary sex characteristics of the new sex. It should be carefully explained that hormonal treatment after gonadectomy will be lifelong to avoid well described metabolic and physiological problems secondary to steroid deficiency. Treatment regimens include various forms of oestrogens, progestins, and/or androgens reported by different clinical centres. No randomized trials are available (Moore *et al*., 2003).

Male-to-female

Anti-androgens

Several agents are available to inhibit androgen secretion or action. Anti-androgens are theorised to lower serum levels of testosterone by suppression of gonadotropins or to block testosterone binding to the androgen receptor, thereby decreasing masculine secondary characteristics. In Europe the most widely used drug is cyproterone acetate (usually 50 to 100 mg daily), a progestational compound with anti-androgenic properties. If this is not available, medroxyprogesterone acetate, 5 to 10 mg per day is an alternative although less effective. Non-steroidal anti-androgens, such as flutamide, are also used, but they increase gonadotropin secretion causing increased secretion of testosterone and oestradiol; the latter is a desirable effect in this context. Spironolactone (100 mg twice daily), a diuretic with anti-androgenic properties, has been said to have similar effects (Prior *et al*., 1989). Long-acting GnRH agonists, used as monthly injections, also inhibit gonadotropin secretion and have been considered by some to increase oestrogen effects when risk factors limit the dose of oestrogen. Finasteride 1 mg, a 5-alpha-reductase inhibitor, or local minoxidil might also be considered if the person is distressed by scalp hair loss.

GnRH agonists have been used in adolescent transsexual persons to delay puberty, to allow cross-sex hormones to be postponed until adulthood, with less psychological stress to the individual (Cohen-Kettenis & van Goozen, 1998).

Oestrogens

Oestrogens are the cornerstone for feminisation of the male-to-female transsexual persons. Hormonal replacement therapy includes ethinyloestradiol in different doses, conjugated equine oestrogens, or oral and transdermal 17-beta-oestradiol. Most centres use oral delivery. Transdermal 17-beta-oestradiol is systematically given once patients reach the age of 40 because this mode of administration might be associated with a lower risk of thrombo-embolic events in older transsexual persons (VU Amsterdam guidelines) (Asscheman *et al*., 1989; van Kesteren *et al*., 1997). Intramuscular formulations are rarely reported. Higher doses of oestrogens may not lead to more rapid or dramatic clinical changes. Ethinyloestradiol doses of 500 μg demonstrated the same degree of testosterone suppression as 100 μg in a small observational

case-controlled study in male-to-female transsexual persons (Meyer *et al*., 1981). However, breast growth was enhanced with higher oestrogen levels. Higher doses are avoided to minimize adverse effects. As insufficient oestrogen doses and possibly also excessive oestrogen may be associated with vasomotor symptoms, adjustment of the dose in both directions may be necessary.

Oestrogen treatment will result in breast growth, distribution of body fat to approximate a female body habitus, decreased upper body strength, softening of the skin, decrease in body hair, slowing or stopping the loss of scalp hair, decrease of fertility and testicular size and less firm erections. Breast tissue increase starts almost immediately after initiation of oestrogen administration. Androgens have an inhibitory effect on breast development and, therefore, oestrogens will be most effective in a milieu devoid of androgen action. After two years of oestrogen administration, no further development can be expected. Breast formation is quantitatively satisfactory in 40 to 50 % of the subjects (Gooren, 1999). The attained size can be disproportional to the male dimension of the chest and height of the subject, so the subject may desire surgical breast augmentation. Adult male beard growth is very resistant to inhibition by combined hormonal intervention, and in Caucasian subjects additional measures to eliminate facial hair are necessary. Sexual hair growth on other body parts responds more favourably (Giltay & Gooren, 2000). Oestrogens (and anti-androgens) have no effect on the properties of the voice so male-to-female transsexuals may wish to consult a specialised phoneatric centre for speech therapy (Van Borsel *et al*., 2001).

Progestins

Although there is no absolute requirement for progestins, some male-to-female transsexual persons find that their libido is maintained and breast growth augments by the addition of dydrogesterone (such as Duphaston, 10 mg twice a day) or the likely more virilizing medroxyprogesterone acetate (Provera, 5 to 10 mg daily). Attempts to mimic the menstrual cycle by prescribing interrupted oestrogen therapy or substituting progesterone for oestrogen during part of the month are not necessary to achieve feminisation.

Female-to-male

The goal of treatment in female-to-male transsexual persons is to induce virilisation, including a male pattern of sexual hair and male physical contours and to stop menses. The principal hormone treatment is a testosterone preparation. The commonly used preparations are testosterone esters in doses of 200 to 250 mg intramuscularly every two to three weeks. Alternative options for androgen treatment include injectable and transdermal delivery systems. Treatment principles are equal to those of the hypogonadal male patient. Androgen administration induces the following permanent changes: a deepening of the voice after six to ten weeks, clitoral enlargement of variable degree, mild breast atrophy, increased libido, acnea, increased facial and body hair and male pattern baldness. Other changes include increased upper body strength, weight gain, increased social and sexual interest and arousability, and decreased hip fat. Occasionally menstrual bleeding does not stop with this regimen and addition of a progestational agent is needed. If a transdermal testosterone preparation is used, addition of a progestational agent is nearly always necessary.

1.2.2.3. Hormonal treatment post-gonadectomy

Some centres recommend lowering the dose of oestrogens to half the pre-surgical levels (Futterweit, 1998; van Kesteren *et al*., 1997). Alternatively, other centres apply a constant hormonal dose before and after surgery (Michel *et al*., 2001). The rationale for continuing oestrogen included maintenance of female features and bone mineral density. After bilateral oophorectomy androgen therapy must be continued to maintain virilisation and prevent osteoporosis. Suppression of the serum LH concentration to within the normal range is sometimes used to indicate the adequacy of androgen administration (van Kesteren *et al*., 1998).

1.2.2.4. Risks of cross-sex hormone treatment

The comparatively small numbers of transsexual persons and the inevitable lack of placebo-controlled prospective trials complicate the distinction between the risks of gender dysphoria itself (Kenagy, 2002; van Kesteren *et al*., 1997) and the risks attributable to cross-sex hormone treatment. In a retrospective, descriptive study of 816 male-to-female transsexuals and 293 female-to-male transsexuals (total exposure 10.152 patient years) there was no increase in mortality overall or mortality that could be attributed to cross-sex hormone treatment compared to an age and gender adjusted general Dutch population (van Kesteren *et al*., 1997). In male-to-female transsexual persons an increased risk of suicide and HIV-infection was reported but no change or a slight reduction in malignancy risk, myocardial infarction, chronic obstructive pulmonary disease, and pancreatitis. Morbidity risks in this group related to hepatitis B and cholelithiasis, a possible increase in risk of stroke and no detected change in risk of hypertension or prostatic carcinoma. As only two deaths were observed in the female-to-male transsexual group relative mortality resulting from male hormone treatment in the genetic females remains unclear. In an earlier retrospective study of 303 male-to-female transsexuals treated with cross-sex hormones a five times higher death rate due to increased numbers of suicide and death of unknown course was reported, compared to a reference population (Asscheman *et al*., 1989). Depressive mood changes were 15 times more common and thrombo-embolic events were increased 45-fold compared to general population. Androgen treatment in 122 female-to-male transsexuals, in the same study, was associated with weight increase and acne. In both groups it was not clear whether persistent liver enzyme abnormalities should be attributed to hepatitis B and alcohol abuse rather than the effects of cross sex steroids. However, much of the morbidity was minor (see also **Chapter 6**) and reversible with appropriate therapy or temporary discontinuation of hormone treatment. Polycythaemia was reported a rare complication in female-to-male transsexual persons.

Venous thrombo-embolism is the most significant cross-sex hormone treatment risk. The studies above report a highly significant excess in absolute risk in a much younger population, compared to post-menopausal women, who may in addition be subject to the additional risk of major pelvic surgical procedures. Venous thrombo-embolism mainly occurs in the first year of oestrogen administration and predominantly in subjects over 40 years of age. In vitro studies show that the thrombogenic effect is typical of ethinyloestradiol but not of oral 17-beta oestradiol valerate or transdermal oestrogens (Toorians *et al*., 2003). The risk should be considered against the potential hazards of inadequately treated gender dysphoria, and morbidity associated with sex hormone deficiency, such as hot flushes and osteoporosis. Because immobilisation is

also a risk factor for venous thrombo-embolic events, oral oestrogen administration should be discontinued three to four weeks before elective surgical interventions. Once subjects are fully mobilised again oral oestrogen therapy may be restarted. Smoking cessation, weight reduction, exercise and appropriate diet are critical elements for preventive health in transsexual persons. Minimising the dose of oestrogens, especially in older persons and those with co-morbidities is of critical importance.

Self-examination of the breast must be part of the monitoring of oestrogen administration, following the same guidelines that exist for other women. There are case reports (n = 5) of male-to-female transsexuals who were found to have breast carcinomas while receiving oestrogen treatment (Ganly & Taylor, 1995; Grabellus *et al*., 2005; Pritchard *et al*., 1988; Symmers, 1968). No cases of breast carcinoma were found in the Amsterdam population.

Oestrogen induced prolactinomas have been reported in male-to-female transsexuals after self-administration of excessive doses of oestrogens (Gooren *et al*., 1988), however, a prolactinoma in a male-to-female transsexual person treated with more conventional oestrogen doses has also been described (Bunck *et al*., 2004). Nevertheless, causality between oestrogen administration and the development of a lactotroph adenoma has not been established.

Transsexual persons who wish transition to their desired gender have to undergo hormonal and surgical treatment, which lead to irreversible loss of their reproductive potential. Male-to- female transsexual persons who have no children may be given the option to store spermatozoa before they start hormonal treatment, so that their gametes can be used in future relationships. There is an argument that the same options should be available as to any other patient who risks losing their sperm cells (De Sutter, 2001; De Sutter, 2003; Lubbert *et al*., 1992; Schulze, 1988). The chance of a successful pregnancy resulting from the use of unfertilised ova is believed to be very low. Preliminary studies have shown that long term androgen treatment does not compromise follicular survival in cryopreserved ovarian cortical tissue. Cryopreserved primordial follicles from young female-to-male transsexual persons could be used for later procreation (Van Den Broecke *et al*., 2001-05).

Because of a lack of gonadotropin stimulation, the testes become atrophic and may enter the inguinal canal, which may cause discomfort

Limited case reports of prostate cancer in male-to-female transsexual individuals taking oestrogen can be found in the literature (Thurston, 1994; Van Haarst *et al*., 1998; van Kesteren *et al*., 1996a). Possibly these cancers were present before oestrogen administration and became hormone independent. Transient dribbling following micturition may be caused by atrophy of the prostate.

Liver abnormalities require careful evaluation for rare complications such as hepatic adenomas, cysts, and hepatocellular carcinoma, similar to those found rarely in some women using contraceptive steroids.

1.2.2.5. Contra-indications for cross-sex hormone treatment

Hormonal treatment is contra-indicated in certain situations. A strong family history of breast cancer or the presence of a prolactinoma may be contra-indications to oestrogen therapy. Lipid disorders accompanied by cardiovascular complications may be a contra-indication for androgen use. Severe diastolic hypertension, significant cardiac dysfunction or ischemic cardiac episodes, thrombophlebitis or thrombo-embolic disease, cerebrovascular disease, hepatic dys-

function, brittle or poorly controlled diabetes mellitus and marked obesity are contra-indications against the use of higher doses of either sex steroid (Futterweit, 1998).

Risk-benefit ratios should be considered collaboratively by the patient and the prescribing physician (Michel *et al*., 2002). The usual transsexual person is mostly a rather young and healthy person and, therefore there are rarely absolute or relative contra-indications against cross-sex hormone administration.

1.2.2.6. Post-transition follow-up

Post-operative patients may sometimes exclude themselves from follow-up by the physician prescribing hormones, not recognizing that these physicians are best able to prevent, diagnose and treat possible long term medical conditions that are unique to hormonally and surgically treated patients. Post-operative patients should undergo regular medical screening according to recommended guidelines for age. Close monitoring and yearly re-evaluation of treatment are also important to minimize the adverse effects while maximizing the benefits. After reassignment surgery, including orchiectomy, hormone treatment must be continued. Continuous oestrogen therapy is required to avoid symptoms of hormone deprivation and, most importantly, to prevent osteoporosis. After bilateral oophorectomy, androgen therapy must be continued to maintain virilisation and prevent osteoporosis (van Kesteren *et al*., 1998). Continued contact with the initial psychiatrist or psychologist is advisable.

1.2.2.7. Surgical sex reassignment

The procedures differ depending upon the direction of the sex change (Monstrey *et al*., 2001).

Male-to-female:

A neo-vagina is surgically constructed, usually using the penile skin for vaginal lining and scrotal skin for the labia. If breast development is judged to be insufficient, the breasts may be surgically augmented.

Female-to-male:

The breasts, uterus and ovaries are surgically removed. In rare cases the hypertrophied clitoris may serve as a phallus. In other cases a so-called metadoioplasty may be performed (Hage *et al*., 1993). Free flaps transposed from arms or legs can be used to construct a neophallus. These surgical interventions allow the person to urinate standing. From the labiae majorae a scrotum can be constructed in which testicular prostheses can be implanted. An erection prosthesis can be optional (Hoebeke *et al*., 2003). Sex reassignment surgery can cause minor changes in urinary habits (Hoebeke *et al*., 2005). The quality of surgical construction of the genitalia is crucial for all transsexuals to permit them to adopt credibly the role of a member of the new sex.

1.2.3. Specific Biological Effects of Cross Sex Hormone Treatment

Optimal hormone treatment for transsexual persons is guided by the development of the desired mental changes and by the onset and maintenance of a physical state that the transsexual person finds acceptable. Induction of breast formation, a more female fat distribution, elimination of sexual hair growth are desired by male-to-female transsexual persons, while a complete stop of menses, a male pattern of sexual hair and increased muscle mass are the goal of treatment in female-to-male transsexuals. There are a variety of consequences of hormonal therapy:

1.2.3.1. Bone

No differences in bone volume or surface, trabecular thickness, osteoclast numbers or eroded surface between transsexuals and controls was seen in histomorphometric studies of trans- ilial bone biopsies from 23 male-to-female transsexual persons (mean age 38 year) following 8-10 months treatment with ethinyloestradiol 100 μg daily and cyproterone acetate 100 mg daily (Lips *et al.*, 1989). Bone mineral density loss induced by testosterone deprivation was prevented in 20 male-to-female transsexual persons exposed to cross-sex hormones for 28 to 63 months (ethinyloestradiol 100 μg daily + cyproterone actetate 100 mg daily; 50 to 100 μg oestradiol without cyproterone after gonadectomy) (van Kesteren *et al.*, 1998). Other smaller studies (Reutrakul *et al*., 1998; Schlatterer *et al*., 1998) confirmed these results, suggesting that bone mineral density is maintained by oestrogen treatment for two years, compared to untreated healthy adult male controls.

In a recent study of 50 female-to-male transsexual individuals a prospective observation over a two-year period was performed. A significant increase in mean BMD of 7.8 % at the femoral neck and a non-significant increase in mean BMD of 3.1 % at the spine over two years was reported (Turner *et al*., 2004).

Intact trabecular bone structure and increased cortical thickness with low bone turnover indices in a bone histomorphometric study of 15 female-to-male transsexuals (mean age 30 years who had undergone hysterectomy and bilateral ovariectomy) compared to 11 healthy men and 8 post-menopausal women was reported (Lips *et al*., 1996). These 15 female-to-male transsexual persons had been treated for an average of 39 months with 250 mg testosterone i.m. every two weeks. The absence of adverse events of long term cross sex hormone treatment after native sex hormone reduction was confirmed in a study of 10 female-to-male transsexuals (Schlatterer *et al.*, 1998). However, a study of 35 female-to-male transsexuals treated for one year (19 were followed-up for 28 to 62 months) suggested that testosterone was unable to prevent bone mineral loss associated with decline in oestrogen levels (van Kesteren *et al*., 1996).

The numbers studied are generally small and individual changes in bone mineral density are highly variable, creating the need for more long-term data on osteoporosis risk in the female-to-male transsexual group. An inverse relationship between serum LH concentrations and bone mineral density has been described in these subjects (van Kesteren *et al*., 1998).

1.2.3.2. Muscle mass and strength

Recent information shows convincingly that levels of circulating testosterone determine

largely muscle mass and strength, with considerable individual diversity. In a study with 19 male-to-female transsexuals and 17 female-to-male transsexuals, androgen deprivation in biological males decreased muscle mass, with maintenance of still significantly higher mean muscle mass compared to female-to-male persons. Androgen administration in female-to-male transsexuals increased muscle mass, without inducing an advantage over non-treated male-to-females (Gooren & Bunck, 2004). It has been shown by the same group that in 20 male-to-female transsexuals treatment with ethinyloestradiol induced a significant decrease in thigh muscle area. Testosterone administration in female-to-male transsexual persons markedly increased thigh muscle area (Elbers *et al*., 1999). Limited research on the topic, and the wish to avoid discrimination, has led to the decision of the International Olympic Committee, November 2003, that transsexual athletes who have undergone a sex-change operation are now allowed to compete in the Olympic games. This was an important decision improving the social status of the transsexual athlete.

1.2.3.3. Adipose tissue

A significant increase in subcutaneous and visceral fat depots and a decrease in thigh muscle area was described by magnetic resonance imaging (MRI) analysis of regional fat deposition in 20 male-to-female transsexuals studied before and one year after cross-sex hormone treatment (Elbers *et al*., 1999). In 17 female-to-male transsexuals a transient reduction in subcutaneous fat, but an increase in visceral fat was seen. Conversely, increased fat around the upper thighs in male-to-female transsexual persons may not be sufficient to induce a true female habitus.

Cross-sex hormone administration induced a reversal of the sex difference in serum leptin levels (Elbers *et al*., 1997). Preliminary data from a study with 8 female-to-male transsexual persons showed that testosterone exerts suppressive effects on adiponectin secretion (Armillotta *et al*., 2005).

1.2.3.4. Haematopoiesis

In female-to-male transsexuals, androgen treatment consistently induces an increase in the haematocrit (Michel *et al*., 2001). Men have higher haematocrit and haemoglobin concentrations than women (Morris *et al*., 1956), in all likelihood due to the higher plasma testosterone levels. Hypogonadal men have lower than normal haematocrit and haemoglobin concentrations and testosterone treatment increases those variables to normal (Jockenhovel *et al*., 1997; Tenover, 1992). The observation that in hypogonadal men, within 3 months, administration of transdermal testosterone dramatically increases haematocrit from mildly anaemic to midnormal (Snyder *et al*., 2000) supports the concept of a role for testosterone. Also, changes in circulating testosterone concentrations induced by GnRH agonist and testosterone administration in healthy young men are associated with testosterone dose- and concentration-dependent changes in haemoglobin (Bhasin *et al*., 2001).

It has been postulated that the administration of androgens to man and laboratory animals results in an increase in plasma erythropoietin activity. However, it has also been shown in recent years that there is no increase in erythropoietin levels in androgen-induced erythrocytosis (Dickerman *et al*., 1998- 99). Induction of androgen deprivation with a luteinizing hormone-

releasing factor (LHRH) agonist did not result in changes in serum erythropoietin levels (Weber *et al*., 1991). Even though there is a significant difference in their testosterone and haemoglobin levels, there is, interestingly, no difference in serum erythropoietin levels between men and women (Miller *et al*., 1985). So, it seems that the androgen-mediated increases in haemoglobin levels and haematocrit are not exclusively mediated by erythropoietin, and testosterone may have a direct effect on bone marrow stem cells (Krabbe *et al*., 1978; Krauss *et al*., 1991; Mooradian *et al*., 1987; Shahidi, 1973).

Iron transport in the plasma is carried out by transferrin, an 85-kDa protein with two iron-binding sites. Uptake of iron by cells is mediated by a membrane-associated transferrin receptor (TfR), a glycoprotein (fig. 2.1.). The amount of transferrin receptor expressed on a cell is proportional to the cell's need for iron. A proteolytic product of the transferrin receptor circulates in plasma as soluble transferrin receptor (sTfR) (see also **Chapter 5**). The concentration of circulating sTfR is proportional to the total concentration of cellular TfR. Since most cellular iron utilization is by erythroid precursor cells –mostly erythroblasts and to a lesser extent reticulocytes- circulating sTfR is proportional to erythroid precursor mass (i.e., rate of erythropoiesis), and it is elevated in iron deficiency, when cells must be more competitive for obtaining their iron requirement. The mechanism of iron uptake by TfR involves a selective affinity of the receptor for diferric transferrin. The TfR-transferrin complex is internalized and cycled through endosomes, where the iron is released to the cytosol, with the TfR-apotransferrin complex returning to the extracellular surface. At the surface the apotransferrin dissociates to be replaced by diferric transferrin, repeating the cycle. A low concentration of intracellular iron leads to increased expression of TfR (Ponka & Lok, 1999).

Transferrin receptor is a transmembrane, disulfide-linked dimer of identical 85-kDa polypeptides (McLelland *et al*., 1984). The two polypeptide chains are covalently linked by disulfide bonds at residues 89 and 98, immediately extracellular of the membrane. Just distal to the second disulfide bond, an Arg-Leu bond is susceptible to proteolysis to yield a short transmembrane protein with only 11 residues extending from the outside of the membrane plus a 660-amino-acid protein, sTfR, a soluble truncated monomer, that circulates in plasma (Shih *et al*., 1990) (fig. 2.2.). The level of circulating sTfR is proportional to the amount of total cellular TfR (Beguin *et al*., 1988).

Eighty percent of metabolic iron is for the synthesis of haemoglobin by erythroid precursors, (Brock *et al*., 1994) and as a consequence, 80% of total TfR is on erythroid precursor cells. Thus, total TfR is roughly proportional to the erythroid precursor mass (i.e., the rate of erythropoiesis). In addition to diagnosis of iron deficiency, serum sTfR has been used to monitor the rate of erythropoiesis (Beguin *et al*., 1995).

In conclusion, marrow erythropoietic activity appears to be the most important determinant of sTfR levels (R'Zik & Beguin, 2001). In situations characterized by diminished erythropoietic activity soluble TfR levels are decreased. When erythropoiesis is stimulated soluble TfR is increased. Measurements of sTfR are useful to monitor the erythropoietic response to various forms of therapy. Predicting an early therapeutic response by measuring sTfR is possible when changes in haemoglobin are not yet apparent (Beguin, 2003).

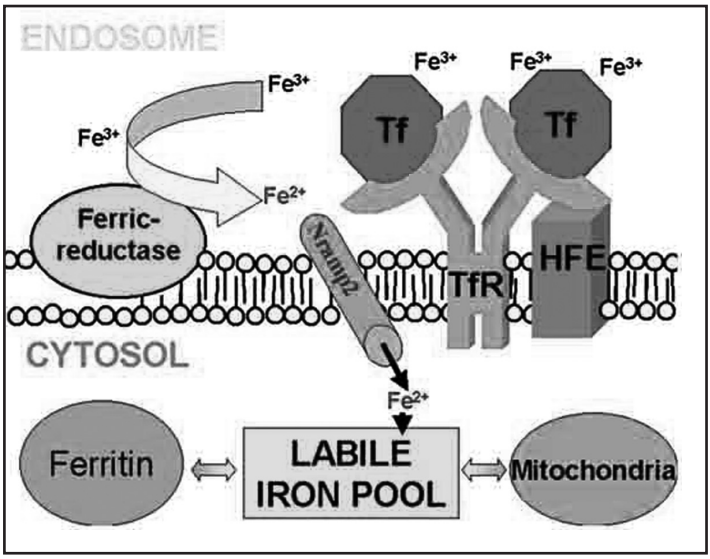

Fig 2.1. Cellular iron uptake from transferrin. Ferric iron (Fe^{3+}), bound to transferrin (Tf), is released in the late endosomal compartment, following binding of Tf to its receptor (TfR). Its membrane association with a protein 'HFE' negatively regulates TfR binding of Tf. Fe^{3+} is converted to ferrous iron (Fe^{2+}) by an endosomal membrane-bound ferric-reductase and is then transported into the cytosol via natural resistance-associated macrophage protein 2 (Nramp2), which is a divalent metal ion transporter. Fe^{2+} enters the cytosolic labile iron pool and is then shunted either into storage in cytosolic ferritin, or to the mitochondria.

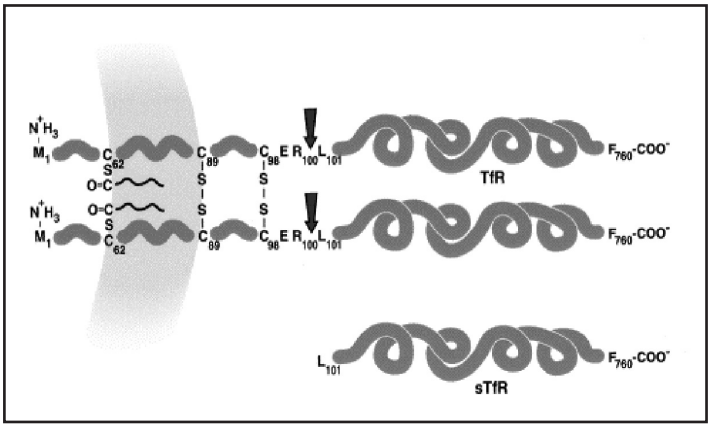

Fig. 2.2. Diagrammatic representation of the human TfR molecule showing the change leading to sTfR. (C, cysteine; E, glutamate; L, leucine; M, methionine; F, phenylalanine; R, arginine). Arrows indicate the site of proteolytic cleavage.

1.2.3.5. Breast

After the start of female sex hormone treatment in male-to-female transsexual individuals, increase in breast size usually begins 2-3 months after the start of female sex hormone treatment, and continues for two years (Meyer *et al*., 1986). Androgen administration may decrease glandular activity of the breasts, but does not reduce their size.

1.2.3.6. Prolactin

Modest increases in prolactin are thought to be universal in oestrogen and cyproterone treated male-to-female transsexuals (Asscheman *et al*., 1988). Variable increases in circulating prolactin but no specific risk of inducing autonomous prolactin secretion was found in a study of 142 male-to-female transsexuals treated with oestrogen and cyproterone acetate for six months to nine years (Gooren *et al*., 1985).

1.2.3.7. Ovary

Ovaries of female-to-male transsexual individuals taking androgens show similarities with polycystic ovaries (Amirikia *et al*., 1986). Data suggest that increased blood levels and presumably increased ovarian concentrations of testosterone may produce the morphological features of polycystic ovarian disease (Futterweit & Deligdisch, 1986). It was concluded that exogenous androgen can thicken the tunica albuginea and basal membrane and that these histologic changes are similar to those seen in PCO-ovaries under excess of endogenous androgen production. Although the association between polycystic ovarian disease and the risk of endometrial and ovarial malignancy is still debated (Balen, 2001), in our genderteam it has been the policy to perform hysterectomy and bilateral salpingoophorectomy, generally carried out around six to twelve months after the start of testosterone treatment.

1.2.3.8. Testis

Little information is available on the effects of oestrogen treatment on semen quality. Morphological changes in human testicular tissue following prolonged oestrogen administration have been described: narrow seminiferous cords surrounded by an extensively thickened lamina propria, containing Sertoli cells and spermatogonia with exclusively characteristic features of pale type-A spermatogonia; Sertoli cells have transformed into immature cells, resembling precursors prior to puberty and no typical Leydig cells can be found (Schulze, 1988, Thiagaraj *et al*., 1987). In a single patient study the time- and dose-dependent effects of ethinyl oestradiol on semen quality has been described (Lubbert *et al*., 1992).

1.2.3.9. Clitoris

To a variable extent, the desired clitoral enlargement occurs in all female-to-male transsexuals. A mean clitoral length of around 4 to 4.5 cm is achieved one year after the start of treatment with testosterone (200 mg every two weeks, i.m.) (Meyer *et al*., 1981). Metaidoioplasty uses the clitoris, overdeveloped by hormonal treatment, to construct a microphallus (Hage, 1996)

1.2.3.10. Penis

In a study of 5 male-to-female transsexual persons chronically (at least two years) treated with oestrogens and cyproterone acetate undergoing sex reassignment surgery, the corpora cavernosa were studied following phallectomy. PDE-5 (phosphodiesterase type 5) mRNA and

functional activity were significantly reduced (Morelli *et al*., 2004).

1.2.3.11. Liver

In approximately 3 % of male-to-female transsexuals treated with oestrogens a transient elevation of liver enzymes can be found (Van Kesteren *et al*., 1997). Metabolic effects of oral and transdermal oestrogens may differ implicating hepatic mechanisms: approximately 60 % of orally administered ethinyloestradiol is inactivated by the liver via a first-pass effect through the entero-hepatic circulation. Orally administered ethinyloestradiol (with a strong hepatic impact) compared to transdermally administered 17-beta-oestradiol (with less hepatic effect) may produce different effects on the somatotropic axis. It is known that in post-menopausal woman oral ethinyloestradiol has a strong hepatic effect with impaired hepatic IGF-1 production that causes increased growth hormone secretion through reduced feed-back inhibition (Ho *et al*., 2003). This was earlier also shown in the study of van Kesteren *et al*. in transsexuals (1996).

Combined treatment with oral ethinyloestradiol and cyproterone acetate but not with transdermal 17-beta oestradiol and CA lowered plasma tissue-type plasminogen activator (tPA) because of increase in hepatic tPA clearance, leaving endothelial tPA synthesis unchanged (Giltay *et al*., 2000).

1.2.3.12. Insulin sensitivity and cardiovascular risk factors

The effects of cross-sex hormone treatment in healthy young transsexual subjects did not show that female sex steroids given in large amounts to male subjects, have beneficial effects on cardiovascular profile, or that high dose testosterone administration to female subjects is detrimental with respect to cardiovascular risk (Elbers *et al*., 2003). In another experiment it was shown that short term administration of oestrogens and anti-androgens increase femoral and brachial artery stiffness in men, and that fasting insulin levels are a stronger determinant of arterial stiffness in women than in men (Giltay, 1999). An experiment using a hyperinsulinemic-euglycaemic clamp before and after four months of hormone administration showed that testosterone treatment in females and ethinyloestradiol treatment in males can induce insulin resistance in healthy subjects (Polderman *et al*., 1994).

Plasma levels of homocysteine, an independent cardiovascular and cerebrovascular risk factor, are reduced in hormone treated male-to-female transsexual individuals and increased in androgen treated female-to-male transsexuals (Giltay *et al*., 1998).

It has been shown that testosterone treatment induces a decrease in serum HDL-cholesterol levels after three months of treatment in female-to- male transsexuals (Asscheman *et al*., 1994; Goh *et al*., 1995). Exogenous oestrogens administered in male-to-female transsexuals result in a female pattern of lipid-lipoprotein-cholesterol (Damewood *et al*., 1989). In the study by Elbers *et al*. (2003), oestrogens and anti-androgens increased HDL-cholesterol and decreased LDL-cholesterol. Nonetheless, this combination also increased triglycerides, blood pressure, subcutaneous fat and visceral fat and decreased the LDL-particle size, and insulin sensitivity. Testosteron treatment induced lower HDL-cholesterol levels, smaller LDL-particle size and elevated triglyceride levels and had neutral effects on blood pressure and insulin sensitivity, but created an increased ratio between visceral and subcutaneous abdominal fat.

There was no relationship shown between androgen-induced male-pattern baldness and indicators of increased coronary heart disease risk (Giltay *et al*., 2004).

1.2.3.13. Hair

Administration of oestrogens and anti-androgens affects length and diameter of hair at different rates. After four months the decrease in shaft diameter has reached its maximum and

does not progress further. Skin sebum production falls rapidly to almost undetectable levels. Androgen treatment in female-to-male transsexuals results in the induction of facial hair growth and increase sebum production and continues to develop beyond one year (Giltay & Gooren, 2000). Male-to-female hair reduction is invariably inadequate with hormonal treatment alone. Laser hair removal or electrolysis are necessary (facial hair). Sexual hair growth on other parts of the body responds more favourably. Effective treatment of genital hair is also required prior to penoscrotal inversion vaginoplasty.

1.2.3.14. Brain

The initiation of hormonal therapy by itself has a calming effect on most patients (Futterweit, 1998). There is evidence that cross-sex hormones directly and quickly affect the gender specific behaviours. The administration of androgens to females was clearly associated with increasing aggression, sexual arousability and spatial ability performance; it had a deteriorating effect on verbal fluency tasks. The effects of cross sex hormones were just as pronounced in the male-to-female group: upon androgen deprivation and oestrogen treatment anger and aggression proneness, sexual arousability and spatial ability decreased whereas verbal fluency improved (Van Goozen *et al*., 1994 -95). In contrast, a later study described that testosterone had an enhancing, and not quickly reversible effect on spatial ability performance but no deteriorating effect on verbal fluency in female-to-male transsexual individuals. In contrast also, anti-androgen treatment in combination with oestrogen therapy did not decrease spatial ability, neither enhanced verbal fluency in male-to-female transsexuals (Slabbekoorn *et al*., 1999).

New research involving male-to-female transsexuals lends further credence to the theory that sex hormones are involved in migraine generation (Pringsheim & Gooren, 2004).

1.2.4. Regrets of Sex Reassignment

Prospective controlled studies specifically designed to assess outcome and its prognostic factors are difficult because of the low prevalence of this condition. It is estimated that one to two percent will have regrets (Pfäfflin, 1992). A major obstacle in conducting follow-up studies is that it is very difficult to find transsexuals post-surgery and even more difficult to find persons who will accept reassessment (Hunt & Hampson, 1980; Kuiper & Cohen-Kettenis, 1988; Stein *et al*., 1990; Stürup, 1976). The participants in follow-up studies do probably not constitute a representative sample of the population of transsexuals. In a great majority of cases transsexuals are satisfied with their transformation with about 10 % of subjects being unsatisfied (1 to 2 % regrets and 10 % temporary dissatisfaction). Limited social skills or their appearance, or the quality of surgical construction of the genitalia are important factors. A lower percentage of dissatisfaction is found in female-to-male transsexual individuals (Blanchard *et al*., 1989; Green & Fleming, 1990; Lawrence, 2003; Lindemalm *et al*., 1987; Pauly, 1981). Follow-up studies have been carried out to evaluate the effectiveness of sex reassignment (Cohen-Kettenis & Gooren, 1999; Lawrence, 2003). Success percentages in recent studies are 87 % for male-to-female and 97 % for female-to-male transsexual individuals, but outcomes differ between studies due to study design and methodology. The post-operative regrets to the point of a second role reversal is estimated to be 1 to 2 %.

From studies to adult transsexuals it seems that psychopathology, poor social support and physical appearance may be the pre-treatment factors that are most likely associated with poor post-operative functioning. Quality of the surgical results is an important post-operative factor. Studies show that there are a little more than 1 % of suicides among post-operative transsexuals (Michel *et al*., 2002).

1.2.5. Juvenile Gender Dysphoria

Sex reassignment for individuals with extreme gender identity disorder (GID) has long been restricted to adults. Prospective studies have shown that most GID-children under 12 will not grow up to become transsexuals (Cohen-Kettenis, *et al*. 2000). Homosexuality will be more often the outcome; only about 20 % will pursue hormonal and surgical treatment beyond adolescence (Cohen-Kettenis, 2001). Because of this, hormonal or any other medical intervention is never considered in pre-pubertal children. However, for some adolescents applying for sex reassignment, medical interventions may be a treatment option. The delay of such treatment until after the development of secondary sex characteristics has obvious drawbacks for transsexual individuals (Cohen-Kettenis & van Goozen, 1998). Suppressing spontaneous puberty (with development of the unwanted sex characteristics) gives these adolescents an advantage in later life because they are able to pass effortless as someone of the desired sex. This treatment, however, is not without criticism. It is argued that the adolescent should experience some of his – her own puberty. Without such experience they would never be able to really appreciate the effects of their own puberty and perhaps be at greater risk for post-treatment regret. It is advised to start suppression of endogenous puberty, if in an experts' opinion a child's cross sex gender identity will not change during long term follow-up, by treatment with depot-forms of GnRH analogues, following the regimen in children with precocious puberty. Pubertal development will be delayed until an age that a balanced and responsible decision can be made to transition to the other sex (Gooren, 2004).

The introduction on 'Transsexualism' was partly based upon L. Gooren's 'Transsexualism: Biologic considerations; definition; and diagnosis' and 'Treatment of transsexualism'. UpTo Date online 13.2.

1.2.6. References

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PART I

CHAPTER 2

COMPARATIVE ASSESSMENT IN YOUNG AND ELDERLY MEN OF THE GONADOTROPIN RESPONSE TO AROMATASE INHIBITION.

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Comparative Assessment in Young and Elderly Men of the Gonadotropin Response to Aromatase Inhibition

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Context: Aging in men is associated with a decline in serum testosterone (T) levels.

Objective: Our objective was to assess whether decreased T in aging might result from increased estradiol (E_2) negative feedback on gonadotropin secretion.

Design and Setting: We conducted a comparative intervention study (2004) in the Outpatient Endocrinology Clinic, Ghent University Hospital.

Participants: Participants included healthy young and elderly men (n = 10 *vs.* 10).

Interventions: We used placebo and letrozole (2.5 mg/d) for 28 d, separated by 2 wk washout.

Main Outcome Measures: We assessed changes in serum levels of free E_2, LH, and FSH, free T, SHBG, and gonadotropins response to an iv 2.5-μg GnRH bolus.

Results: As assessed after 28 d of treatment, letrozole lowered E_2 by 46% in the young men ($P = 0.002$) and 62% in the elderly men ($P < 0.001$). In both age groups, letrozole, but not placebo, significantly increased LH levels (339 and 323% in the young and the elderly, respectively) and T (146 and 99%, respectively) (P value of young *vs.* elderly was not significant). Under letrozole, peak LH response to GnRH was 152 and 52% increase from baseline in young and older men, respectively ($P = 0.01$).

Conclusions: Aromatase inhibition markedly increased basal LH and T levels and the LH response to GnRH in both young and elderly men. The observation of similar to greater LH responses in the young compared with the elderly does not support the hypothesis that increased restraining of LH secretion by endogenous estrogens is instrumental in age-related decline of Leydig cell function. (***J Clin Endocrinol Metab* 90: 5717–5722, 2005**)

AGING IN MEN is accompanied by a gradual decline in androgens that becomes more apparent after the age of 50 yr. Between the ages of 25 and 75 a modest decline of mean serum testosterone (T) levels up to 20–30% can be seen. The fall of the biologically active free T (FT) and non-SHBG-bound, or so-called bioavailable, T in serum is, however, of greater magnitude, with a reduction by 50% over the same age range (1–7). The decline in T production is underlaid by testicular changes and altered neuroendocrine regulation of LH secretion (8) with blunted circadian rhythms (9) and increased responsiveness to sex steroid hormone feedback compared with young men (10–12). Estrogens contribute substantially to the negative feedback regulation of gonadotropin secretion (13, 14). As a result of increasing aromatase activity with age and the age-associated increase in fat mass (15, 16), the decrease in T levels is not paralleled by a similar decline of plasma estradiol (E_2) levels (4, 15), with a consequent age-related decrease of the plasma ratio of the T over E_2 levels. Pharmacological inhibition of aromatase activity results in increased levels of gonadotropin and T levels, both in young and elderly men (13, 17, 18).

In the present clinical study, the hypothesis is tested that decreased T in aging men might result from increased E_2 negative feedback. To this end, we compared in young and elderly men the effect on gonadotropin and T secretion of aromatase inhibition by administration of letrozole, a specific and potent fourth-generation aromatase inhibitor. Letrozole, currently indicated as a treatment for breast cancer, reduces systemic E_2 concentration in males by 30–50% (19). The premise was that if increased E_2 negative feedback were instrumental in the age-related decline of T levels, aromatase inhibition would result in a greater gonadotropin response in elderly men compared with young men.

Subjects and Methods

Subjects

Ten healthy young men [mean age, 25.9 ± 4.6 yr (range, 20–33 yr); mean body mass index (BMI), 24.2 ± 2.9 kg/m^2 (range, 19.4–28.1)] and 10 elderly men [mean age, 76.1 ± 5.0 yr (range, 68–81 yr); mean BMI, 24.5 ± 2.6 kg/m^2 (range, 18.8–27.5)] gave their written informed consent to participate in this study, which was conducted according to the principles of the Declaration of Helsinki and approved by the Ethical Review Board of the University Hospital Ghent. Medical history, physical examination (male habitus, virilization, and testis size), biochemical measures of hematological, hepatic, renal, and metabolic function and fasting concentrations of T_4, TSH, T, E_2, prolactin, LH, and FSH were within the normal range at screening. Exclusion criteria included active

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Abbreviations: E_1, Estrone; E_2, estradiol; FE_2, free E_2; FT, free T; T, testosterone.

smoking, excessive alcohol or substance abuse, major psychiatric disease and dementia, exposure to psychotropic or neuroactive drugs, use of glucocorticoids or sex hormones, history of sleep apnea, significant cardiopulmonary disease, recent weight loss or gain, transmeridian travel, shift work, untreated prostatic disease or prostate-specific antigen greater than 4 ng/ml, and unwillingness to provide written informed consent.

Study design

Figure 1 depicts the overall design of the study. The study design was a randomized, double-blind, placebo-controlled crossover intervention. Patients were first screened on d 14 before participation in the protocol. Placebo or letrozole (2.5 mg daily, Femara; Novartis AG, Stein, Switzerland) orally taken on awakening were administered in random order each day for a 28-d period separated by a 14-d treatment-free washout period (start of treatment on d 1 and 43, respectively). In both age cohorts, the same number of subjects (n = 5) started with the aromatase inhibitor and placebo, respectively. Blood sampling in the first phase was at d 1 (before dosing) and d 28 and in the second phase at d 43 (before dosing) and d 70. At the conclusion of the visits on d 1 and 43, sufficient letrozole or placebo was provided to last until the following visit 4 wk later; compliance was assessed by pill counting.

Sampling procedure

An iv catheter was placed in the antecubital vein of supine subjects after an overnight fast and 10 min of bed rest between 0800 and 1000 h. Blood samples for assay of serum T, E_2, SHBG, LH, and FSH were withdrawn at 0 and 20 min. At d 28 and 70, an iv 2.5-μg GnRH (Relefact, Aventis Pharma BV, Hoevelaken, The Netherlands) bolus was injected. Earlier studies of iv bolus injection of GnRH in small physiological doses have shown consistent responsiveness of the gonadotropins to this dose in both young and elderly men (20). Blood samples for serum LH and FSH determination were taken via the iv indwelling catheter at 40, 20, and 0 min before (mean of the three samples taken as baseline for calculation of response to GnRH) as well as at 10, 20, and 40 min after the bolus injection.

Hormone assays

Serum was stored at −80 C until assay; all samples from the same subject were assayed in a single assay run. Commercial immunoassays were used to determine the serum concentrations of E_2 (Incstar, Stillwater, MN) (adapted protocol with use of double amount of serum), estrone (E_1) (Bio Line, Brussels, Belgium), T and SHBG (Orion Diagnostica, Espoo, Finland), and LH and FSH (Elecsys LH and FSH immunoassay; Roche, Mannheim, Germany). Intra- and interassay coefficients of variation for the E_2 assay were 3 and 9% with a detection limit of 2 pg/ml, respectively; intra- and interassay coefficients of variation for all other assays were less than 10 and 15%, respectively. The T/E_2 ratio was used as an indirect indicator of aromatase activity. Serum FT and free E_2 (FE_2) were calculated from the total serum hormone concentrations, serum SHBG, and serum albumin using a validated equation derived from the mass action law (21, 22). For all considered hormonal variables, basal values for each sampling day are the mean of the result for two samples obtained at a 20-min interval.

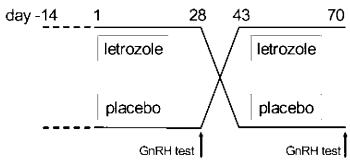

FIG. 1. Schematic representation of the study design. After the baseline visit, both young (n = 10) and elderly (n = 10) subjects were randomized into one of two groups, beginning with either placebo or letrozole for 28 d, followed by the alternative treatment after a 2-wk washout.

Data analysis

The primary end points of this study were between-age group differences in changes of biochemical hormonal values. Baseline characteristics of the groups were compared according to Student's *t* test. LH and FSH were ln-transformed to meet the model requirements. To examine the absolute changes from baseline for each hormone, the statistical significance of the Pearson correlation coefficients was evaluated using a Student's *t* test. Data are expressed as mean ± SD. A level of 0.05 was used to indicate statistical significance. All analyses were done using SPSS software (version 12.0).

Results

Sex steroid levels

As shown in Table 1, at baseline, serum T, E_2, FE_2, LH, and FSH levels did not significantly differ between young and elderly subjects; SHBG levels were higher and FT concentrations lower in the elderly. No differences between subjects in the subgroups starting with placebo or letrozole were observed for either BMI or sex steroid levels. In subjects starting with active treatment, on d 43 at the start of the placebo phase, T concentrations were markedly higher compared with d 1 both in young men ($P = 0.006$) and elderly men ($P < 0.05$) (data not shown). Because of this unanticipated but prominent carryover effect from letrozole administration at d 43, additional results will summarize the data for n = 20 subjects (n = 10 young and n = 10 elderly men) who started with letrozole intake on d 1 or 43, and the results of n = 10 subjects (n = 5 young and n = 5 elderly men) who commenced the study with placebo on d 1. The data for the participants using placebo in the second phase of the study were excluded from statistical analysis.

Letrozole was well tolerated and lowered serum E_2 by 46% in the young men ($P = 0.002$) and by 62% in the elderly men ($P < 0.001$) (Table 2) and comparably over the two study periods; serum E_1 was lowered by 31% ($P = 0.01$) and by 50% ($P < 0.001$) in the young and the elderly, respectively. The decreases in E_2 and FE_2 were significantly greater in elderly men compared with the young ($P = 0.03$ and $P = 0.02$, respectively). LH, FSH, and FT concentrations increased significantly in young and elderly men, with a mean increase of serum LH, FSH, and T of 339, 204, and 146% (SD, 72%) in

TABLE 1. Hormonal characteristics of young and elderly men at screening

	Young men (n = 10)	Elderly men (n = 10)	Significance
SHBG (nmol/liter)	23.0 (9.1)	47.9 (12.9)	<0.001
T (ng/dl)	523.8 (157.3)	509.5 (82.3)	0.80
FT (ng/dl)	13.0 (3.3)	8.7 (2.0)	0.003
E_2 (pg/ml)	18.0 (4.4)	20.7 (4.4)	0.20
FE_2 (pg/ml)	0.340 (0.056)	0.342 (0.062)	0.93
E_1 (pg/ml)	32.4 (6.0)	34.9 (8.6)	0.46
LH (IU/liter)[a]	3.70 (1.61)	5.33 (1.79)	0.14
FSH (IU/liter)[a]	4.59 (1.74)	7.44 (2.00)	0.10
T/E_2[b]	2.96 (0.74)	2.52 (0.47)	0.13
FT/FE_2[b]	3.80 (0.57)	2.59 (0.62)	<0.001

All values are the mean (SD) for two samples obtained at a 20-min interval. Means of the two baseline blood samples (0 and 20 min) were analyzed. To convert E_2 to pmol/liter, multiply by 3.676, and to convert T to nmol/liter, multiply by 0.0347. Significance levels are according to Student's *t* test.

[a] For LH and FSH, geometric means are given.

[b] Divided by 100.

TABLE 2. Hormonal values before and after treatment

	Treatment					
	Letrozole			Placebo		
	d 1/42	d 28/70	Significance[a]	d 1	d 28	Significance[a]
Young men	n = 10	n = 10		n = 5	n = 5	
SHBG (nmol/liter)	21.1	17.5	0.003	18.7	19.5	0.61
T (ng/dl)	514.0	1198.8	<0.001	557.4	562.2	0.92
FT (ng/dl)	13.5	37.5	<0.001	14.6	14.8	0.88
E_2 (pg/ml)	18.9	10.2	0.002	17.5	18.9	0.54
FE_2 (pg/ml)	0.362	0.226	0.001	0.360	0.386	0.64
E_1 (pg/ml)	30.3	20.9	0.01	27.2	35.1	0.15
LH (IU/liter)[b]	4.12	16.40	<0.001	3.42	3.98	0.22
FSH (IU/liter)[b]	4.54	12.94	<0.001	3.29	3.43	0.55
T/E_2[c]	2.97	13.24	<0.001	3.13	3.05	0.75
FT/FE_2[c]	3.83	18.72	<0.001	4.04	4.00	0.88
Elderly men	n = 10	n = 10		n = 5	n = 5	
SHBG (nmol/liter)	47.2	40.8	<0.001	48.8	48.0	0.74
T (ng/dl)	512.7	973.5	<0.001	505.2	529.8	0.46
FT (ng/dl)	8.84	21.8	<0.001	8.46	9.06	0.52
E_2 (pg/ml)	20.4	7.8	<0.001	19.8	19.9	0.98
FE_2 (pg/ml)	0.341	0.148	<0.001	0.326	0.330	0.88
E_1 (pg/ml)	32.4	15.9	<0.001	32.2	30.9	0.43
LH (IU/liter)[b]	5.53	22.18	<0.001	5.75	4.72	0.01
FSH (IU/liter)[b]	7.16	19.75	<0.001	6.75	6.86	0.73
T/E_2[c]	2.53	12.83	<0.001	2.55	2.65	0.61
FT/FE_2[c]	2.64	14.81	<0.001	2.59	2.74	0.48

All values are the mean for two samples obtained at a 20-min interval.
[a] According to paired Student's *t* test.
[b] For LH and FSH, geometric means are given.
[c] Divided by 100.

young *vs.* 323, 182, and 99% (SD, 61%), respectively, in elderly men (P value for young *vs.* elderly was not significant). In both the young and elderly group, SHBG levels decreased during letrozole treatment ($P = 0.003$ and <0.001, respectively; P value for young *vs.* elderly was not significant). The ratios T/E_2 and FT/FE_2 were significantly higher after letrozole treatment in both groups ($P < 0.001$). In a multivariate analysis of differences in sex steroid levels and gonadotropins, the treatment effect was shown to be independent of age.

Response to GnRH administration

Under letrozole treatment, the peak LH response to stimulation by administration of 2.5 μg GnRH (after 20 min) was 152 and 52% increase from baseline in young and older men, respectively ($P = 0.01$) (Fig.2); under placebo, the increase was 221% in young men and 140% in elderly men ($P = 0.22$). As for FSH, peak response to GnRH stimulation under letrozole was 30 and 5% increase from baseline in young and older men, respectively ($P = 0.01$); the response under placebo was 13% in young men and 10% in elderly men ($P = 0.42$).

Discussion

In the present comparative study, aromatase inhibition with letrozole during 28 d induced a remarkable increase of gonadotropin and T serum levels both in young and older men. The observed similar response to aromatase inhibition in the young and the elderly men does not support the tested hypothesis that the altered gonadotropin secretion in the elderly (8) and its attendant decline of T production are the result of an increased restraining action of estrogens.

The major increase of gonadotropin levels under aromatase inhibition seen in the present study with letrozole, as well as in previous studies in men with the aromatase inhibitor anastrozole (17, 18, 23), illustrates the important contribution of estrogens to the sex steroid feedback inhibition of gonadotropin secretion in men. The restraining action of estrogens on gonadotropin secretion is also revealed by el-

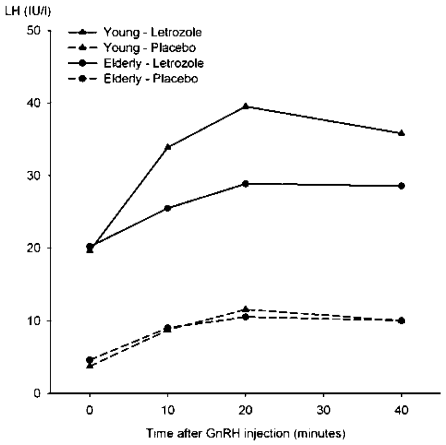

FIG. 2. LH response to stimulation with iv administration of 2.5 μg GnRH; baseline is the average for LH at 40, 20, and 0 min before GnRH injection.

evated gonadotropin levels in men with congenital aromatase deficiency (24–26) or lack of functional estrogen receptor α (27). In agreement with the observations in the present study of stimulation by aromatase inhibition of both basal gonadotropin levels and the response to exogenous GnRH, the restraining action of estrogens on gonadotropin secretion in men has been shown to be exerted both at the pituitary and at the hypothalamic levels (13–14).

Few studies in men are available in which letrozole was chosen as aromatase inhibitor. One study in elderly men made use of letrozole, however, in combination with a long-acting GnRH agonist (28). In studies in early and midpubertal boys, letrozole 2.5 mg has been used in combination with T (29). Short-term letrozole treatment with a daily dose of 2.5 mg or 2.5 mg three times a week in severely obese men with hypogonadotropic hypogonadism normalized serum T levels in all participants (30). The decrease of serum estradiol by a mean of 46% in young men and 62% in elderly men after 28 d of treatment with 2.5 mg letrozole daily in the present study is on the same order of magnitude as previously reported decreases of 50% after 10 wk treatment with 1 mg anastrozole daily in young men (23), of 30% after 9 wk treatment with 2 mg anastrozole daily in eugonadal men over 65 yr (18), and of 40% after 12 wk treatment with a daily dose of 1 mg anastrozole in elderly men with initially low serum T (17). Observed decreases of serum E_1 levels in the present study with letrozole and in previous studies with anastrozole were somewhat greater but still on the same order of magnitude as for E_2. In studies with anastrozole in young and elderly men, treatment induced substantial increases in serum gonadotropin and T levels, but the observed increases were generally smaller than seen in the present study. An increase of serum total T by 58% after 10 wk of 1 mg anastrozole in young men was reported (23) compared with the increase of 146% in the young men in the present study; an increase of up to 50% was seen in elderly men treated for 9 wk with 2 mg anastrozole (18) or with 1 mg anastrozole daily for 12 wk (17) compared with an increase of 99% observed by us in the elderly men. In the present study, treatment with 2.5 mg letrozole daily for 28 d increased serum T levels in elderly men to or above the upper limit of the normal range for young men. This confirms the previous reports (17, 18) indicating that aromatase inhibition in elderly men can increase T serum levels with achieved serum values in the upper normal range for young men.

In accordance with previous observations (17, 18), aromatase inhibition results in a slight but significant decrease of SHBG serum levels. Nevertheless, the marked age-related difference in SHBG concentrations is maintained under aromatase inhibition. As a result of the decrease of SHBG, the increases of the bioavailable, non-SHBG-bound fractions of T under aromatase inhibition are even more marked than for total T.

The increase of T serum levels in men treated with aromatase inhibitors is explained by LH stimulation of T secretion with increase of both basal LH levels and LH response to GnRH. The increases in gonadotropin levels observed in the present study under 2.5 mg letrozole daily are generally of greater amplitude than previously described under anastrozole treatment at doses of 1 or 2 mg daily (17, 18, 23). The plasma terminal elimination half-life of letrozole is approximately 2 d (31). The powerful effect of aromatase inhibition with letrozole on gonadotropin and T levels is illustrated by the marked carryover effect we have seen in both young and older study subjects initially treated with letrozole, with still marked elevation of gonadotropin and T levels at the beginning of the placebo phase, 14 d after discontinuation of letrozole administration. It is remarkable that in the present as well as in previous studies with anastrozole, a marked increase of gonadotropin and T levels is being achieved even though the decrease in circulating estrogens is of rather modest amplitude. This might suggest that the restraining action of estrogens on gonadotropin secretion in men is at least in part dependent on local aromatization of T to estrogens in the hypothalamus and pituitary gland, as previously suggested by Winters *et al.* (32). However, the design of the present study does not allow differentiating between effects on gonadotropin secretion of blood-borne and locally produced estrogens. Also, one cannot exclude the possibility that technical limitations in terms of assay sensitivity and specificity may have resulted in an underestimation of the reduction of serum estradiol levels during aromatase inhibition in studies in men.

The primary focus of interest in the present study was the comparison of the effects of aromatase between young and elderly men. Notwithstanding a somewhat greater reduction of estrogen serum levels in the elderly compared with the young, in young men the increase of basal gonadotropin levels in response to aromatase inhibition was comparable to that in elderly men. The response to a challenge with low-dose GnRH under aromatase inhibition was greater in the young compared with the elderly. Thus, aromatase inhibition did not uncover a state of increased inhibitory tone by endogenous estrogens in the elderly compared with the young, which would have been expected to result in a greater gonadotropin response in the elderly. The greater response to GnRH under aromatase blockade in the young compared with the elderly could indicate a greater responsiveness of the gonadotropes and/or a lower frequency of endogenous GnRH stimulation with build up of a greater LH releasable pool in the young under these experimental conditions. The observation of Veldhuis and Iranmanesh (33) of a greater disorderliness of spontaneous LH secretion in the elderly compared with the young under treatment with an aromatase inhibitor might be relevant to this context. The responses for T and FT in the elderly, although not significantly different from those in young men, tended to be somewhat smaller. This is as expected in view of the known moderate decrease in responsiveness of the Leydig cells to LH in the elderly (34, 35).

We are aware of one other side-to-side comparison of the effects of aromatase inhibition on gonadotropin and T levels in young and elderly men. In the latter study by Veldhuis and Iranmanesh (33), administration of 10 mg anastrozole daily for 5 d increased 24-h mean LH concentrations significantly and equivalently in young and older men, whereas the T response in the elderly was only limited compared with that in young men. In the elderly, there was a diminished incremental LH pulse amplitude and area, failure to further accelerate LH pulse frequency, and a more disorderly secretory

pattern of LH compared with young men. Except for the limited T response in the elderly in this short-duration study compared with the marked T increase observed during longer-duration aromatase inhibition, the results of the latter study are in general agreement with the present study. Earlier, a comparable response of LH levels and a smaller increase of serum T in elderly, compared with young men, were observed after administration of the selective estrogen receptor modulator clomiphene citrate (36). Finally, also relevant to the present discussion are the observations by Winters *et al.* (11) that elderly men respond with a greater inhibition of gonadotropin secretion than young men to infusion of dihydrotestosterone and T, but not of estradiol, suggesting that gonadotropin secretion in the elderly is not more sensitive to suppression by E_2 in the young.

From the whole of these data it can be concluded that, whereas elderly men are more responsive to the inhibitory action of exogenously administered T on LH secretion (11, 12), the age-related decline in serum T levels is not the result of increased restraining activity by endogenous estrogens. Previously, it has been reported that these age-related changes in regulation of LH secretion that are situated at the hypothalamic level (20), with evidence of diminished GnRH secretion (37), are neither the consequence of an increased opioidergic tone (38) or from relative leptin deficiency (39). The exact mechanisms of the age-related changes in regulation of LH secretion in men are yet to be uncovered and may involve loss of GnRH neurons, intrinsic or regulatory functional changes in the GnRH neurons, and/or less effective coordinated recruitment of GnRH neurons needed for the intermittent release of an adequate bolus of GnRH into the pituitary portal circulation. Limitations of the present study are the relatively small number of subjects and the fact that the carryover effect seen after initial letrozole treatment did not allow for the benefits of an analysis according to a crossover design as was initially intended. However, this was compensated to a large extent by the consistent and robust responses observed during aromatase inhibition.

In conclusion, aromatase inhibition with 2.5 mg letrozole daily for 28 d produced a remarkable and comparable elevation of gonadotropin serum levels in young and elderly men, with also a marked T response in both groups. The results of this study, together with the results of a previous side-to-side comparison of the effects of aromatase inhibition (35), allow us to reject the tested hypothesis that increased restraining of LH secretion by endogenous estrogens is instrumental in the age-related decline of Leydig-cell function.

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CHAPTER 3

PERCEPTION OF MALES' AGING SYMPTOMS, HEALTH AND WELL-BEING IN ELDERLY COMMUNITY-DWELLING MEN IS NOT RELATED TO CIRCULATING ANDROGEN LEVELS

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Perception of males' aging symptoms, health and well-being in elderly community-dwelling men is not related to circulating androgen levels

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Abstract

Aging in men is associated with a progressive but variable decline in androgen production. In aging men there is also an increased occurrence of symptoms such as lack of concentration, nervousness, impaired memory, depressive mood, insomnia, lack of energy and general sense of well-being, decreased libido and erectile dysfunction, periodic sweating, bone and joint complaints, reduction of strength and increased adiposity. This ill-defined male climacterium syndrome is often referred to as "andropause", with the underlying implication that it is at least in part related to (relative) androgen deficiency. Recently an "aging males" symptoms' (AMS) rating scale was developed aimed at a more systematic description of severity of symptoms related to a clinically defined "male climacteric". We studied the relationship of male climacteric symptoms as assessed by the AMS with androgen levels and other questionnaires assessing the perception of health and well-being. Serum levels of sex steroids, sex hormone binding globulin and gonadotropins were measured in blood samples of 161 healthy, ambulatory, elderly men, aged 74–89 years who also completed the AMS scale. Mean value of total, free and bioavailable testosterone in this group was 401.6, 6.8 and 151.4 ng/dl, respectively, with 24.7, 32.4 and 52.2% of the values under the normal range for young men. The results of the AMS scores mostly suggested mild psychological and mild to moderate somatovegetative symptoms. However, clear sexual symptoms were reported in 88% of cases. None of the

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three AMS domain scale scores significantly correlated with testosterone, free testosterone or bioavailable testosterone. Significant correlations were observed between results for the AMS scores and those for other health questionnaires, but none of the subscores for the latter questionnaires correlated with androgen serum levels. In conclusion, the results of this study have shown that, as assessed by the AMS, healthy ambulatory elderly males over 70 had a high perception of sexual symptoms with mild psychological and mild to moderate somatovegetative symptoms. These data failed to support the view that in healthy elderly men, "climacteric symptoms" can predict androgen levels.

Keywords: Aging male; Male climacteric; Symptoms; Testosterone; Aging males' symptoms; Questionnaire

1. Introduction

Aging in men is accompanied by a gradual decline in androgens that becomes more apparent after the age of 50 years (Gray et al., 1991; Swerdloff and Wang, 1993; Tenover, 1994; Vermeulen, 2001). Between the ages of 25 and 75, a 30% decline of mean serum testosterone levels can be seen. As serum levels of sex-hormone binding globulin (SHBG) increase with age, the fall of the biologically active free testosterone and non-SHBG bound or bioavailable testosterone in serum is of even greater magnitude, with a reduction by 50% over the same age range (Ferrini and Barrett-Connor, 1998; Vermeulen et al., 1996). There is, however, considerable inter-individual variability in androgen levels. With advancing age, a progressively larger proportion of men have serum testosterone levels below the reference range for young men (more than 20% after age 60 years). This also means that a majority of elderly men have well preserved serum testosterone levels. Awaiting more convincing documentation that androgen requirements may change during aging, the lower limit of the reference range in young men is most commonly used as a cut-off for defining (relative) hypoandrogenism in elderly men, and has been considered as an indicative threshold for intervention in recent studies assessing the potential benefits of testosterone treatment in elderly men (Vermeulen, 2001).

Postpubertal hypoandrogenism is manifested by changes in psychosocial function (e.g. depressed mood, anxiety, irritability, reduced cognitive capacity; Sternbach, 1998), loss of sense of well-being, decreased muscle mass and strength, sexual dysfunction (decreased libido and erectile dysfunction), and can be accompanied by "hot flushes" (Vermeulen, 2001). Onset is gradual and the symptomatology often insidious, within the older age groups symptoms being attributed to the aging process (Tenover, 1998). The ill-defined male climacteric syndrome is often referred to as "andropause", implying that it is at least in part related to (relative) androgen deficiency. Undoubtedly many signs and symptoms of aging in males are reminiscent of the symptomatology of androgen deficiency in young males. As reviewed by Vermeulen (2001) many studies have shown significant, albeit often only modest, correlations between (free or bioavailable) testosterone levels and various clinical variables such as muscle mass and strength, bone mass, fat mass and libido. Hence,

it appears plausible that the decreased (free or bioavailable) testosterone levels may contribute to the symptomatology of the aging male. In the absence of useful objective clinical parameters of androgen deficiency in aging, the latter should be diagnosed on the basis of both the clinical symptomatology and decreased (free or bioavailable) testosterone levels.

If this approach is valid, androgen supplementation should alleviate at least part of these symptoms. Although the effects of androgen supplementation are more pronounced in young hypogonadal men than in elderly men, androgen substitution of the latter has generally been shown to result in an increase in muscle mass and less consistently muscle strength. Also a limited increase in bone mass (at least at some skeletal sites), a decrease of fat mass, and at least in some studies, in an increase in libido, general well-being, mood, energy, friendliness and cognition have been described (Morley et al., 1993; Wang et al., 1996, 2000). It appears that at least in physiological doses, androgen supplementation decreases total and low-density lipoprotein cholesterol, high-density lipoprotein cholesterol remaining unchanged or decreasing slightly (Morley and Perry, 1999; Morley et al., 2000; Tenover, 1998).

As to the relation between testosterone levels and mood in elderly men, a recent large study by Barrett-Connor et al. (1999) showed a significant inverse correlation between bioavailable testosterone and a depressed mood score.

There is evidence supporting the idea that relative androgen deficiency may contribute to the clinical changes in aging men. Conversely, it remains controversial whether a constellation of clinical symptoms in aging, in particular those related to psychological well-being, may indicate low androgen levels. The aim of the present study was to correlate clinical symptoms tentatively described as andropause with biochemical measures of androgen status, the usefulness of questionnaires to detect hypoandrogenism in the elderly being dependent on their ability to predict (subnormal) androgen levels.

In this context we examined the relation between (free and bioavailable) testosterone levels and dehydroepiandrosterone sulphate (DHEA-S) with the results of health questionnaires in a community-based population of ambulatory older men participating in an observational study on the relationship between androgen status and bone mineral density.

2. Methods

2.1. Subjects

The elderly ambulatory men in this cross-sectional study performed in the year 2000 ($n = 210$) have been recruited in 1996 from the population register of a semi-rural community of 20 000 inhabitants close to the University Hospital of Ghent. They constitute a subgroup of a study population included in an ongoing longitudinal observational study on the relationship between androgen status and bone mineral density. The questionnaires discussed in this study were completed by these men in the year 2000. The initial selection criteria were at inclusion in 1996 the age between

70 and 85 years and willingness to participate. Initial participation rate in 1996 was 47.1% (n = 352 out of 748), 69 subjects were excluded because of potentially interfering diseases or treatments (subjects may be counted more than once): current or previous long-lasting systemic glucocorticoid treatment (n = 21); diabetes treated with insulin (n = 7); treatment with phenytoin (n = 13), androgens or anti-androgens (n = 13), Vitamin D (n = 11), biphosphonates (n = 7); orchidectomy; Paget's disease (n = 5); hyperthyroidism (n = 1); adrenal insufficiency (n = 1); malignancy (n = 3); inflammatory rheumatic diseases (n = 5); calcemia $>$ 2.65 mmol/l (n = 1); fasting glycemia $>$ 8.33 mmol/l (n = 13); serum Creatinine $>$ 177 μmol/l (n = 4). Descriptive details can be found in the paper by Goemaere et al. (2001). Of these 283 eligible subjects included in 1996, 210 were still available for evaluation in 2000. Reasons for loss of follow-up are death, institutionalization, withdrawal of consent and occurrence of exclusions, such as diseases or treatments considered affecting androgen levels. In 2000, of the 210 recruited 161 men completed the questionnaires and allowed blood samples to be taken. A standardized interview was used to obtain information on current lifestyle. The marital status identified four major categories: never married, married, divorced or widowed. Educational level was described as nursery school and kindergarten, elementary school, high school, and college or equivalent professional school. Living arrangements were categorized as non-institutionalized living alone or living with spouse, living in a nursing home or home for retired, or living with relatives. Physical activity was assessed as being involved in any activity of sports, including walking or bicycling in the recent past. Sleep disturbance was assessed indirectly through identifying the use of sleep medication.

Demographics and medical information was reviewed together with each subject with both a study nurse and a medical doctor.

All participants gave a written informed consent for participation in this study, approved by the Ethical Committee of the Ghent University Hospital.

2.2. Hormone assays

Sex steroids were measured in each subject in order to evaluate these hormones that may affect the clinical variables. Gonadotropins were measured to detect more subtle variations in the hypothalamo-pituitary-gonadal axis. Venous blood was obtained between 0800 and 1000 h, after an overnight fast and serum was stored at −80 °C until assay. To avoid effects of seasonal variation blood collection was completed in a period of 2 months. For the present study hormone assays on blood samples obtained in 1996 and 2000 were considered (assayed in the same assay-run). Commercial kits for radioimmunoassay were used to determine the serum concentrations of testosterone (Medgenix Diagnostics, Fleurus, Belgium) and oestradiol (Incstar, Stillwater, MM, USA). Commercial kits for immuradiometric assays were used for determinations of serum SHBG (Orion Diagnostica, Espoo, Finland), LH and FSH (Medgenix Diagnostics); the latter hormone levels having been assessed because of their potential ability to reveal changes in gonadal function (Deslypere et al., 1987). Serum samples from 1996 and 2000 for a same study subject were

assessed within the same assay run. For testosterone intra-assay coefficient of variation (CV) ranged between 5.4 and 7.6% over the whole concentration range; inter-assay CV at 400 ng/dl was 5.5%. Intra- and inter-assay CV for all other assays were below 10 and 15%, respectively (Van Den Saffele et al., 1999). Serum free and bioavailable testosterone (T) and oestradiol were calculated from the total serum hormone concentrations, serum SHBG, and serum albumin using a validated equation derived from the mass action law (Vermeulen et al., 1999).

2.3. Questionnaires

2.3.1. Aging male symptoms (AMS) questionnaire

The AMS scale was originally developed and standardized in Germany using factor analytical methods (Heinemann et al., 1999). In this 17 item-scale three dimensions of symptoms were identified: a psychological, a somatovegetative and a sexual factor. The AMS uses a Likert response scale for all questions. Reference values of the three dimensions were defined to be used in daily practice. The factor psychological symptoms aggregates symptoms or complaints of a psychological nature in aging men: discouragement, depression, irritability, anxiety and nervousness (score$\leq$5 no, 6–8 mild, 9–12 moderate, $\geq$12 severe impairment). The somatovegetative dimension describes a complex of somatic and vegetative symptoms: pain in muscles or joints, sweating (hot flushes), increased need for sleep and sleep disturbances, impaired general well-being, decrease in muscular strength, and decreased energy (score$\leq$8 no, 9–12 mild, 13–18 moderate and $\geq$19 severe impairment). The sexual complaints dimension consists of basically five symptoms: disturbances of potency, decrease in morning erections, decrease in libido and sexual activity, decrease in beard growth and "the impression of having past the zenith of life" (score$\leq$5 no, 6–7 mild, 8–10 moderate and $\geq$11 indicating severe impairment). The global score of the three dimensions may be used, but this has not originally been described (Heinemann et al., 1999). The AMS scale was selected because it was suggested as a valuable tool for assessing symptoms in the aging male that was easily applicable in daily practice.

Test–retest comparisons of the final scales' total scores were done in many countries and showed acceptable reliability with correlation coefficients ranging from r = 0.80–0.93. At present time the validity of this tool for clinical assessment of the aging male is under investigation (Heinemann, personal communication). The degree of internal consistency of the AMS psychological, somatovegetative and sexual domain demonstrated with the present study was, respectively, 0.64, 0.52 and 0.66 (Cronbach's Alpha).

The AMS scale has been translated in a standardized way into English for use in North America and the UK. The AMS list we used in 2000 was a Flemish translation of the German original, but this version was not a standardized, pretested translation. However, from our experience using our translation misinterpretation of the items in the scale appears unlikely.

2.4. SF-36

The SF-36 was developed as part of the Medical Outcomes Study (MOS) and the Health Insurance Experiment (HIE) of the Rand Corporation. It is available in two versions: one measures acute episodes and the other follows chronic conditions. This study utilized the chronic conditions version (Ware and Sherbourne, 1992). The SF-36 Health Survey is a widely used patient-based health status survey worldwide.

The SF-36 includes one multi-item scale that assesses eight health concepts: (1) limitations in physical activities because of health problems; (2) limitations in social activities because of physical or emotional problems; (3) limitations in usual role activities because of physical health problems; (4) bodily pain; (5) general mental health (psychological distress and well-being); (6) limitations in usual role activities because of emotional problems; (7) vitality (energy and fatigue); and (8) general health perceptions. More useful summary scores were developed recently, a functional status score and a well-being score (PCS, Physical Component Summary; MCS, Mental Component Summary). Respondents answer each statement reflecting their health at present and the previous 4 weeks. A higher score on the SF-36 indicates better health status, with individual minimum and maximum scores for each of the eight items. The SF-36 uses a Likert response scale for most questions. This produces a user-friendly instrument, which can discriminate among the various levels of health and detects changes in health status over time. Many studies using the SF-36 support its content and construct validity, test–retest reliability and responsiveness to change (Hobson et al., 1997; McHorney et al., 1992)

2.5. RDRS-2

The Rapid Disability Rating Scale (RDRS) was developed as a research tool for summarizing the functional capacity and mental status of elderly chronic patients. A revised scale of 18 items was published as the RDRS-2 in 1982. Details on reliability and validity can be found in the original paper: two nurses independently rated 100 patients; item correlations ranged from 0.62 to 0.98. Rating of 845 men was used to predict subsequent mortality using multiple regression and discriminant function analysis; 20% of variance in mortality was explained, correctly classifying 72% of patients who eventually died (Linn and Linn, 1982).

There are eight questions on activities of daily living, three on sensory abilities, three on mental capacities and one question on each of dietary changes, continence, medications and confinement to bed. The scores range from 18 to 72 with higher values indicating greater disability. This is a broad scale that rates specifically the amount of assistance required in 18 activities, broader in scope than most activities of daily living (ADL) scales.

2.6. Statistics

Descriptive statistics given are means and standard deviations. Most variables in the analysis turned out to be positively skewed. In order to meet the necessary model

assumptions, a natural logarithmic transformation in these analyses was used for hormonal parameters, except for the normally distributed testosterone and free testosterone. Model assumptions were evaluated by visualization of the Pearson residuals. Spearman correlation coefficients were calculated for studying univariate associations for ordinal data and not normally distributed data. In order to evaluate the independent contribution of androgens in explaining the variability of questionnaire results multiple regression analyses were performed with additional correction for age, body mass index (BMI), educational level, physical activity, marital status, living arrangements and sleep disturbances. Comparison of the distribution of study variables between subgroups of AMS scores (AMS categories made up using the scoring system) was performed using ANOVA. To account for multiple testing Bonferroni corrections were used. Internal consistency of questionnaires, based on the average inter-item correlation, was evaluated using Cronbach's alpha. All analyses were performed using SPSS software (version 10.0).

3. Results

3.1. Subject characteristics

The patients studied in 2000 were between the ages of 75 and 89 with a mean age of 79. Mean BMI was 26.5 kg/m^2 (SD 3.5). Most of these men were married (73.8%) while 23.8% were widowers. As for educational level 7.7% had completed only nursery school and kindergarten, 43.5% elementary school, 37.8% high school and 11% college or equivalent professional school. The majority of these men were non-institutionalized (23.3% lived alone, 74.8% lived with spouse). Physical activity was reported as never (5%), sometimes (20%), less than 1 h/week (12%), 1–2 h/week (19%) and more than 2 h/week (44%). Sleep medication was used by 25.5% of men.

In 2000, total serum testosterone in this group of patients showed a mean value of 401.6 ng/dl (SD 156.2); 24.7% had a value of less than 319 ng/dl, the lower limit of the normal range in our laboratory (Vermeulen, 2001). Free testosterone mean was 6.8 ng/dl (SD 2.2), with 32.4% below the young reference range (<6.5 ng/dl), and mean bioavailable testosterone was 151.4 ng/dl (SD 50.1), with as many as 52.2% of the values below the young reference range (<152 ng/dl) (Table 1).

Remarkably, the results of the AMS scores mostly suggested mild psychological and mild to moderate somatovegetative symptoms; however, clear sexual symptoms were reported in 88% of cases (Table 2).

3.2. Association between AMS scores and sex steroid levels

None of the three AMS domain scale scores correlated significantly with testosterone, free testosterone or bioavailable testosterone as shown in Table 3. There was no relation between either the longitudinal change in androgen levels (difference between androgen levels 1996–2000, $r = 0.77$) or the mean values for 1996 and 2000 with the AMS score. Also, when comparing the AMS scores in men with

Table 1
Characteristics of the study cohort of 161 ambulatory men, clinical and hormonal profiles

	Mean	SD
Clinical characteristics		
Age (years)	79	4
Height (m)	1.68	0.6
Weight (kg)	75	12
BMI (kg/m^2)	26.5	3.5
Hormonal levels		
Testosterone (ng/dl)	401.6	156.2
Free testosterone (ng/dl)	6.8	2.2
Bio testosterone (ng/dl)	151.4	50.1
SHBG (nmol/l)	46.6	18.5
Oestradiol (ng/dl)	2.07	0.58
LH (IU/l)	8.6	6.4

Table 2
Results of AMS scores in 161 ambulatory elderly men

AMS scores	No	Mild	Moderate	Severe
Psychological factor	(≤5) 37.4%	(6–8) 40%	(9–12) 19.5%	(≥12) 3.2%
Somatovegetative factor	(≤8) 11.8%	(9–12) 41.1%	(13–18) 40.5%	(≥19) 7.1%
Sexual factor	(≤5) 2.6%	(6–7) 1%	(8–10) 8.4%	(≥11) 87.9%

Table 3
Association between testosterone, gonadotropin levels, DHEA-S and AMS score, SF-36 summary scores and RDRS-2

Correlation coefficient	Testosterone	Free testosterone	Bioavailable testosterone	LH	FSH	DHEA-S
AMS psychological factor	−0.07	−0.08	−0.09	−0.01	−0.04	0.12
AMS somatovegetative factor	−0.05	−0.10	−0.11	0.13	0.09	−0.13
AMS sexual factor	0.04	0.03	0.04	0.18*	0.19*	−0.03
SF-36 PCS	0.08	0.07	0.08	−0.01	−0.04	0.13
SF-36 MCS	0.03	0.08	−0.08	−0.08	0.02	−0.06
RDRS-2	−0.08	−0.13	−0.14	0.05	0.04	−0.10

*Correlation significant at the 0.05 level. Entries are Spearman's rank correlations. PCS, Physical Component Summary Scale; MCS, Mental Component Summary Scale. n = 161.

normal or subnormal androgen levels no significant differences were detected (data not shown).

Change in body weight between 1996 and 2000 (mean −0.8 kg, range −15 to +17.7 kg) had no influence on the scores. There was also no significant association of serum levels of oestradiol, free oestradiol, bioavailable oestradiol, SHBG, DHEA-S or the ratios of serum testosterone and oestradiol and free testosterone and free oestradiol with the AMS scores (data not shown, except for DHEA-S). A significant association was found between the results of the AMS sexual subdomain score with both LH and FSH (correlation coefficients 0.18 and 0.19, respectively, $p < 0.05$). However, when considering subgroups of patients according to their AMS score level (no, mild, moderate, severe symptoms) there were no significant differences for either LH or FSH, and also no differences for testosterone, bioavailable or free testosterone. In a multiple regression analysis including BMI, age, educational level, physical activity, marital status, educational level, living arrangements and sleep disturbances as possible confounders, again no relationship could be established between testosterone, free or bioavailable testosterone and the AMS scores.

3.3. AMS versus other questionnaires

The scores obtained with the different health questionnaires, were variably inter-correlated. Absolute values of the SF-36 and RDRS-2 are presented in Table 4. The eight SF-36 subscales and RDRS-2 correlated with psychological symptoms, somatovegetative symptoms and sexual complaints as assessed by the AMS (correlation coefficients ranging between −0.59 and 0.28, $p < 0.05$ and $p < 0.001$, except for the sexual symptoms scale and bodily pain, social functioning and emotional role and the Mental Component Summary Scale as assessed by SF-36; Table 5). The MCS subscale of the SF-36 is most strongly correlated with the psychological symptoms scale of the AMS ($r = -0.44$). Similarly, the PCS subscale is most strongly correlated with the somatovegetative subscale of the AMS ($r = -0.59$). The inter-relationships between results of the health questionnaires remained

Table 4
Absolute values of the SF-36 and RDRS-2 in 161 ambulatory elderly men

SF-36 measures (raw scores)	Median	Inter-quartile range	Minimum	Maximum	Raw score range
Physical functioning	25	20–28	10	30	10–30
Physical role	8	5–8	4	8	4–8
Bodily pain	50	39–60	11	60	11–60
General health	18	15–20	8	25	5–25
Vitality	19	16–20	5	24	4–24
Social functioning	10	8–10	3	10	2–10
Emotional role	6	5–6	3	6	3–6
Mental health	25	22–28	9	30	5–30
RDRS-2	21	20–23	18	39	18–72

Table 5
Association between AMS scores and Quality of Life scales SF36 and RDRS-2

	AMS psychological symptoms	AMS somatovegetative symptoms	AMS sexual symptoms
SF-36 PF	−0.17*	−0.46***	−0.28***
RP	−0.25**	−0.43***	−0.16*
BP	−0.22*	−0.49***	−0.20
GH	−0.26**	−0.46***	−0.22*
VT	−0.46***	−0.52***	−0.22**
SF	−0.35***	−0.41***	−0.09
RE	−0.26**	−0.27***	−0.12
MH	−0.52***	−0.32***	−0.24***
PCS	−0.15*	−0.59**	−0.24**
MCS	−0.44**	−0.22**	−0.10
RDRS-2/72	0.22**	0.27***	0.28***

$*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Entries are Spearman's rank correlation coeffcients. PF, physical functioning; RP, role physical; BP, bodily pain; GH, general health; VT, vitality; SF, social functioning; RE, role-emotional; MH, mental health; PCS, Physical Component Summary Scale; MCS, Mental Component Summary Scale.

essentially unchanged when correction for an effect of age was applied. Neither RDRS-2 nor SF-36 correlated significantly with testosterone, free testosterone, bioavailable testosterone or DHEA-S; there was also no correlation with LH, FSH, (bioavailable) oestradiol (data not shown).

4. Discussion

The results of the present study appear to suggest that in community-dwelling ambulatory old men, male climacteric symptoms are not predictive of the prevailing androgen status as assessed by serum (free and bioavailable) testosterone. Similarly, there is no indication for a relationship between climacteric symptoms and either androgen levels or serum DHEA-S.

Currently there is no gold standard for clinically defining "male climacteric" and relatively little information is available in the literature on inter-relations between age-related decrease in hormone serum concentrations and symptoms in elderly males. In the original report on the description of the AMS by Heinemann et al. (1999), the severity of symptoms assessed using the AMS scale was found to be related to a clinically defined male climacteric in their study population. One expert assessed all clinical information relating to the original 116 subjects and defined a probability that some of the symptoms are "likely" or "unlikely" to be suggestive of the male climacteric. This judgement was entirely based on clinical grounds and blinded to hormonal data. Clearly, this clinical validation did not intend to validate the questionnaire as a tool to predict low serum testosterone in elderly men. Therefore, it seemed important to correlate the findings for AMS with serum androgen

levels. Indeed, the question whether climacteric symptoms can predict serum androgen levels in elderly men has obvious practical clinical implications.

The prevalence of subnormal (free) testosterone levels in our study group was in agreement with previous findings (Ferrini and Barrett-Connor, 1998; Kaufman and Vermeulen, 1997; Vermeulen, 2001; Vermeulen et al., 1996). Nevertheless, we could not establish any relationship between androgen status and the different AMS subscales in our study population. Perry et al. (2001) described a similar result in a study where in male outpatients 55 years or above, bioavailable testosterone was not an important determinant of cognitive, psychological, or sexual functioning or of quality of life. A similar result is described by Dunbar et al. (2001) who could not establish a relation between SF-36 and bioavailable testosterone in a cross-sectional survey of 93 men, aged 70 or higher.

In our study, there was also no relation with any of the individual questions included in the AMS (data not shown), indicating that different combinations of questions are unlikely to result in a different outcome. Although the AMS questionnaire may help to differentiate and more systematically describe the patient's symptomatology, the importance of the information provided should not be overestimated in view of the lack of association with hormone levels. A major reason for the lack of predictive value of clinical symptoms may lie in the fact that signs and symptoms of aging in men are undoubtedly multifactorial in origin. Aging is accompanied by a decrease in almost all physiological functions and as far as the endocrine system is concerned, by a decrease of not only of gonadal and adrenal androgen secretion but also of growth hormone secretion (Martin et al., 1997), amongst other changes. Moreover, age associated changes in lifestyle may be important as indicated by the impact of the decrease in physical activity, the decrease in muscle mass and bone mineral density (Rudman et al., 1994). Furthermore, androgen requirements may vary between subjects and in a same subject according to the considered physiological functions (Vermeulen, 2001)

Our results suggest that the ambulatory elderly males have a high perception of sexual symptoms, without experiencing much psychological and mostly mild to moderate somatovegetative problems according to the AMS scale. This agrees with previous findings in a study performed in the same community (Mak et al., 2002).

We must emphasize that the present study specifically addresses the situation on an elderly population with mean age over 79 years. This is of interest because there is little information available for such populations but the results may not be extrapolable to younger populations.

In fact, it is quite conceivable that in a younger age group symptomatology may be more specific and that the AMS scores or similar questionnaires may better predict androgen levels. Smith et al. (2000) developed a self-administered eight item screener for testosterone deficiency (hypogonadism) in aging men—40–79 years at baseline—based on age, BMI, occurrence of diabetes, asthma, headaches, sleep pattern, dominance preferences and smoking status. The screener performed significantly better than chance in identifying men with low testosterone levels. The latter questionnaire is based on the prevalence of low testosterone levels in subjects with co-morbidity, which is a different issue than the one addressed here. Morley et al. (1999) developed

the ADAM screening questionnaire for androgen deficiency in aging males, addressing issues as decrease in libido, lack of energy, decrease in strength, loss of height, decrease in enjoyment of life, feeling sad and grumpy, less strong erections, deterioration in the ability to play sports, falling asleep after dinner and deterioration in work performance. Authors reported on 88% sensitivity and 60% specificity of the questionnaire in a group of men aged 40–62 years. This questionnaire has not been tested in our age group.

Barrett-Connor et al. (1999) have reported on a relation between depressed mood (as assessed by the Beck Depression Inventory) and bioavailable testosterone in a group of men aged 50–89 years who attended a clinic visit.

In our population of community dwelling men aged 74 years and above there was a variable correlation of AMS with the results of the non-disease specific health measures SF36 and RDRS-2, suggesting the AMS scale may be a specific Quality of Life scale for problems in aging males. Again, neither SF-36 nor RDRS-2 did relate to hormonal parameters.

In conclusion, our findings for the AMS scale suggest that in ambulatory elderly men the perception of symptoms of male climacterium does not predict androgen status as assessed by serum androgen levels. However, because our elderly population was relatively healthy, due mainly to the application of our stringent exclusion criteria, the results of the present study should not be extrapolated to less healthy populations as they should not be to younger age groups. Also, the fact that clinical symptom questionnaires cannot predict androgen levels certainly does not exclude the possibility that relative androgen deficiency may contribute to the multifactorially defined clinical changes in aging men.

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CHAPTER 4

SEX STEROID LEVEL, ANDROGEN RECEPTOR POLYMORPHISM, AND DEPRESSIVE SYMPTOMS IN HEALTHY ELDERLY MEN.

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Sex Steroid Level, Androgen Receptor Polymorphism, and Depressive Symptoms in Healthy Elderly Men

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OBJECTIVES: To determine the prevalence of depression in a cohort of elderly men as assessed using a 30-item Geriatric Depression Scale (GDS) score and to describe the association between this score and sex steroids, androgen receptor (AR) polymorphism, and general health status.

DESIGN: Observational study on the relationship between sex steroid status and health-related parameters.

SETTING: Community-based.

PARTICIPANTS: Ambulatory men (n = 236 in 1997, n = 192 in 2000) aged 70 and older at inclusion in 1996, interviewed in 1997 and 2000.

MEASUREMENTS: Serum levels of testosterone, estradiol, sex hormone binding globulin (SHBG), dehydroepiandrosterone-sulfate (DHEAS), cortisol, and the AR gene cytosine, adenine, guanine (CAG)-repeat length polymorphism were determined. Free testosterone and free estradiol were calculated. Questionnaires included GDS, 36-item Short Form, and Rapid Disability Rating Scale—2.

RESULTS: Median age was 75.3 years (interquartile range = 73.5–78.5). A GDS score of 11 or greater was found in 30 (12.7%) men. Age and GDS score were significantly interrelated ($P < .01$), as were all health-assessment scores. GDS scores were not related to (free) testosterone or AR polymorphism in 1997 or 2000. In 1997 only (n = 236), higher GDS scores were related to higher estradiol, free estradiol, and DHEAS levels.

CONCLUSION: The data did not support a role for testosterone in depression in elderly community-based men as assessed using the GDS. **J Am Geriatr Soc 53:636–642, 2005.**

Key words: aging male; depression; testosterone; Geriatric Depression Scale; androgen receptor polymorphism

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A substantial body of literature exists characterizing a decline of physical, emotional, and sexual functioning in elderly men. A gradual decline in androgens that becomes more apparent after the age of 50 accompanies aging in men.[1–3] Between the ages of 25 and 75, a 30% decline of mean serum testosterone levels can be seen. As serum levels of sex hormone binding globulin (SHBG) increase with age, the fall of the biologically active free testosterone and non-SHBG bound, or bioavailable testosterone, in serum is of even greater magnitude, with a 50% reduction over the same age range.[4,5] There is, however, considerable interindividual variability in androgen levels. With advancing age, a progressively larger proportion of men have serum testosterone levels below the reference range for young men (more than 20% after age 60), although a majority of elderly men have serum testosterone levels well within the normal range for young men. Androgen action is mediated through the androgen receptor (AR), whose transactivation capacity appears to correlate with the length of a cytosine, adenine, guanine (CAG)-repeat polymorphism of the AR gene.[6]

Symptoms, such as changes in psychosocial function (e.g., depressed mood, anxiety, irritability, reduced cognitive capacity, loss of sense of well-being, decreased muscle mass and strength, decreased libido, erectile dysfunction and hot flushes), might be related to declining serum testosterone levels.[7,3] However, for the ill-defined male climacteric syndrome—often referred to as "andropause," implying that it is related to (relative) androgen deficiency—the observed association between andropause symptoms and declining testosterone may be causal, or rather merely coincidental. The decline in functioning might be more directly related to age, health, and other factors. Mild depressive symptoms are common in elderly men, as is hypogonadism, or relative testosterone deficiency.[8] Moreover, the psychiatric symptoms of hypogonadism overlap with symptoms of depression.[9] The literature on the relationship between testosterone levels and depression, depressive symptoms, and depressive mood in elderly men has not been unequivocal. In the Rancho Bernardo Study, using the Beck Depression Inventory in 856 men aged 50 to 89,

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endogenous bioavailable, but not total, testosterone or estradiol levels were reported to be inversely associated with depressive mood.[10] In the latter study, assessment of serum estradiol gave no indication for a role of aromatization of estrogens in the observed testosterone-mood association. In a retrospective cohort study, hypogonadal men aged 45 and older showed a greater incidence of depressive illness and a shorter time to diagnosis of depression.[11] In a study of 754 selected men aged 50 to 70 who participated in a screening program on prostate cancer and "andropause," there was an inverse correlation between free testosterone and depressive symptoms assessed using the Carroll Rating Scale, but serum free testosterone was not related to the prevalence of depression.[12] Clinically, it has been suggested that the age-related decline in testosterone levels, persisting over years, may lead to mild depressive illness, or dysthymia,[13] but in contrast to these findings, it has been reported that declining bioavailable testosterone levels were associated with lower levels of depressive symptoms using the Hamilton Depression Scale in men aged 55 to 76.[14] In the Massachusetts Male Aging Study (MMAS), 1,709 community-based men aged 40 to 70 were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D).[15] Serum testosterone levels were not associated with CES-D–diagnosed depression. In further MMAS analyses, neither testosterone level nor the AR genotype alone were associated with CES-D–defined depression, but in a statistical model combining the three variables, AR polymorphism and testosterone together predicted depression.[16] Parameters of depressive symptoms, selected from the Aging Males' Symptoms (AMS) Scale were reported to correlate positively with the length of AR CAG repeats.[17]

The present study focused specifically on community-dwelling older men. The aims of the present study were to determine the prevalence of depression, as assessed using the Geriatric Depression Scale (GDS), in a community-based population of 236 ambulatory men aged 70 and older participating in an observational study on the relationship between age-related clinical and hormonal changes and to assess possible relationships between scores for depression, health-related quality of life and functionality, sex steroid levels, and an AR-gene polymorphism.

METHODS

Subjects

The elderly ambulatory men in this observational study that has been performed from 1996 and onwards (n = 283) have been recruited from the population register of a semirural community of 20,000 inhabitants close to the University Hospital of Ghent. They constitute a subgroup of a population of Caucasian study subjects included in a longitudinal observational study on the relationship between androgen status and bone mineral density.[18,19] All participants gave a written informed consent for participation in this study, which the ethical committee of the Ghent University Hospital approved.

These men completed the questionnaires discussed in this study in 1997 and 2000. The initial selection criteria were, at inclusion in 1996, aged 70 to 85 and willingness to participate. Initial participation rate in 1996 was 47.1% (N = 352 of 748); 69 subjects were excluded because of diseases or treatment potentially interfering with bone metabolism (subjects may be counted more than once): current or previous long-lasting systemic glucocorticoid treatment (n = 21); fasting glycemia greater than 8.33 mmol/L (n = 13); diabetes mellitus treated with insulin (n = 7); treatment with phenytoin (n = 13), androgens or antiandrogens (n = 13), vitamin D (n = 11), or bisphosphonates (n = 7); orchiectomy (n = 5); Paget's disease (n = 5); inflammatory rheumatic diseases (n = 5); serum creatinine greater than 177 μmol/L (n = 4); malignancy (n = 3); hyperthyroidism (n = 1); adrenal insufficiency (n = 1); and calcemia greater than 2.65 mmol/L (n = 1). Of these 283 eligible subjects included in 1996, 210 were still available for evaluation in 2000. Reasons for loss to follow-up were death, institutionalization, withdrawal of consent, and occurrence of exclusions, such as diseases or treatments considered to affect androgen levels. In 1997 and 2000, 236 and 192 men, respectively, completed the questionnaires on depression and quality of life. A standardized interview was used to obtain information on current lifestyle, including smoking and alcohol intake. Educational level was described as elementary school, high school, and college or equivalent professional school. Type of former employment was described as manual labor, in between, and no manual labor. Sleep disturbance was assessed indirectly through identifying the use of sleep medication. Antidepressant use was queried.

A study nurse and a medical doctor reviewed demographics and medical information with each subject.

Hormone Assays

An extensive baseline hormonal status in this study population was assessed using blood samples obtained in 1996. Venous blood was obtained between 8:00 a.m. and 10:00 a.m. after an overnight fast, and serum was stored at − 80°C until assay. Measurement of total serum testosterone was also performed on a serum sample obtained in 1997 and in 2000, together with a repeated measurement on the 1996 sample in a single assay run to allow reliable estimates of changes in serum levels between 1996 and 2000. Serum total estradiol was determined in the 1996 and 2000 samples only. To avoid effects of seasonal variation, blood collection was completed over a period of 2 months. For the present study, hormone assays on blood samples obtained in 1996, 1997, and 2000 were considered. Commercial kits for radioimmunoassays were used to determine the serum concentrations of testosterone (Medgenix Diagnostics, Fleurus, Belgium) and estradiol (Incstar, Stillwater, MN); commercial kits for immunoradiometric assays were used for determinations of serum SHBG (Orion Diagnostica, Espoo, Finland), cortisol (DiaSorin, Stillwater, MN), and dehydroepiandrosterone-sulfate (DHEAS) (DSL Inc., Webster, TX). The testosterone/estradiol ratio was used as an indirect indicator of aromatase activity. Intra- and interassay coefficients of variation for all assays were below 10% and 15%, respectively. Serum free and bioavailable testosterone and estradiol were calculated from the total serum hormone concentrations, serum SHBG, and serum albumin using a validated equation derived from the mass action law.[20,21]

Use of a single sample for determination of androgen levels should be an acceptable approach for estimation (categorization) of androgen status in this type of study.[22] Moreover, it has been documented in this study population that single-point determination of testosterone at 1-year intervals was highly intercorrelated (correlation coefficient $(r) > 0.8$).[23]

Determination of AR CAG-Repeat Length

Genomic deoxyribonucleic acid was extracted from blood treated with ethylene diamine tetra-acetic acid using a commercial kit (Qiagen Midi Kit; QiagenInc, Valencia, CA). Polymerase chain reaction was used to amplify exon 1 of the AR gene with primers 5′AGCCTGTTGAACTCTT CTGAGC3′ (sense) and 5′CTGCCTTACACAACTCCTT GGC3′ (antisense). After ethanol precipitation, the amplified fragment was directly sequenced on an ABI Prism 310 sequencer (ABI Prism, Perkin-Elmer Applied Biosystems, Foster City, CA), using BigDye Terminator Cycle Sequencing Reaction Kit (ABI Prism). The CAG-repeat length ranges normally from six to 39 repeats, with a mean of 22.[24]

Questionnaires

Geriatric Depression Scale

The GDS, developed in 1983, has 30-and 15-item versions and was designed in a yes/no format for self-or caregiver administration, making it easy to use.[25] In this study, the 30-item version was administered. The GDS minimizes questions about somatic and vegetative symptoms, which can overlap with symptoms of concurrent medical illness. The GDS has long-standing success in identifying major depression in psychiatric and hospital settings and demonstrates accuracy in primary care.[26] Sensitivity and specificity ranged from 79% to 100% and 67% to 80%, respectively. A GDS score between 0 and 10 is viewed as within the normal range, and 11 or greater is a possible indicator of depression. The GDS scale was selected because it is a valuable tool for assessing depression symptoms in aging men that was easily applicable in daily practice. The degree of internal consistency of the GDS demonstrated with the present study was 0.83 in 1997 (Cronbach alpha). The GDS list used was a Flemish variant of a Dutch translation of the English original. This Dutch version, although not the Flemish variant, was a standardized, pretested translation,[27,28] but the slightly changed wording is better adapted to everyday language for the elderly Flemish population than the validated Dutch version.

Short Form-36

The principle quality-of-life measure was the widely used SF-36 Health Survey. The SF-36 was developed as part of the Medical Outcomes Study and the Health Insurance Experiment of the Rand Corporation. It is available in two versions; one measures acute episodes, and the other follows chronic conditions. This study used the chronic-conditions version.[29]

The SF-36 includes one multiitem scale that assesses eight health concepts: limitations in physical activities because of health problems, limitations in social activities because of physical or emotional problems, limitations in usual role activities because of physical health problems, bodily pain, general mental health (psychological distress and well-being), limitations in usual role activities because of emotional problems, vitality (energy and fatigue), and general health perceptions. Respondents answer each statement reflecting their health at present and during the previous 4 weeks. A higher score on the SF-36 indicates better health status, with individual minimum and maximum scores for each of the eight items. The SF-36 uses a Likert response scale for most questions. This produces a user-friendly instrument that can discriminate between the various levels of health and detects changes in health status over time. Many studies using the SF-36 support its content and construct validity, test-retest reliability, and responsiveness to change.[30]

Rapid Disability Rating Scale-2

The Rapid Disability Rating Scale (RDRS) was developed as a research tool for summarizing the functional capacity and mental status of elderly chronic patients. A revised scale of 18 items was published as the RDRS-2 in 1982. Details on reliability and validity can be found in the original paper.[31] There are eight questions on activities of daily living (ADLs), three on sensory abilities, three on mental capacities, and one question each on dietary changes, continence, medications, and confinement to bed. The scores range from 18 to 72, with higher values indicating greater disability. This is a broad scale that rates specifically the amount of assistance required in 18 activities, broader in scope than most ADL scales.

Statistics

Descriptive statistics given are medians and interquartile range. Most variables in the analysis turned out to be positively skewed. To meet the necessary model assumptions, a natural logarithmic transformation in these analyses was used for hormonal parameters, except for the normally distributed testosterone and free testosterone. Model assumptions were evaluated using visualization of the Pearson residuals. Spearman correlation coefficients were calculated for studying univariate associations for ordinal data and not normally distributed data. To evaluate the independent contribution of steroid levels in explaining the variability of questionnaire results, multiple regression analyses were performed with additional correction for age, alcohol intake, and professional status. Internal consistency of questionnaires, based on the average interitem correlation, was evaluated using Cronbach alpha. All analyses were performed using SPSS software, version 10.0 (SPSS, Inc., Chicago, IL).

RESULTS

Subject Characteristics

The subjects studied in 1996 were aged 70 to 85, with a median age of 75.3 (Table 1). Median body mass index (BMI) was initially 26.3 kg/m^2 (range 24.2–28.6). At the start of the study, most were married (73.8%); 23.8% were widowers. Almost half (49.2%) had completed elementary school, 39.0% high school, and 11.9% college or equivalent professional school. Most were noninstitutionalized (23.3% lived alone, 74.8% lived with a spouse). Almost one-quarter (23.9%) used sleep medication, and 3.4% used antidepressants.

Table 1. Selected Demographic and Hormonal Characteristics of the Analysis Sample of 236 Healthy Elderly Men

Characteristic	Baseline*	2000
Age, median (IQR)	75.3 (73.5–78.5)	78.0 (76.0–81.0)
BMI (kg/m^2), median (IQR)	26.3 (24.2–28.6)	26.5 (24.1–28.8)
Smoking, % (n/N)		
Never	19.6 (46/235)	
Stopped	62.6 (147/235)	
Active	17.9 (42/235)	
>2 units/d alcohol, % (n/N)		
Never	28.9 (68/235)	
<1×/mo	14.9 (35/235)	
1–2×/mo	17.0 (40/235)	
1–2×/wk	17.0 (40/235)	
3–4×/wk	8.1 (19/235)	
Daily	14.0 (33/235)	
Education, % (n/N)		
<high school	49.2 (116/236)	
High school graduate	39.0 (92/236)	
>high school	11.9 (28/236)	
Employed, % (n/N)		
Manual labor	32.3 (74/229)	
In between	36.7 (84/229)	
No manual labor	31.0 (71/229)	
Geriatric Depression Scale score, % (n/N)		
0–10	87.3 (206/236)	83.1
11–20	11.9 (28/236)	13.5
21–30	0.8 (2/236)	3.49
Use of antidepressants, % (n/N)	3.4 (8/236)	
Use of sleep medication, % (n/N)	23.9 (54/234)	
SF-36 score, median (IQR)		
Physical functioning	85 (65–95)	
Social functioning	100 (87.5–100)	
Physical role	16 (12–16)	
Emotional role	100 (100–100)	
Mental health	84 (72–92)	
Vitality	75 (65–85)	
Bodily pain	89.8 (67.4–100)	
General health	65 (55–80)	
Health change	50 (50–50)	
Rapid Disability Rating Scale-2 (score/72), median (IQR)	20 (20–22)	
Hormonal levels, median (IQR)		
Testosterone, ng/dL	437.8 (347.2–513.5)	403 (293.2–508.0)
Free testosterone, ng/dL	7.54 (6.22–8.70)	6.63 (5.2–8.39)
Estradiol, ng/dL	1.8 (1.5–2.2)	2.02 (1.66–2.38)
Free estradiol, ng/dL	0.029 (0.025–0.036)	0.035 (0.029–0.041)
Testosterone/estradiol	230.0 (189.9–278.2)	
DHEA-S, μg/dL	71 (48.5–95)	54.6 (34.9–78.6)
Sex hormone–binding globulin, nmol/l	41.8 (30.9–55.1)	47.5 (34.5–57.4)
Cortisol, μg/dL	19.3 (16.4–22.6)	
Cortisol/DHEA-S	0.273 (0.184–0.417)	

*Hormonal data, 1996; questionnaires, 1997.
DHEA-S = dehydroepiandrosterone sulphate; IQR = interquartile range; BMI = body mass index.

Cross-Sectional Analyses

At inclusion in 1996, median total serum testosterone in this group of patients was 437.8 ng/dL (range 347.2–513.5). Median free testosterone was 7.54 ng/dL (range 6.22–8.70). A total testosterone value less than 319 ng/dL, the lower limit of the normal range for young men in the study laboratory, was found in 25.8% of men. Median total estradiol was 1.8 ng/dL (range 1.5–2.2), free estradiol was 0.029 ng/dL (range 0.025–0.036), and serum cortisol was 19.3 μg/dL (range 6.4–22.6). The median AR CAG-repeat length was 23 repeats (range 16–32) (25th percentile to

75th percentile: 21–25). As previously reported, in a multiple linear regression model with age and BMI as covariates, no significant role for the AR CAG-repeat polymorphism was established in the determination of (free) testosterone, SHBG, or estradiol.[32]

In the analysis sample in 1997, there were 30 men (12.7%) with possible depression (GDS ≥11). GDS scores increased with age and use of sleep medication and antidepressants. SF-36 physical and social functioning, physical role, vitality scores, and RDRS-2 were negatively related to age. Most SF-36 subscores showed a negative relationship with the use of sleep medication. The scores obtained with the different health questionnaires were intercorrelated. Absolute values of the 1997 SF-36 and RDRS-2 scores are presented in Table 1. The SF-36 subscales correlated with GDS scores (correlation coefficients ranging between −0.15 and −0.55; $P < .05$ to $P < .001$). The interrelationships between results of the health questionnaires remained essentially unchanged when correction for an effect of age, alcohol intake, and professional status was applied.

There was no relationship between the GDS score of 1997 and free or total testosterone levels as assessed in 1997 ($P > .05$). The GDS scores did not correlate with AR gene CAG-repeat length ($P > .05$). No association was detected between CAG-repeat polymorphism and serum (free) testosterone within the subgroups with positive and negative GDS score (results not shown). The GDS of 1997 correlated significantly with (free) estradiol ($r = 0.20$, $P < .05$) and with DHEAS ($r = 0.23$, $P < .001$). According to quartiles of sex steroid levels, men with free estradiol in higher quartiles had higher GDS scores ($P = .002$). When only considering the subgroup of possibly depressed elderly (GDS ≥11), the odds ratio of having a GDS score of 11 or greater when presenting a higher free estradiol was 3.67 (4th quartile vs 1st quartile) (data not shown). In addition, a lack of influence of aromatase activity as assessed using the testosterone/estradiol ratio on GDS scores was confirmed. There was no correlation between GDS and SHGB or cortisol levels. SF-36 and RDRS-2 did not correlate significantly with steroid levels, except for a relationship between RDRS-2 and free testosterone, the mental health SF-36 subscore and DHEAS, and the physical functioning SF-36 subscore and testosterone/estradiol.

A repeated cross-sectional analysis on the sample still participating in 2000 confirmed a lack of association between GDS score and androgen levels ($P > .05$) (results not shown). Of 192 participants who completed the GDS in 2000, 17.0% had a score of 11 or greater. GDS scores in 2000 did not correlate with AR gene CAG-repeat length ($P > .05$). The association between GDS and (free) estradiol and DHEAS was not observed in the subgroup (n = 192) of men studied in 2000. A lack of influence of aromatase activity as assessed using the testosterone/estradiol ratio on GDS scores was confirmed.

Longitudinal Analyses

Within this group of elderly men, there was a decrease with age in free testosterone and DHEAS and an increase in SHBG. There was a significant correlation between individual scores on the GDS in 1997 and the results in 2000 ($r = 0.54$, $P = .03$). Considering only those men who were evaluated in both 1997 and 2000, the presence of a GDS score of 11 or greater increased from 11.7% (n = 21) to 18.7% (n = 33). Only four of 33 men with a GDS score of 11 or greater in 2000 had an initially low serum testosterone at baseline, below the normal range for young men (<319 ng/dL). The baseline median GDS score of the subgroup of men who no longer participated in 2000 was 9.95.

Changes in serum (free) testosterone and serum (free) estradiol levels between 1996 and 2000 were not related to GDS score in 2000. Change in body weight between 1996 and 2000 (mean − 0.83 kg, range − 15.0 kg to 17.7 kg) had no influence on the scores.

DISCUSSION

The results of the present study indicate that, in the considered cohort of community-dwelling ambulatory men aged 70 and older, depression as assessed using the GDS is present in 12.7% (in 1997), with an increase from 11.7% to 18.7% in the subgroup followed longitudinally over 3 years. This is in accordance with data from the literature.[15] In the current study, there was no indication of a relationship between GDS and serum androgen levels at different points in time. This finding may seem to contradict those of some previous reports. Endogenous bioavailable, but not total, testosterone, levels were reported to be inversely associated with depressive mood as assessed using the Beck Depression Inventory, but this study considered a broader age group (50–89).[10] Another study found that hypogonadal men showed a greater incidence of depressive illness and a shorter time to diagnosis of depression, but the retrospective design and the broad age range, with subjects as young as 45, were major differences from the present study.[11] In a study of men aged 50 to 70, depressive symptoms assessed using the Carroll Rating Scale showed an inverse correlation with free testosterone, in this study, serum free testosterone was not related to the prevalence of depression.[12] Age-related decline in testosterone levels, persisting over years, may lead to mild depressive illness, or dysthymia, which may remain undetected using the GDS.[13] In contrast with these findings, others have reported that declining bioavailable testosterone levels were associated with lower levels of depressive symptoms on the Hamilton Depression Scale in men aged 55 to 76.[14] In a study based on selected questions from the AMS Scale in 172 men aged 41 to 70, depressed mood and "wish to be dead" did not correlate with hormone levels, but there was a positive correlation with the length of AR CAG repeats.[17] The lack of association between the AMS domain scores or individual questions and sex steroid levels in men aged 70 and older has been reported previously,[19] although the validity of selecting items out of a group of questions that are part of a domain score (AMS was developed as such) seems questionable. In the MMAS, serum testosterone levels were not associated with CES-D–diagnosed depression. In a statistical model combining the three variables, AR polymorphism and testosterone together predicted depression, whereas neither testosterone level nor AR genotype alone was associated with CES-D-defined depression.[16] In the current study, depression was not associated with low serum (free) testosterone levels in men with shorter AR gene CAG

polymorphism in 1997 or 2000, a finding that has to be interpreted with caution, because the number of patients with a GDS score of 11 or greater was small. It seems likely that differences in study population and methodology largely underlined the apparent disparities between studies, the homogenous age range of a population-based sample being the essential feature of the present study.

The prevalence of subnormal (free) testosterone levels in this study group was similar to previous findings.[5,33] Nevertheless, no relationship could be established between androgen status in 1997 or 2000 with GDS or SF-36 scores in this study population. A similar result is described in another study that could not establish a relationship between SF-36 and bioavailable testosterone in a cross-sectional survey of 93 men aged 70 and older.[34] In addition, a baseline testosterone and longitudinal changes in testosterone did not predict the occurrence of depression.

The complete absence of an association between circulating estradiol and depressed mood scores in the Rancho Bernardo study did not support the hypothesis that aromatization to estradiol could mediate the observed testosterone-mood association. In the current study, in the baseline assessment, there was an association between free estradiol and GDS but not SF-36, with a greater odds ratio for a GDS score of 11 or greater in the men in the higher quartile of free estradiol, but these findings were not confirmed in the 2000 data. Moreover, GDS did not correlate to BMI (data not shown) or aromatase activity as assessed using the testosterone/estradiol ratio, which may suggest, but not establish, that this was a spurious finding. This observation deserves further investigation.

A major reason for the lack of association with sex steroid levels may lie in the fact that depression in elderly men is undoubtedly multifactorial in origin.[12] A decrease in almost all physiological functions and a decrease in not only gonadal and adrenal androgen secretion, but also of growth hormone secretion, amongst other changes, accompanies aging.[35] Moreover, age-associated changes in lifestyle may be important, as indicated by the effect of the decrease in physical activity or the decrease in muscle mass.[36] Furthermore, androgen requirements may vary between subjects and in the same subject according to the considered physiological functions.[37]

The present study specifically addresses the situation in an elderly population with a median age of 75 and older. This is of interest because there is little information available for such populations, but it may not be possible to extrapolate the results to younger populations. Second, because this elderly population was relatively healthy, due mainly to the application of stringent exclusion criteria, the results of the present study should also not be extrapolated to less-healthy populations. The study sample cohort consisted of ambulatory men with general good health, which is not representative of the whole cross-section of men of this age.

Nevertheless, in this population of community-dwelling men, there was a consistent correlation between GDS and the results of the nondisease-specific health measures SF-36 and RDRS-2, suggesting that, in this relatively healthy group, the GDS is related to some extent to other health-related variables affecting quality of life, but again, a few SF-36 subscores related to some hormonal parameters.

In conclusion, these findings for the GDS Scale suggest that, in ambulatory elderly men, the perception of symptoms related to major depression is not associated with androgen status as assessed using serum androgen levels.

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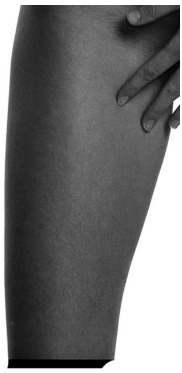

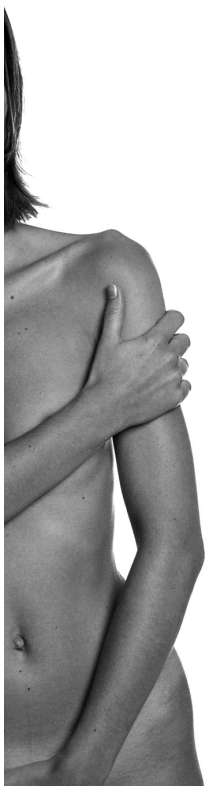

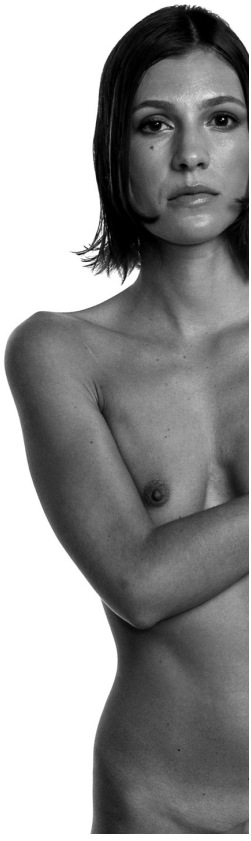

PART II

CHAPTER 5

INFLUENCE OF EXOGENOUS OESTROGEN OR (ANTI-) ANDROGEN ADMINISTRATION ON SOLUBLE TRANSFERRIN RECEPTOR IN HUMAN PLASMA.

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Influence of exogenous oestrogen or (anti-) androgen administration on soluble transferrin receptor in human plasma

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Abstract

The objective of this investigation was to study the effects of sex steroids on levels of haemoglobin (Hb) and haematocrit (Hct) and to analyse whether these effects can be related to levels of the soluble transferrin receptor (sTfR), a marker of erythropoietic activity. Nineteen male-to-female transsexuals were randomly assigned to either oral ethinyl oestradiol (EE) ($n=12$) or transdermal 17β-oestradiol (E2) ($n=7$); both treatments included the anti-androgen cyproterone acetate (CA). Six male-to-female transsexuals were treated with CA only. Fifteen female-to-male transsexuals were treated with i.m. testosterone esters. The Hct, and levels of Hb, IGF-I, GH and sTfR were measured before and after 4 months of hormone administration. Androgen administration significantly increased the sTfR concentration by 31·5% ($P=0·008$) and increased levels of Hct, Hb and IGF-I. Both regimens of CA with oral EE and transdermal E2 reduced plasma testosterone similarly to castrate values and decreased Hb and Hct. The CA+oral EE combination induced a decrease in sTfR of 19·0% ($P=0·002$) which was not the case with CA+transdermal E2 ($P=0·27$). This cannot be explained by the profound decline in plasma testosterone which was similar with both regimens, but this difference could be related to the different effects of the two regimens on plasma IGF-I. This assumption is supported by the positive correlation that was found to exist between plasma sTfR and IGF-I after the interventions ($P<0·05$).
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Introduction

Men have higher haematocrit (Hct) and haemoglobin (Hb) concentrations than women (Morris *et al.* 1956), in all likelihood due to their higher plasma testosterone. Men who are hypogonadal have lower than normal Hct and Hb concentrations and testosterone treatment increases those variables to normal (Tenover 1992, Jockenhovel *et al.* 1997). In support of a role of testosterone is the observation that, in hypogonadal men, administration of transdermal testosterone dramatically increases Hct, from mildly anaemic to midnormal within 3 months (Snyder *et al.* 2000). Also, in healthy young men changes in circulating testosterone concentrations induced by gonadotrophin-releasing hormone (GnRH) agonist and testosterone administration are associated with testosterone dose- and concentration-dependent changes in Hb (Bhasin *et al.* 2001). The effects of testosterone on the Hct have been documented in several small and short-term studies, as reviewed by Hajjar *et al.* (1997). The mechanisms by which androgens exert these effects have not been elucidated. It has been suggested that androgen action on erythropoiesis is mediated by a nuclear androgen receptor (Claustres & Sultan 1988).

Administration of testosterone has been successfully used for the treatment of refractory anaemia in males (Piedras *et al.* 1998). It has been postulated that the administration of androgens to man and laboratory animals results in an increase in plasma erythropoietin activity. However, it has also been shown in recent years that in androgen-induced erythrocytosis, there is no increase in erythropoietin levels (Dickerman *et al.* 1998, 1999). Induction of androgen deprivation with a luteinizing hormone-releasing factor (LHRH) agonist did not result in changes in serum erythropoietin levels (Weber *et al.* 1991). Interestingly, there is no difference in serum erythropoietin levels between men and women, even though there is a significant difference in their testosterone and Hb levels (Miller *et al.* 1985). So, it seems that the androgen-mediated increases in Hb levels and Hct are not exclusively mediated by erythropoietin, and testosterone may have a direct effect on bone marrow stem cells (Shahidi 1973, Krabbe *et al.* 1978, Mooradian *et al.* 1987, Krauss

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et al. 1991). Our study monitored the effects of sex steroids on Hb and the Hct in transsexuals undergoing cross-sex hormone administration, making use of a quantitative assay of bone marrow erythropoietic activity, the soluble transferrin receptor (sTfR).

Iron transport in the plasma is carried out by transferrin. The interaction with a specific membrane receptor, the TfR allows iron to be included in cells. In both animal and human serum a soluble form of the TfR (sTfR) has been identified (Beguin 2003). Marrow erythropoietic activity appears to be the most important determinant of sTfR levels (R'Zik & Beguin 2001). In situations characterized by diminished erythropoietic activity, sTfR levels are decreased. When erythropoiesis is stimulated sTfR is increased. Measurements of sTfR are useful for monitoring the erythropoietic response to various forms of therapy. Predicting an early therapeutic response by measuring sTfR is possible when changes in Hb are not yet apparent (Beguin 2003).

To our knowledge, this is the first paper describing the effects of exogenous oestrogens and (anti-) androgens on the levels of the sTfR.

Materials and Methods

Subjects

We included 25 male-to-female (M→F) and 15 female-to-male (F→M) Caucasian transsexuals. Additional details of this study group can be found in Giltay *et al.* (2000). Psychological criteria for the diagnosis and treatment followed the guidelines provided by the Harry Benjamin International Gender Dysphoria Association (Walker *et al.* 1985). Nineteen M→F transsexuals were open-label-randomised to receive either oral ethinyl oestradiol (EE) (Lynoral, 100 μg/day; Organon, Oss, The Netherlands; $n=12$) or transdermal 17β-oestradiol (E2) (Estraderm TTS 100, 100 μg twice a week; CIBA-Geigy, Basel, Switzerland; $n=7$), both in combination with cyproterone acetate (CA) (Androcur, 100 mg/day; Schering, Berlin, Germany) which is a progestational compound with androgen receptor-blocking properties. Because the effects of the administration of CA alone on sTfR levels are unknown in men, we also studied six M→F transsexuals who received CA alone during the first 4 months of cross-sex hormone administration. F→M transsexuals were treated with testosterone esters (Sustanon, 250 mg/2 weeks, i.m.; Organon). All F→M transsexuals had had regular menstrual cycles (28–31 days) before cross-gender sex hormone administration. There was no evidence of iron deficiency, hypertension, cardiovascular disease, thromboembolism, diabetes mellitus or use of sex hormones in any of the subjects tested. Due to the availability of blood samples, the number of participants in this freezer study is smaller than in the study of Giltay *et al.* 2000 where 30 M→F transsexuals receiving CA+oral or transdermal oestrogens, 10 M→F transsexuals receiving CA alone, and 17 F→M transsexuals were described.

Smoking status (yes or no) and body mass index (BMI, weight/height^2) were also assessed. Informed consent was obtained from all subjects, and the study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethical Review Board of the University Hospital Ghent and the Free University Amsterdam.

Assays

Each subject served as his or her own control, with samples drawn before and during hormone administration. In F→M transsexuals, blood was drawn at baseline between days 5 and 9 of the follicular phase of the menstrual cycle. During testosterone treatment, blood was drawn within 5–9 days after the previous testosterone injection. An intravenous catheter was placed in the antecubital vein of supine subjects after an overnight fast and 10 min of bed rest. Because of the travel time to the clinic, the time of blood sampling was between 0830 h and 1330 h. Within-subject time of sampling was, however, comparable before and after 4 months of hormone administration: mean 1055 h (95% confidence interval (CI), 1037–1112 h) versus mean 1037 h (95% CI, 1019–1055 h) respectively, $P=0{\cdot}10$. The mean intraindividual variation was 50 min (95% CI, 40–70 min). Blood was collected without a tourniquet into evacuated tubes (Diatube H CTAD, i.e. citrate, theophylline, adenosine and dipyridamole; Becton Dickinson, Rutherford, NJ, USA). Samples were immediately placed on ice and centrifuged at 3500 ***g*** for 30 min at 4 °C to obtain platelet-poor plasma. Plasma was separated and snap-frozen within 1 h and stored at −70 °C until analysis. The samples underwent one freeze–thaw cycle before serum sTfR, iron, transferrin and ferritin were measured. Serum sTfR was measured by a commercially available ELISA (R&D, Minneapolis, MN, USA). Serum ferritin was measured by the nephelometry method (Behring Nephelometer Analyzer; Dade Behring Marburg Co. Ltd, Marburg, Germany) following the manufacturer's instructions. Iron and transferrin were measured by standard methods (Modular; Roche). Standardized radioimmunoassays were used to measure serum levels of E2 (Double Antibody; Sorin Biomedica, Saluggia, Italy) and testosterone (Coat-A-Count; Diagnostic Products Corp., Los Angeles, CA, USA). Serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by immunometric luminescence assays. Commercial assays were used for determinations of growth hormone (GH) (Immulite 2000; Diagnostic Products Corp.) and insulin-like growth factor (IGF-I) (extraction method, DSL-5600; Diagnostic System Laboratories, Webster, TX, USA). The effects of transdermal administration of E2 were compared with those of oral EE, both in combination with oral CA.

Table 1 Baseline characteristics of the study cohort

	M→F transsexuals					F→M transsexuals	
	Oral ethinyl oestradiol* plus CA (n=12)	Transdermal 17β-oestradiol* plus CA (n=7)	P†	CA** (n=6)	P‡	Testosterone-esters (n=15)	P§
Age (years)	31 ± 7	27 ± 5	0·144	32 ± 3	0·861	27 ± 6	0·093
BMI (kg/m^2)	23·1 ± 2·8	20·3 ± 2·0	0·035	21·5 ± 2·4	0·255	23·7 ± 4·4	0·113
sTfR (μmol/l)	18·4 ± 3·2	20·8 ± 6·8	0·326	24·2 ± 5·0	0·009	21·3 ± 5·4	0·632
Haematocrit (%)	0·42 ± 0·04	0·40 ± 0·02	0·400	0·44 ± 0·04	0·657	0·37 ± 0·02	<0·001
Haemoglobin (mmol/l)	9·1 ± 1·0	9·3 ± 0·5	0·652	9·2 ± 0·5	0·774	7·9 ± 0·5	<0·001
Serum iron (μg/dl)	129·5 ± 36·2	133·2 ± 50·5	0·855	181·2 ± 55·7	0·036	116·1 ± 57·0	0·160
Ferritin (ng/ml)	122·1 ± 74·8	154·3 ± 68·2	0·369	126·2 ± 75·2	0·919	55·8 ± 40·4	0·001
Transferrin (g/l)	2·57 ± 0·48	2·65 ± 0·33	0·701	2·46 ± 0·43	0·696	2·65 ± 0·46	0·583
IGF-I (ng/ml)	352·6 ± 119·6	386·9 ± 72·5	0·503	357·8 ± 120·5	0·936	390·5 ± 149·8	0·527
GH (ng/ml)	3·05 ± 4·75	1·35 ± 1·71	0·902	4·09 ± 4·40	0·679	4·56 ± 5·20	0·248

Data are means ± S.D. M→F, male-to-female; F→M, female-to-male. *randomised; **non-randomized; †P value by Student's t-test comparing males treated with oral ethinyl oestradiol versus transdermal 17β-oestradiol; ‡P value by Student's t-test comparing males treated with oral ethinyl oestradiol versus those treated with CA only; §P value by Student's t-test comparing all males and females.

Statistics

Most variables in the analysis turned out to be positively skewed. In order to meet the necessary model assumptions, a natural logarithmic transformation in these analyses was used for hormonal parameters and sTfR. All data are given as (geometric) means (± S.D. or 95% CI). Student's t-tests for independent samples or χ^2 tests were used to compare baseline differences. ANCOVA was used for intergroup comparisons after adjusting for possible confounders. Baseline values were correlated with the Pearson correlation coefficient.

An ANOVA for repeated measurements or a Student's t-test for paired samples was used to analyse the effects of cross-gender sex hormones. An ANCOVA was used to compare effects of different treatment regimens and to adjust for potential covariates. Proportional changes, after 4 months of cross-sex hormone administration were correlated with the Spearman correlation coefficient. If values were below the lower limit of detection, the value of that lower limit was used for statistical analysis (1·0 nmol/l for testosterone, 0·3 IU/l for LH and 0·5 IU/l for FSH). A two-tailed P<0·05 was considered statistically significant. The software used was SPSS 10·0 for Windows 8·0.

Results

Pre-treatment values

At baseline, all subjects were eugonadal by clinical and laboratory criteria. Iron deficiency as judged from serum iron, ferritin and transferrin serum levels was not present in any of the participants. Baseline characteristics are presented in Table 1. There was at baseline an expected difference between men and women for Hb, Hct and ferritin (P<0·001), but no significant difference for sTfR, iron or transferrin levels. In M→F transsexuals at baseline, there were no significant differences between the two groups except for BMI that was significantly (P=0·035) higher in males randomised to treatment with oral EE compared with males randomised to treatment with transdermal E2. Between M→F transsexuals treated with oral EE and M→F treated with CA only there was a difference in sTfR and serum iron levels at baseline (both P<0·05). There was no significant difference in sTfR between M→F transsexuals treated with transdermal E2 and M→F transsexuals treated with CA only.

Effects of sex steroid administration

In the group treated with CA alone, there were small but significant decreases in serum levels of testosterone and E2 (P<0·05) after 4 months treatment, and no changes in LH and FSH (P=0·72 and 0·22 respectively). Hb, Hct and sTfR levels did not change, while there was a trend for serum IGF-I to increase (P=NS). No significant relation between IGF-I and sTfR was established.

Oral and transdermal oestrogen administration, both with CA, decreased serum levels of testosterone (both P<0·001), LH (P<0·001 and P=0·007 respectively) and FSH (P<0·001 and 0·003 respectively). The combination of CA+EE administration (the latter not measured by the oestradiol assay used) suppressed endogenous oestradiol levels; whereas percutaneous administration of E2 increased plasma levels of E2 up to values typical of the midfollicular phase in women (Table 2).

In the group receiving CA+oral EE, Hb, Hct, IGF-I and sTfR levels decreased, whereas the increase in GH

Table 2 Laboratory data before and after 4 months of cross-gender sex hormone administration in M→F transsexuals, randomised for administration of oral ethinyl oestradiol in combination with CA or transdermal 17β-oestradiol in combination with CA

	M→F transsexuals (*n*=12), oral ethinyl oestradiol+CA			**M→F transsexuals (*n*=7), transdermal 17β-oestradiol+CA**		
	Baseline	4 months	*P*	Baseline	4 months	*P*
17β-oestradiol (pmol/L)	99·9 ± 33·4	24·7 ± 4·4	<0·001	82·0 ± 17·60	191 ± 152	0·121
Testosterone (nmol/L)	22·6 ± 6·5	1·0 ± 0·1	<0·001	22·1 ± 6·5	1·1 ± 0·2	<0·001
LH (IU/L)	3·2 ± 1·7	0·3 ± 0·0	<0·001	2·4 ± 1·1	0·4 ± 0·3	0·007
FSH (IU/L)	3·2 ± 1·5	0·5 ± 0·0	<0·001	2·0 ± 0·8	0·5 ± 0·0	0·003
Haematocrit (%)	0·42 ± 0·04	0·36 ± 0·02	<0·001	0·43 ± 0·02	0·39 ± 0·01	<0·001
Haemoglobin (mmol/l)	9·1 ± 1·0	8·0 ± 0·4	0·001	9·3 ± 0·5	8·6 ± 0·3	0·001
sTfR (μmol/l)	18·4 ± 3·2	14·9 ± 1·7	0·002	20·8 ± 6·8	19·3 ± 4·0	0·265
IGF-I (ng/ml)	352·6 ± 119·6	244·2 ± 100·7	0·034	386·9 ± 72·5	458·9 ± 78·3	0·004
GH (ng/ml)	3·05 ± 4·75	4·69 ± 4·04	0·415	1·35 ± 1·71	1·63 ± 2·38	0·412

Values are means ± S.D. Ethinyl oestradiol, which suppresses endogenous 17β-oestradiol, cannot be detected in conventional 17β-oestradiol assays.

was not significant. Soluble TfR levels correlated with IGF-I (r=0·643, P=0·024) at month 4. In the group receiving transdermal E2 and CA, there was a decrease in Hb and Hct after 4 months treatment (both P<0·001); the decrease in sTfR was not significant (P=0·27), while serum levels of IGF-I rose (P=0·040). There was a correlation between sTfR levels and IGF-I (r=0·821, P=0·023) at month 4.

After testosterone administration to F→M transsexuals, serum levels of testosterone increased markedly (P<0·001), whereas serum levels of E2, LH and FSH decreased (P=0·03, 0·01 and 0·005 respectively) (Table 3). The F→M transsexuals had significantly higher testosterone levels after 4 months of treatment, compared with the 3 groups of M→F transsexuals at baseline (P<0·001). Hb, Hct, sTfR and IGF-I rose significantly after testosterone administration in F→M transsexuals. There was no significant relation between IGF-I and sTfR in this group at month 4. However, the relation between IGF-I and sTfR at month 4 was confirmed when including all participants, both female and male (r=0·689, P=<0·01). Plasma iron concentrations were unaffected by the different hormonal treatments (data not shown).

Discussion

This study addressed the effects of sex steroids on Hb, Hct and the sTfR. The latter is a marker of marrow erythropoietic activity and is useful as an early indicator of diminished/increased erythopoietic activity (Beguin 2003). At baseline, males and females, in spite of their significant differences in plasma testosterone, Hb and Hct, did not show differences in values of sTfR. The possible interpretation of this observation is that differences in plasma testosterone may produce sex differences in values of Hb and Hct, but once baseline erythropoietic activity is stable, levels of sTfR are comparable in males and females; sTfR being an indicator of increases/decreases in erythropoietic activity.

A clear example of an effect of androgens was provided by testosterone administration to females. The dose of

Table 3 Laboratory data before and after 4 months of cross-gender sex hormone administration in F→M transsexuals receiving testosterone and in M→F transsexuals receiving CA only

	F→M transsexuals (*n*=15), testosterone			**M→F transsexuals (*n*=6), CA only**		
	Baseline	4 months	*P*	Baseline	4 months	*P*
17β-oestradiol (pmol/L)	176·8 ± 77·3	130 ± 34·6	0·03	77·3 ± 15·7	44·8 ± 17·7	0·023
Testosterone (nmol/L)	2·0 ± 0·8	34·6 ± 7·8	<0·001	20·3 ± 4·4	10·2 ± 7·3	0·029
LH (IU/L)	5·8 ± 3·6	2·6 ± 2·1	0·01	2·5 ± 1·2	2·6 ± 1·1	0·717
FSH (IU/L)	4·3 ± 1·0	2·9 ± 1·1	0·005	2·9 ± 2·3	2·6 ± 2·5	0·224
Haematocrit (%)	0·37 ± 0·02	0·40 ± 0·03	0·001	0·44 ± 0·04	0·42 ± 0·04	0·205
Haemoglobin (mmol/l)	7·9 ± 0·5	8·4 ± 0·9	0·016	9·4 ± 0·6	8·7 ± 1·0	0·234
sTfR (μmol/l)	21·3 ± 5·4	28·0 ± 8·4	0·008	24·2 ± 5·0	26·1 ± 3·9	0·350
IGF-I (ng/ml)	390·5 ± 149·9	446·5 ± 145·1	0·050	357·8 ± 120·8	433·6 ± 64·4	0·088
GH (ng/ml)	4·56 ± 5·20	5·18 ± 5·19	0·686	4·09 ± 4·35	2·10 ± 3·03	0·184

Values are means ± S.D.

Table 4 Overview of changes in levels of testosterone, IGF-I, haemoglobin (Hb) and sTfR between baseline and 4 months of cross-gender sex hormone administration

	Testosterone	IGF-I	sTfR	Hb
M→F transsexuals, oral EE+CA	↓	↓	↓	↓
M→F transsexuals, transdermal 17β-oestradiol+CA	↓	↑	=	↓
M→F transsexuals, CA only	↓	=	=	=
F→M transsexuals, parenteral testosterone esters	↑	↑	↑	↑

testosterone administered to females induced plasma testosterone levels in the high-normal or above-normal range for men and after 4 months increased Hb, Hct and sTfR, together with higher serum IGF-I levels (Table 4), but there was no demonstrable correlation between levels of IGF-I and sTfR. However, a reduction in circulating testosterone to approximately 50% of baseline by CA only in males – which also has antiandrogenic effects at the level of the androgen receptor – had no effect on Hb, Hct and sTfR values. A non-significant increase in serum IGF-I was seen in this group, with a non-significant decrease of serum GH levels. In this group, no correlation could be established between levels of IGF-I and sTfR.

The findings in males receiving CA+oestrogens were very remarkable. Both treatment regimens (CA+oral EE and CA+transdermal E2) equally suppressed plasma testosterone levels to castrate values and reduced values of Hb and Hct similarly, but only oral EE significantly reduced levels of sTfR. It should be noted that the effect of transdermal E2+CA on Hb and Hct showed a tendency to be smaller than in the other group receiving CA+oral EE, although not significantly. The difference in effect on sTfR between the two oestrogen regimens cannot be explained by their effects on plasma testosterone levels: both regimens decreased plasma testosterone similarly to castrate values. The quantitative difference in sTfR between oral and transdermal oestrogen may have been related to the different potencies of the two oestrogen regimens. EE is much more biopotent than E2. However, another mechanism to consider may be the impact of the route of oestrogen administration on the somatotropic axis. Previous studies have documented that metabolic effects of oral and transdermal oestrogens may differ, implicating hepatic mechanisms or effects on serum IGF-I in treatment with oral oestrogens (Chetkowski *et al.* 1986, De Lignieres *et al.* 1986, Lisset & Shalet 2003). Approximately 60% of orally administered EE is inactivated by the liver via a first-pass effect through the enterohepatic circulation. Orally administered EE (with a strong hepatic impact) compared with transdermally administered E2 (with less hepatic effects) might produce a different effect on the somatotropic axis. It is known that in post-menopausal women oral EE has a strong hepatic effect with impaired hepatic IGF-I production that causes increased GH secretion through reduced feedback inhibition (Ho *et al.* 2003). This was earlier also shown in the study of Van Kesteren *et al.* (1996) in transsexual persons. In the study by Ho *et al.* (2003) transdermal administration of E2 resulted in a slight increase in serum IGF-I but no change in mean 24 h GH levels. High-dose transdermal oestrogen (200 μg/day) in postmenopausal women did not affect basal levels of IGF-I in the study by Lissett and Shalet (2003). In our study, transdermal E2+CA resulted in a significant increase in IGF-I levels whereas oral EE+CA resulted in a significant decrease of IGF-I levels. CA was part of both oestrogen regimens. There were no demonstrable effects of CA only on Hb, Hct and sTfR, though a tendency of IGF-I to increase with a tendency of GH to decrease were noted. The most likely interpretation is that oral CA, a potent progestagen, increases hepatic IGF-I production with a negative feedback effect on pituitary GH production. The addition of transdermal E2 to CA probably had little or no additional effect on the increase of serum IGF-I observed with administration of CA only. This is consistent with earlier observations on transdermal E2 on the IGF-I/GH axis. In agreement with data from the literature, oral EE treatment may have counteracted the tendency to IGF-I increase which was seen with CA only administration. The significant decrease of IGF-I following administration of CA+EE substantiated this assumption.

Androgen therapy leading to supraphysiological plasma testosterone levels produced a stimulation of bone marrow erythropoietic activity, possibly through a direct effect of testosterone on erythropoiesis, but possibly in part mediated through IGF-I induction. The administration of CA only resulted in an approximately 50% reduction in plasma testosterone but had no effect on Hb, Hct or sTfR, possibly explained by the concomitant rise of IGF-I. There was no correlation between levels of IGF-I and of sTfR in this group. Transdermal E2 combined with CA had similar increasing effects on IGF-I levels as CA only, without a demonstrable effect on sTfR, but there was a correlation between levels of IGF-I and sTfR. The decrease in Hb and Hct must probably be ascribed to the profound fall in plasma testosterone. However, administration of oral EE combined with CA reduced sTfR levels significantly, with a correlation between levels of IGF-I and of sTfR. The effects of the decrease in testosterone and IGF-I levels may be responsible for the significant decrease in sTfR levels upon administration of CA with oral oestrogens, while after transdermal

oestrogens plus CA – in spite of a decline of plasma testosterone – there was no decrease of sTfR; this can probably be explained by the rise of IGF-I with the latter regimen. This speculation is further substantiated by the significant correlation of plasma sTfR and IGF-I following both oestrogen regimens.

A larger number of participants and the measurement of other potential confounders, such as erythropoietin levels, would have strengthened the interpretation of these data. This study paves the way for other larger studies that are needed to assess these interactions further. Evaluating the various effects in shorter intervals and for a longer period of time would be interesting in order to see if described effects do or do not persist.

In conclusion, our results show that profound alterations in plasma testosterone levels from male to female values, and vice versa, are associated with significant changes in Hb and Hct of the order of 10%. A reduction of plasma testosterone of the order of 50% had no impact on Hb and Hct. We tested whether these changes in Hb and Hct could be related to levels of sTfR, a marker of diminished/increased erythropoietic activity. Indeed, the increase in Hb/Hct upon testosterone administration was associated with an increase in sTfR, while there was no change in sTfR levels with lack of change of Hb/Hct upon administration of CA. The fall in Hb/Hct levels upon administration of CA combined with either oral EE or transdermal E2 had disparate effects on levels of sTfR: a decline with CA+oral EE, but no effect on levels of sTfR was observed with CA+transdermal E2. This disparity cannot be explained by the effects these two hormone regimes had on plasma testosterone. Earlier studies have indicated that administration of oral EE reduces plasma IGF-I, which is not the case with transdermal E2. In addition, indeed, in our study a correlation between plasma IGF-I and sTfR could be demonstrated after the intervention with the two modes of oestrogen administration and for the total group of patients. This assumption is not fully supported by the results of testosterone administration: this resulted in a rise of both plasma IGF-I and sTfR but these increases did not correlate.

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CHAPTER 6

SEXUAL AND PHYSICAL HEALTH AFTER SEX REASSIGMENT SURGERY.

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ENDOCRINE TREATMENT OF TRANSSEXUAL PEOPLE: A REVIEW OF TREATMENT REGIMENS, OUTCOMES AND ADVERSE EVENTS.

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Sexual and Physical Health After Sex Reassignment Surgery

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A long-term follow-up study of 55 transsexual patients (32 male-to-female and 23 female-to-male) post-sex reassignment surgery (SRS) was carried out to evaluate sexual and general health outcome. Relatively few and minor morbidities were observed in our group of patients, and they were mostly reversible with appropriate treatment. A trend toward more general health problems in male-to-females was seen, possibly explained by older age and smoking habits. Although all male-to-females, treated with estrogens continuously, had total testosterone levels within the normal female range because of estrogen effects on sex hormone binding globulin, only 32.1% reached normal free testosterone levels. After SRS, the transsexual person's expectations were met at an emotional and social level, but less so at the physical and sexual level even though a large number of transsexuals (80%) reported improvement of their sexuality. The female-to-males masturbated significantly more frequently than the male-to-females, and a trend to more sexual satisfaction, more sexual excitement, and more easily reaching orgasm was seen in the female-to-male group. The majority of participants reported a change in orgasmic feeling, toward more powerful and shorter for female-to-males and more intense, smoother, and longer in male-to-females. Over two-thirds of male-to-females reported the secretion of a vaginal fluid during sexual excitation, originating from the Cowper's glands, left in place during surgery. In female-to-males with erection prosthesis, sexual expectations were more realized (compared to those without), but pain during intercourse was more often reported.

KEY WORDS: transsexualism; gender identity disorder; sexual functioning; orgasm; sex reassignment surgery.

INTRODUCTION

Hormonal treatment and sex reassignment surgery (SRS) are both considered the treatment of choice for transsexual persons. Evaluations of these treatments are still needed. In this study, a long-term follow-up investigation of 55 patients post-SRS was carried out to evaluate sexual and general health outcome. Since the start of the multidisciplinary Ghent Genderteam, we have always used a dual-phase hormonal schedule, with a first reversible part where sex specific features are suppressed, together with starting the real-life test. In the second part, cross-sex hormones were given, resulting in irreversible feminization and masculinization. In some centers, spironolactone (Prior, Vigna, & Watson, 1989) or cyproterone acetate (van Kesteren, Asscheman, Megens, & Gooren, 1997) are routinely added to estrogen treatment at the beginning of the hormonal treatment, whereas in other centers cross-sex hormonal treatment is started as a unique treatment. No randomized studies are as yet

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available to determine the optimal dosage of hormones or the preferred regimen for the treatment of transsexuals. A variety of compounds are used in high dosages, increasing the risk of side effects (Asscheman & Gooren, 1992). The Amsterdam group reported a high incidence of depressive mood changes, hyperprolactinaemia, and thromboembolic events, compared to a normal population (Asscheman, Gooren, & Eklund, 1989).

The first aim of this study was therefore to evaluate the long-term safety of the Ghent hormonal treatment regimen. Secondly, where most studies on transsexual people focus on long-term psychological, surgical, and physical health (Eldh, Berg, & Gustafsson, 1997; Pfäfflin & Junge, 1998), a surprisingly small number of studies have focused on the sexual life of postoperative transsexuals, although adequate sexual functioning is universally acknowledged as an important component of mental health. Little attention has been given to this subject and, indeed, the vast majority of follow-up studies investigated the sexual functioning only as part of the psychological or the surgical outcome.

Although there is general agreement that female-to-male transsexuals are mostly attracted to females, discordant findings where female-to-male transsexuals were attracted to men have been reported by Chivers and Bailey (2000), indicating that this group may not be homogenous. Also Coleman, Bockting, & Gooren (1993) reported on nine female-to-male transsexuals sexually attracted to men. The group of male-to-females is known to be more heterogeneous and figures vary from 23 to 58% of male-to-females attracted to women (De Cuypere, Jannes, & Rubens, 1995; Smith, 2002). Until recently, it was also assumed that the sexual orientation of transsexual people did not change during transition. Daskalos (1998) reported on 6 male-to-females (out of 20) who reported that their sexual orientation had switched from attraction to females to attraction to males. The respondents themselves explained these changes as part of their emerging female gender identity. Before SRS, they had tended to conform as "normal males," which implied being attracted to women. Most male-to-females look for a new partner after SRS, whereas female-to-males tend to remain with the same partner (Bodlund & Kullgren, 1996; Köckott & Fahrner, 1988; Steiner & Bernstein, 1981). It is remarked (Eldh et al., 1997; Köckott & Fahrner, 1988; Pfäfflin & Junge, 1990) that not all transsexual people wish to inform their new partners about their transsexual past and more male-to-females (up to one-third) than female-to-males manage to keep silent about their past.

Few studies deal with the topic of masturbation. Female-to-males are supposed to masturbate more frequently than male-to-females and more frequently than before SRS (Kuiper, 1991; Smith, 2002; Sorensen, 1981a, 1981b). The data about reaching orgasm after SRS are very inconsistent throughout the literature. Lindemalm, Korlin, and Uddenberg (1986) reported that 54% of male-to-females were not able to reach orgasm. Blanchard, Legault, and Lindsay (1987) described that the capacity for orgasm in male-to-females decreased after SRS, which is contradicted by the data by Kuiper (1991). Pfäfflin and Junge (1990) and Eicher, Schmitt, and Berger (1991) described that 70–80% of the male-to-females were capable of orgasm even during intravaginal intercourse. Although not all postoperative transsexual people are orgasmic, there is a much wider sexual satisfaction after transition. It is possible to change one's body image and be sexually satisfied, despite inadequate sexual functioning (Lief & Hubschman, 1993). Recent studies show good genital sensitivity, probably as a result of advances in surgical techniques where, for example, in phalloplasty one forearm nerve is anastomosed to one of the dorsal clitoral nerves (Monstrey et al., 2001).

Our center is presently among those with extensive experience in phalloplasty (P.H and S.M). This gives us the opportunity to focus on the sexual consequences of this particular surgical intervention. As the number of male-to-females and female-to-males was similar and both groups had followed a similar procedure—screened and treated by the same professionals—we were able to compare the male-to-female and the female-to-male transsexuals for different topics of sexual health. Finally, this descriptive study also focused on lubrication during sexual arousal, as some postoperative male-to-female patients spontaneously mentioned this.

METHOD

Participants

In 1985, the Ghent Genderteam started treating persons with gender identity disorder using a multidisciplinary approach. The first surgery (a vaginoplasty) took place in 1988. Our surgeons have offered phalloplasty since 1993.

A total of 107 Dutch speaking patients who underwent SRS between 1986 and 2001 were contacted to participate in this study. We selected only Dutch speaking patients because they followed the same treatment procedure and also because of practical reasons (distance from the hospital and standardization of translated questionnaires). A minimum delay period of 1 year after SRS was respected. This year is often called the honeymoon period and therefore does not represent a realistic picture of

long-term emotional stability, sexual, and psychological status. Of the 107 patients who were eligible for the study, 30 could not be contacted (in majority male-to-females) and 15 (mainly female-to-males) declined to participate. Seven patients wanted to cooperate only if it did not involve attending the hospital visit. The other 55 participants (32 male-to-females and 23 female-to-males) completed the questionnaires and were subsequently interviewed on a face-to-face basis by a sexologist (R.B.), endocrinologist (G.T.), and examined by a surgeon (G.S.). None of the researchers had been involved in the initial assessment or treatment of the patients. All of the male-to-females underwent vaginoplasty, 21 female-to-males had a phalloplasty whereas 2 female-to-males had not yet made up their minds about having one. Most of the female-to-males chose to undergo a phalloplasty, as the outcome is mostly very satisfying. A total of 28 male-to-female transsexuals and 20 female-to-male patients agreed to additional blood testing (examination of hormonal parameters, lipids, glucose, and liver and renal function).

At our center, when the transsexual patient has passed the first diagnostic phase, she/he is referred to the endocrinologist for a general health survey and hormonal therapy. The treatment regimen used at our center is somewhat particular. The transsexual patient will first undergo a (reversible) chemical castration for approximately 1 year, before receiving hormones of the opposite sex, after which irreversible changes occur. The first reversible phase includes anti-androgens (e.g., cyproterone acetate 50–100 mg daily) in male-to-female transsexuals and progestins (e.g., lynestrenol 5 mg daily) in female-to-male transsexuals. The reversible phase is considered an important phase of the real-life test, in which gender-specific features, such as erections or menstrual bleeding, are suppressed. The dual-phase hormonal therapy gives the patient more opportunity for reflection and adaptation to the new sex. In the second part, cross-sex hormones are given for 1 year, resulting in irreversible feminization or masculinization. The administration of these hormones may lead to side effects, all the more so because the cross-sex hormones are administered life-long (Moore, Wisniewski, & Dobs, 2003). Surgical sex correction is considered after 2 years of hormonal therapy during which the patient has to pass *the real-life test or experience*: the patient has to live in the opposite sex role within her/his own personal and professional life when cross-sex hormones are started. Most gender teams have adopted the Standards of Care of the Harry Benjamin International Gender Dysphoria Association, which have clearly defined the indications and methods for hormonal and sex reassignment surgery of gender dysphoric patients (Meyer et al., 2001). The policy of our Genderteam implies a longer waiting period for cross-sex hormones compared to other centers, increasing the risk of self-medication. However, explanation of the rationale behind the protocol usually prevents patients from doing so.

The male-to-females were significantly older than the female-to-males at the time of SRS, $t(52) = -4.7$, $p < .001$, as well as at the time of research, $t(53) = -3.6$, $p = .001$ (Table I). The mean follow-up period was different for both groups, with a longer period for the female-to-males, $t(52) = 2.6$, $p = .013$. Regarding the duration of relationship, no significant difference between the two groups was found. For both male-to-females and female-to-males, there were no significant differences in age of the patient and their respective partners. A

Table I. Description of Study Participants

	Male-to-females ($n = 32$)		Female-to-males ($n = 23$)			
	M	*SD*	*M*	*SD*	t or χ^2	*p*
Age at time of SRS (years)	37.8	8.9	26.9	7.2	−4.7	<.001
Age at time of interview (years)	41.5	9.1	33.2	7.0	−3.6	.001
Duration of follow-up period (years)	3.8	2.7	6.2	4.2	2.6	.013
Duration of relationship (years)	5.1	5.1	5.2	5.2		*ns*
Age of partner (years)	41.1	10.0	29.0	3.5	−3.1	.006
Height (m)	1.76	0.08	1.65	0.07	−4.8	<.001
Weight (kg)	73.4	13.2	63.9	9.0	−2.8	.008
Body Mass Index (kg/m^2)	23.7	3.9	23.4	2.8	−2.8	*ns*
Alcohol intake on a weekend/nonworking day (g)	43.4	40.6	45.9	37.6	0.2	*ns*
Alcohol intake on a working day (g)	26.3	10.0	26.5	17.2	0.2	*ns*
Active smoking (*n*)	15	46.9%	4	17.4%	7.3	.026
Homosexual orientation (*n*)	18	56.3%	21	91.3%	8.1	.004

homosexual orientation was reported in 56.2% of male-to-females and in 91.3% of female-to-males, $\chi^2(1, n = 54) = 6.12$, $p = .013$. At the time of the interview, there was a significant difference in age between nonhomosexual and homosexual male-to-females (45.6 and 37.5 years, respectively, $t(31) = -2.804$, $p = .009$.

All participants gave written informed consent for participation in this study, approved by the Ethical Committee of the Ghent University Hospital.

Measures

General Health

As sex steroid treatment is known to be associated with several side effects, selected post-surgery data were collected from this sample. Current and past hormonal treatment, smoking and drinking habits, other medical treatment, and general health issues, such as thrombogenic accidents, heart conditions, hypertension, depression, hyperprolactinaemia, thyroid problems, hyperlipidemia, liver function problems, and osteoporosis, were addressed. This information was discussed in detail with the patient, and confirmed by physical examination (e.g., blood pressure), laboratory assessment (e.g., prolactin levels), review of medication intake, and systematic review of the medical records. It is, however, possible that patients gave incorrect information about, for example, use of alcohol and tobacco to present themselves favorably to their clinicians.

Biographical Questionnaire for Transsexuals and Transvestites

Data were derived from an extensive, structured interview (BVT, Biographical Questionnaire for Transsexuals and Transvestites; Verschoor & Poortinga, 1988) in which each question has fixed response categories. This interview is used in all Dutch gender clinics as part of the intake procedure at the initial diagnostic assessment (Doorn, Poortinga, & Verschoor, 1994). This descriptive questionnaire contains items referring to sociodemographic information, gender development during adolescence and adulthood, preadolescent gender behavior, transvestite practice, sexuality, and medical antecedents (250 items). Selected items were reapplied in this study: stable sexual relationship (e.g., "Do you have a stable sexual relationship?"), sex of the partner, sexual satisfaction with the partner, frequency of orgasm (during intercourse), frequency of masturbation, and frequency of sexual arousal. In this way, pre- and posttreatment data were obtained. Items as start of relationship, duration of relationship, age of partner, sexual satisfaction in general, improvement/worsening of sex life, frequency of orgasm during masturbation, change in orgasmic feelings, lubrication, meeting the expectation of SRS, pain during intercourse, and general satisfaction describing the postsurgical condition were incorporated in a self-developed questionnaire with fixed response categories. If applicable, the categories were quantified from 1 = never to 4 = (almost) always.

Body Image Scale

The Body Image Scale (BIS; Lindgren & Pauly, 1975), adapted for a Dutch sample by Kuiper (1991), was used. This scale consists of 30 items divided into three subscales: primary, secondary, and neutral sexual characteristics, with higher scores representing more dissatisfaction (5-point category). The internal consistency was good for the versions for both female-to-male and male-to-female transsexuals, with Cronbach's $\alpha = .88$ and .87, respectively.

Satisfaction with the Surgical Results

The satisfaction with mastectomy and phalloplasty or mammoplasty and vaginoplasty was evaluated by the patient on a 5-point rating scale (1 = *very unsatisfied* to 5 = *very satisfied*).

Hormone Assays

Following the personal interviews, venous blood was obtained between 08:00 and 12:00 hr after an overnight fast, but because of practical reasons, regardless of the timing of the last administration of the hormonal treatment. This was important in female-to-males in regard to long-acting intramuscular testosterone administration. The sex steroid levels were measured in each participant in order to evaluate these hormones that may affect features of virilization or feminization. The gonadotropin levels were measured to detect more subtle variations in the hypothalamo–pituitary–gonadal axis. To avoid effects of seasonal variation, blood collection was completed in a period of 2 months. Commercial kits for radio immunoassay (RIA) were used to determine the serum concentrations of testosterone (Medgenix Diagnostics, Fleurus, Belgium) and estradiol (Incstar, Stillwater, MM, USA); commercial kits for immuradiometric assays were used for determinations of serum sex hormone binding globulin (SHBG; Orion Diagnostica, Espoo, Finland),

Dehydroepiandrosterone-sulphate (DHEA-S; DSL Inc., Webster, TX), LH (luteinizing hormone), and FSH (follicle stimulating hormone; Medgenix Diagnostics); the latter hormone levels were assessed because of their potential ability to reveal changes in gonadal function (Deslypere et al., 1987). Dihydrotestosterone was assayed by an in-house RIA following chromatographic separation.

Serum-free and bioavailable testosterone (T) and estradiol were calculated from the total serum hormone concentrations, serum SHBG, and serum albumin using a validated equation derived from the mass action law (Vermeulen, Verdonck & Kaufman, 1999).

RESULTS

Physical and Endocrinological Parameters

Female-to-males were significantly shorter, $t(46) = -4.8, p < .001$, and weighed less, $t(46) = -2.8, p = .008$, than male-to-females; results that remained unchanged when correction for age was applied (Table I). Body mass index (BMI) was comparable (27 kg/m^2) in both groups. Alcohol intake was mild and similar in both sexes. Nearly 50% of male-to-females compared to only 20% of female-to-males smoked cigarettes, $\chi^2(1) = 7.3, p = .026$. In this postoperative setting, 72% of male-to-females were on estradiol, whereas others were taking conjugated estrogens ($n = 3$), estradiol–cyproterone acetate ($n = 2$), estrogen–progestagen ($n = 1$), or no hormonal treatment ($n = 1$). One patient suffered a stroke, an absolute contraindication for further estrogen treatment. This patient was not included in further hormonal analysis. Sixty-five percent of female-to-males were treated by intramuscular testosterone and 30% by oral testosterone undecanoate. One female-to-male transsexual was not on androgen treatment because of recurrent liver function problems. He was also excluded from further hormonal analysis.

As expected, there was a significant difference in androgen levels between female-to-males and male-to-females, Mann–Whitney test $U = 0.0, p < .001$ (Table II). Hematocrit, partly reflecting androgen exposure, was significantly higher in female-to-males, $t(46) = 5.9$, $p < .001$. However, the median testosterone value in this group was low (285.0 ng/dl), with 25% of patients reaching the cut-off value for hypogonadism of 320 ng/dl. Usually testosterone treatment should aim at testosterone concentrations in the mid-normal male testosterone range. LH and FSH levels were 18.4 and 34.0 mU/ml in male-to-females, and 39.2 and 97.0 mU/ml in female-to-males, higher than normal values (1–9 and 1–12, respectively). Although all male-to-females had total testosterone levels within the normal range for non-transsexual women (10–80 ng/dl) because of the estrogen effect on sex hormone binding globulin, only 32.1% reached normal free testosterone levels (0.2–0.5 ng/dl).

Female-to-male patients rarely reported physical co-morbidity. One patient was treated for hypertension and one patient for depression; one patient had Type 1 diabetes and one patient had been diagnosed with Type 2 diabetes. One patient reported liver problems, already present before hormonal treatment. Twenty-one percent of male-to-female patients developed hypertension (>160/95 mmHg) during sex steroid treatment and 14.3% reported having had a prolactin level above the upper limit of normal, which was confirmed by review of medical records. Prolactin levels in all participants were within the normal limits on blood testing at the time of research. Twenty-five percent of male-to-female patients were treated for depression. Hypothyroidism and hyperlipidemia were mentioned by 7.1% of participants, whereas one patient had suffered a stroke. No malignancies were reported. No further abnormalities were observed on the blood examination (data not shown). We are not aware of any death by suicide in the total group of transsexuals since the initiation of our gender team.

Partner Relation Parameters

More often transsexuals had a stable sexual relationship after SRS (52.7%) compared to before (35.3%), $\chi^2(1, n = 51) = 5.06, p = .025$ (Table III). This was particularly the case in male-to-females, $\chi^2(1, n = 32) = 4.08, p = .043$). The female-to-males had more difficulties in starting a new relationship after transition. Between the two groups, there was no difference in having a stable relationship, $\chi^2(1, n = 55) = .37$, *ns*. Nearly one out of four participants did not have a sexual partner since SRS. Half of the participants were in a relationship before or during transition, whereas the others started a new relationship after surgical reassignment. Before SRS, female-to-males all had sexual partners of the same biological sex, where after SRS one female-to-male chose a male partner. A female partner was chosen by 45.5% of the male-to-females before SRS, whereas after surgery only 26.3% had a female partner, $\chi^2(1, n = 32) = 0.0$, *ns*). All partners, except for one partner of a male-to-female transsexual, had been informed about the transsexual past. After SRS, 80% of all participants expressed their satisfaction with their relational and sexual life. In particular, a tendency

Table II. Hormonal Parameters

	Male-to-females ($n = 28$)	Female-to-males ($n = 19$)	t or U	p
LH (mU/ml)			4.32	<.001
M	18.4	39.2		
SD	12.2	20.3		
FSH (mU/ml)			5.2	<.001
M	34.0	97.0		
SD	27.5	50.3		
Prolactin (ng/ml)			199.0	.09
Mdn	4.7	6.6		
IQR	3.4–6.9	4.1–9.8		
DHEA-S (μg/dl)			116.0	.001
Mdn	105.5	202.0		
IQR	74.0–165.8	124.0–320.0		
Testosterone (ng/dl)			0.0	<.001
Mdn	18.1	285.0		
IQR	12.4–27.8	155.0–823.0		
Free T (ng/dl)			0.0	<.001
Mdn	0.12	5.7		
IQR	0.1–0.3	3.6–19.4		
SHBG (nmol/l)			34.0	<.001
Mdn	98.6	22.1		
IQR	53.0–195.3	11.4–34.2		
DHT (ng/dl)			20.0	<.001
Mdn	6.8	42.4		
IQR	4.5–8.4	23.9–60.6		
Estradiol (pg/ml)			180.0	.037
Mdn	13.1	20.4		
IQR	9.3–31.0	16.3–32.1		
Hematocrit (%)			5.9	<.001
M	40.2	44.9		
SD	2.6	2.8		

Note. T: testosterone; SHBG: sex hormone binding globulin; DHEA-S: dehydroepiandrosterone-sulphate; DHT: dihydrotestosterone.

was noted for more female-to-males to report sexual satisfaction in a relation after SRS compared to before.

Sexual Satisfaction

A total of 5 (9%) out of 55 participants, 3 male-to-females and 2 female-to-males, reported not having any sexual activity. For those who had sexual activity, 30 (60%) participants were very satisfied with their sex life, 18% remained neutral, and 22% were dissatisfied (single-item measure; Table IV). The participants with a partner were more satisfied with their sex life than those who remained single, $\chi^2(1, n = 55) = 3.61$, $p = .058$. A significant correlation between general and sexual satisfaction was found, $r(49) = .49, p < .001$. When evaluating changes in sex life before and after SRS, 75.5% of participants indicated an improvement and 12.3% a worsening. Pain, lack of sensation, and difficulties to relax were reported in this context. A correlation between the improvement in sex life and the satisfaction with the new primary sex characteristics (scored with BIS) was found, $r(47) = .29$, $p = .043$. This correlation was not found when evaluating satisfaction with the secondary or neutral sexual characteristics. After SRS, the participants were more often sexually excited than before. This difference was only statistically significant in the male-to-females, $\chi^2(1, n = 29) = 5.78$, $p = .016$. The more frequently participants experienced sexual excitement, the more they felt their sexual life had improved, $r(49) = .38$, $p = .007$.

Orgasm

Female-to-male participants masturbated more frequently after SRS compared to before, $\chi^2(1, n = 15) = 5.14$, $p = .023$. Before SRS, there was no difference in frequency of masturbation between the two groups,

Table III. Sexual Relationship Parameters Before and After Sex Reassignment Surgery

	Male-to-females			Female-to-males		
	Before	After	*p*	Before	After	*p*
Stable sexual relationship						
N	32	32		19	23	
%	34.4	59.4	.043	36.8	43.5	*ns*
No sexual partners since SRS						
N		32			23	
%		21.9			30.4	*ns*
Start relationship						
N		19			9	
%	52.6	47.4	*ns*	44.4	55.5	*ns*
Sexual partner (*N*)	11	19		9	10	
Man (%)	54.5	73.7		100.0	90.0	
Woman (%)	45.5	26.3	*ns*	0.0	10.0	*ns*
Sexual satisfaction with partner (*N*)	9	19		6	11	
(Very) satisfied (%)	77.7	78.9		50.0	81.9	
Neutral–(very) unsatisfied (%)	22.2	21	*ns*	50.0	18.1	*ns*
Frequency orgasm in sexual intercourse (*N*)	24	28		11	18	
(Almost) always (%)	41.7	50.0		45.5	77.8	
Never–sometimes (%)	58.3	50.0	*ns*	55.5	22.2	*ns*

Note. *p*: *p* value of difference before and after SRS, McNemar test; for χ^2 test between-group differences after SRS, *p* values, all *ns*.

Table IV. Sex Life Before and After SRS

	Male-to-females			Female-to-males		
	Before	After	*p*	Before	After	*p*
Sexual satisfaction (*N*)		29			21	
Satisfied (%)		48.3			76.2	
Neutral (%)		27.5			4.8	
Unsatisfied (%)		24.2			19.0	
Comparison of sex life (*N*)		29			21	
Improvement (%)		75.8			75.0	
Unchanged (%)		10.3			15.0	
Worsening (%)		13.8			10.0	
Sexual arousal (*N*)	29	32		15	23	
(Very) often (%)	17.2	46.9		40.0	60.9	
Never–sometimes (%)	82.8	53.1	.016	60.0	39.1	*ns*
Frequency masturbation (*N*)	29	31		15	23	
(Very) often (%)	34.5	32.3		20.0	78.3	
Never–sometimes (%)	65.5	67.7	*ns*	80.0	21.7	.023
Orgasm during masturbation (*N*)		23			19	
(Almost) always (%)		65.2			94.7	
Never–sometimes (%)		33.8			5.3	
Change in orgasmic feelings (%)		79.2		73.7		
Secretion during excitement (%)		64.3				
Secretion during orgasm (%)		76.0				

Note. *p*: *p* value of difference before and after SRS, McNemar test; χ^2 test for between-group differences after SRS, all *p* values *ns*, except for frequency of masturbation, $p = .02$.

Table V. Satisfaction with Surgical Results (%)

	Male-to-female		Female-to-male	
	Breast augmentation ($n = 21$)	Vaginoplasty ($n = 29$)	Mastectomy ($n = 14$)	Phalloplasty ($n = 19$)
Very satisfied	66.6	48.3	35.7	33.3
Satisfied	28.6	37.9	42.8	55.5
Neutral	4.8	10.3	21.4	11.1
Unsatisfied	0.0	0.0	0.0	0.0
Very unsatisfied	0.0	3.4	0.0	0.0

whereas after surgery, the female-to-males masturbated more, $\chi^2(1, n = 54) = 14.19$, $p = .007$, regardless of having a partner. Seventy-eight percent of the total group was able to reach an orgasm through masturbation. No significant difference was found between female-to-males and male-to-females regarding the ability to reach orgasm during sexual activity with their partner, $\chi^2(1, n = 46) = 1.07$, *ns* (Table III). In our experience, female-to-males' sexual activity preoperatively involved clitoral stimulation rather than vaginal intercourse, whereas following phalloplasty it referred mostly to intercourse with their new penis. On the other hand, in male-to-females preoperative sexual activity occurred through vaginal and anal penetration, and postoperatively mostly by vaginal intercourse.

The majority of the male-to-females and the female-to-males reported changes in their orgasmic feelings. These feelings changed in both groups: a more powerful and shorter orgasm for the female-to-males, and a more intense, smoother and longer orgasm for the male-to-females. More than two-thirds of the male-to-females reported the secretion of a fluid in the neovagina, not only during orgasm but also during sexual excitation.

Expectations

The transsexual persons' expectations (both in female-to-males and male-to-females) were met on the physical, emotional, and social level and less on the sexual level, with satisfaction rates of 81.5, 94.4, 90.7, and 66.7%, respectively.

Surgical Parameters

Most participants were very satisfied with the mammoplasty and vaginoplasty, except for one female with a shallow vagina, rendering intercourse difficult (Table V). There was a correlation between satisfaction with vaginoplasty and sexual life, $r(26) = .43$, $p = .029$. This correlation was not found for breast augmentation alone. Most female-to-males expressed their satisfaction with the mastectomy and the phalloplasty. Nobody was disappointed and two participants remained neutral. One patient suffered a partial necrosis of the neophallus and mild urinary incontinence was the problem in another man. A correlation for the female-to-males between satisfaction with sex life and satisfaction with the surgical results was seen, especially regarding the phalloplasty, $r(17) = .68$, $p = .003$.

Table VI shows the comparison on several sexual items between the female-to-male patients with an erection prosthesis and those without it. Most participants without prosthesis were considering having one in the future. As the number of participants was too small for further statistical testing, we could only notice the following trend. In female-to-males with prosthesis, sexual expectations were more realized. They had more sexual

Table VI. Female-to-Males with and Without Erection Prosthesis

	With prosthesis, % ($n = 12$)	Without prosthesis, % ($n = 10$)
(Nearly) totally realization of expectation	83.3	60.0
More than one partner since SRS	66.7	40.0
Stable sexual relationship	50.0	40.0
(Very) satisfied with sex life	75.0	77.8
Improvement of sex life	83.3	62.5
Sexuality is (very) important	91.7	50.0
(Very) often excited sexually	58.3	60.0
Often preoccupied with provoking sexual fantasies	50.0	13.5
Often (from several times/week until 1 time/month) masturbation	91.7	80.0
(Mostly) always orgasm during masturbation	90.9	100
(Mostly) always orgasm during intercourse	60.0	100
Never pain during intercourse	44.5	100

partners and experienced a considerable improvement of their sex life since SRS. Sexuality was a more important aspect of life, and they were more often preoccupied with sex. Nevertheless, both groups expressed the same degree of sexual satisfaction. Transsexuals with a prosthesis more often experienced pain during intercourse and possibly therefore were less able to reach orgasm during intercourse.

DISCUSSION

The purposes of this study were to describe our specific hormonal treatment regimen, to explain the rationale behind it, to evaluate its long-term safety and, to evaluate sexual health in postoperative transsexual patients.

Contrary to the usual practice, whereby an inclination to maximize hormone dosage by patients as well as physicians is seen, our gender team has always treated patients by a mild dual-model hormonal scheme, aiming at no or minimal side effects. We believe that pharmacological ablation of endogenous sex steroid production prior to the initiation of exogenous cross-sex steroid treatment may be advisable, in order to reduce dosage of administered hormones and thus morbidity. One could expect that a lower dose of cross-sex hormones may then be sufficient because of increased sensitivity of tissues. Administration of estrogens alone will suppress gonadotropin output and by consequence androgen production, but dual therapy with one compound that suppresses androgen secretion or action and a second compound that supplies estrogen is likely to be more effective (T'Sjoen, Rubens, De Sutter, & Gooren, 2004). Of course, these ideas need systematic clinical trials in order to find an ideal treatment, where tailoring for the individual patient will still be necessary. In this study, it was shown that male-to-females reported more frequently a decrease in libido. We recommended they would start hormonal therapy with antiandrogens to let them adapt to low testosterone levels. This phase may prove to be an extra diagnostic test. It is remarkable that in postoperative male-to-females only a minority reached normal free testosterone levels (reference range for women). It may be that male-to-females need a small amount of androgens to be libidinous, as is suggested for women with hypoactive sexual desire disorder (Laughlin, Barrett-Connor, Kritz-Silverstein, & von Muhlen, 2000).

Relatively few and minor morbidities were observed in our patient group that were mostly reversible with appropriate treatment, possibly in part related to response bias. A trend toward more general health problems in male-to-females was seen, which could be explained by the older age and smoking habits of this group. The number of complications reported here was markedly lower than those described by the Amsterdam group, who reported on a much larger group of transsexual patients (van Kesteren et al., 1997). Our treatment schedule of cross-sex hormones is acceptably safe and has some tentative management and diagnostic implications for the hormonal treatment of transsexual persons. The low testosterone values found in female-to-males in our study can be explained by the fact that blood sampling was performed regardless of the moment of the last administration of the hormonal treatment; however, the high gonadotropin levels that were measured led us to optimally adjust the testosterone dosage.

In accordance with other studies, our data showed that male-to-females were significantly older than female-to-males. The age difference at time of SRS could be related to the fact that 43.7% of our male-to-female patients was nonhomosexual compared to 8.6% of our female-to-males patients. This finding is further supported by the age difference between homosexual and nonhomosexual male-to-females at the time of the interview. It is known from other studies that the nonhomosexual natal male patients are significantly older at time of evaluation as well as at time of SRS (Blanchard, Steiner, Clemmensen, & Dickey, 1989).

Female-to-males had more difficulties in establishing a stable relationship after transition. One-third of the female-to-males did not have any sexual partner after transition although libido was not impaired. Despite their masculine presentation and their masculine sex organs, some avoided a relationship with a potential partner, because they felt uncertain and anxious about their maleness. If transsexual patients are able to establish a stable relationship, they are sexually very satisfied, which in turn improves their general satisfaction. In contrast with the data of some researchers (Bodlund & Kullgren, 1996), who reported that male-to-females have, after transition, more frequently a new partner whereas the female-to-males tend to remain with the same partner, we observed no significant difference between the two groups. It is remarked that not all transsexual patients wish to inform their new partners about their natal sex. In a small, dense country as Belgium it is very difficult to hide one's transsexual past. We always give the advice to patients to inform their partners about their past. Mostly this has a positive effect on the relationship.

After surgery, more male-to-females acted upon their attraction to men, as a male sex partner was more often preferred. Daskalos (1998) suggested that male-to-females conform before SRS regarding their sexual orientation and choose a female partner, but we can

assume that some conform after SRS (and then choose a male partner). From the 19 male-to-females who had a male partner after SRS, only 14 reported being exclusively orientated to men. On the other hand, the male-to-female participants who defined themselves as homosexual before sex reassignment more often had a stable relationship after SRS than the nonhomosexual male-to-females, but this difference was not significant (70.6% vs. 46.6%). A question to resolve is if the self-definition of sexual orientation is a reliable measure. Lawrence, Latty, Chivers, and Bailey (2005) questioned reported changes in sexual orientation by examining sexual arousal in male-to-female transsexuals by vaginal photoplethysmography.

In general, most transsexual individuals indicated an improvement in their sex life and more sexual excitement after SRS. Most participants were able to reach orgasm both through masturbation and intercourse. Before surgery, they experienced their body as strange and not belonging to themselves. Often they did not accept being touched by anybody (even by themselves). They were not preoccupied by sex, but were preoccupied by getting rid of the unwanted sex organs. After SRS, sexuality can only improve, on condition they have the right body, with the right genitals. Our data showed that an improvement of sex life and sexual satisfaction was correlated with the satisfaction with the surgical results and the new primary sex characteristics. A correlation between sexual functioning and the anatomy of the neovagina or neophallus has been described (Green, 1998). A dysfunctional vagina is often a reason for sexual dissatisfaction in male-to-females. The phalloplasty surgery appears to be crucial in increasing the body image satisfaction. However, if the surgical results are not optimal, this may actually increase sexual dissatisfaction.

Although the effect of relief of gender dysphoria after SRS is the same for both groups, we noticed a difference regarding some aspects of sexual life. Female-to-males masturbated more frequently (more than before SRS and more than male-to-females), and a trend toward increased arousal and easily reached orgasm (during intercourse as well as when masturbating) was reported. A study with a larger number of participants may clarify these trends. A possible explanation for these group differences may be that before transition, female-to-males suffer even more from gender dysphoria, derived from the younger age at which they seek sex-reassignment. Also, male hormones influence sexual behavior and libido (Mooradian, Morley, & Korenman, 1987). The absence of testosterone possibly explains the effect on the sexual satisfaction rate of the male-to-females, where one out of four remained unsatisfied. Male-to-female transsexuals have to deal with the absence of testosterone, whereas female-to-male transsexuals have to experience the presence of markedly higher androgen levels after SRS, compared to the initial hormonal treatment phase.

A retrospective finding of the current study is the experience of orgasm changes: a female orgasm pattern for the male-to-females and a male orgasm pattern for the female-to-males. This was also reported earlier by Rehman, Lazer, Benet, Schaefer, & Melman (1999).

More than two-thirds of the male-to-females reported the secretion of a vaginal fluid during sexual excitation and during orgasm. The hypothesis is that during sexual excitation this fluid is produced in Cowper's glands (in natal males it is called the preejaculatory penile secretion) whereas during orgasm it originates from the prostate. Cowper's glands are situated in the urogenital diaphragm, beneath the prostate gland, and are not removed during genital surgery. We expect that the production of this fluid will decrease in the absence of androgens or by the administration of estrogens, but further study is necessary. Details are lacking on the nature of the hormonal control of these glands. It is clear from studies on castration that there is a dependence on the testes. Some early studies have addressed the action of estrogens on the accessory genital organs in a variety of animals; these reports were almost exclusively on morphological findings. It was concluded that a small supply of estrogen appears to be favorable for the well-being of the male accessory reproductive organs (Raeside, Christie, & Renaud, 1999). Informing the postoperative transsexual of the continuation of the fluid production is useful. The spontaneous lubrification can create more comfort in the sexual relationship.

Some female-to-male transsexuals did not choose for an erection prosthesis. They were satisfied with phalloplasty alone, and did not wish further surgical interventions. In the group with penile prosthesis, sexual expectations were more realized, but more often pain during intercourse was experienced. During phalloplasty, the free forearm flap is connected with two nerves, one of two dorsal clitoral nerves for erogenous sensation and the ileoinguinal nerve for proprioception (the ability to sense the position, location, orientation, and movement of a body part). Probably this proprioceptive sensation during intercourse is responsible for the pain; however, it has a protective function against perforation of the prosthesis (Hoebeke, De Cuypere, Ceulemans, & Monstrey, 2003). Pain may also be explained by an exaggerated pressure of the erection prosthesis on the free forearm flap or by irritation of the pubic bone at the place of fixation.

The response rate remains a difficult problem in this type of follow-up research. The patients are either difficult

to trace because of the frequent change of residence, or because of unwillingness to participate in interviews of this kind. This implies that researchers can never obtain the profiles of those who fail to respond. This selection bias cannot be avoided. The longer the postoperative period, the lower a response rate will be. The response-rate in the Blanchard et al. (1989) sample was 84.1% with a mean follow-up interval of 4.4 years. Pfäfflin and Junge (1990) reported a response rate of 63% after 5.1 years for male-to-female and 6.7 years for female-to-male transsexuals. Nearly 80% of the population in Smith's (2002) study agreed to cooperate with a mean delay of 22 months. The study of Lawrence (2003) had a 43% response rate, which was comparable to our study (51.3%). A possible explanation for the rather low response rate in the current study could be that all participants were required to be seen on a face-to-face basis, involving an outpatient visit. Other limitations of this research are that data were based on self-reports and thus are subjective. However, the evaluation of SRS can be made mainly on the basis of such subjective data, as SRS is intended to solve a problem that cannot be determined objectively (Lawrence, 2003).

It is clear that the significance and the importance of sexuality have undergone an evolution in our study group. Consequently, we feel that during the preoperative period more attention should be paid to sexual expectations and to possible sexual changes, in order to help the patients cope with these new sensations. Systematic investigation on a larger number of patients is certainly needed to gain more insight into sexual functioning of postoperative transsexuals. A prospective study is needed in male-to-females to further investigate the relation between libido and (subnormal) testosterone levels.

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LETTERS TO THE EDITOR

The Endocrine Care of Transsexual People

To the editor:

I commend the authors and the *Journal* for publishing Clinical Review 161 on the endocrine treatment of transsexual people (1). Despite the central role the endocrinologist plays in the management of these patients, there are few clinical studies or publications by the endocrine community focusing on this area. This is well reflected by the range of hormonal preparations and dosages used by different centers specializing in the treatment of transsexual people (1). Most of these clinical regimens and protocols are empiric and have not been compared or tested in a controlled and rigorous manner. This leaves the clinical endocrinologist with the responsibility of integrating recommendations from the various centers. Moore *et al.* (1) embarked on this difficult task and have drawn general guidelines of their own but pointed to the need for randomized clinical trials. I would like to comment on some of the issues dealt with by the authors:

1) Asscheman *et al.* (2) have reported an incidence of thromboembolic episodes of 2.1% in male-to-female (M→F) patients less than 40 yr of age and in 12% of M→F patients above 40 yr of age under estrogen therapy. This led them to recommend transdermal estrogen administration to subjects over age 40 yr. In a subsequent analysis of 816 M→F transsexuals, the same group reported a substantial decrease in the incidence of thromboembolic events, which could be attributed to their change in clinical practice (3). Because the risk of this severe complication is also significant in younger patients, I would propose the use of transdermal preparations as the first-choice estrogen treatment for all age groups.

2) Hyperprolactinemia is a common finding in M→F transsexual patients, and its degree is positively correlated with the dosage of estrogen (3). Nevertheless, the incidence of prolactinoma is extremely low and probably does not warrant the routine performance of visual field assessment during follow-up, as recommended by the authors (1). Prolactin levels should, in my view, be measured at the initial visit before treatment to exclude the presence of a prolactinoma unrelated to the hormonal treatment. Subsequent prolactin measurements should suffice to detect clearly elevated levels that could raise the possibility of lactotroph hyperplasia, leading to estrogen dose adjustment.

3) Moore *et al.* (1) recommend assessment of bone mass in M→F transsexuals after surgical castration. Female-to-male (F→M) transsexuals are also at risk for osteoporosis. It has been shown that in the M→F population, estrogen treatment prevented bone loss after testosterone deprivation, whereas in the F→M group, testosterone treatment was in general unable to prevent the decrease in bone mass associated with the decline of serum estradiol levels (4). Furthermore, the change in bone mineral density correlated inversely with serum gonadotrophin levels. High LH levels appeared to be the best predictor of bone loss and reflected hormone undertreatment (4). Therefore, it would be wise to recommend performance of densitometry studies for F→M subjects as well and to incorporate measurements of LH to the other variables to be tested during the follow-up of these patients.

4) Endometrial hyperplasia is a serious concern in testosterone-treated F→M transsexuals. Consequently, periodic uterine sonography, which can be performed through the abdominal wall if technically feasible, is advised until hysterectomy is performed. On the other hand, pelvic exams are very demanding for these patients from the psychological point of view. Because most of them do not have sexual contact that involves intercourse, the performance of routine Pap smears, as recommended, is probably not necessary, based on the recommendations of the U.S. Preventive Services Task Force for screening for cervical cancer (5).

5) Preservation of fertility is an important issue that should be discussed with patients. Freezing and storage of sperm should be proposed to M→F transsexuals before hormone therapy is initiated.

In conclusion, much improvement is needed in the quality of treatment that transsexual people receive, together with an empathic and supportive approach that these patients require.

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Authors' Response: The Endocrine Care of Transsexual People

To the editor:

The Clinical Review by Moore *et al.* (1) on endocrine treatment in transsexual people in the August issue of *JCEM* is timely. We wish to offer some nuances to the recommendations made by Moore *et al.* (1).

The authors state that transition should be rapid and complete. However, cross-sex hormonal effects are, to an extent, irreversible. In our view, hormonal treatment should be embedded in the so-called "real-life test". The real-life test is an extended period of full-time living as a member of the desired sex. The real-life test allows the subject and the attending professional to monitor the experience in the new sex status as she/he habituates her/his responses to other people. Without this test of how others react and how she/he reacts to others, the subject knows only her/his private convictions and fantasies of being a member of the opposite sex. The subject should have lived at least 2 yr full-time in the new sex before irreversible surgical reassignment is considered. The real-life test may be prolonged if too many hurdles present themselves during the test period. It is our belief that a slow transition phase of usually 2 yr, rather than a quick one, may be more advisable. Arguments include psychosocial reasons and, furthermore, a more gradual adaptation of the body to a changing hormonal milieu. In this regard, the dual-phase hormonal schedules may be recommendable. The first largely reversible phase includes antiandrogens (*e.g.* cyproterone acetate 50–100 mg daily) in male-to-female transsexuals and progestins (*e.g.* lynestrenol 5 mg daily) in female-to-male transsexuals. This is an important phase of the real-life test, during which sex-specific features of the natal sex such as erections/ejaculations or menstrual bleeding are suppressed. This allows assessment of whether loss of characteristics of the natal sex alleviates the suffering of the candidate and whether induction

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of characteristics of the desired sex will further enhance well-being. If so, this is to be followed by administration of cross-sex hormones, with largely irreversible feminization and masculinization. Pharmacological ablation of endogenous sex steroid production before the initiation of exogenous cross-sex steroid treatment may allow a lower dosage of cross-sex hormones and reduce the risks of side effects and thus morbidity. This is worthy of research, but preliminary results in patients treated following this hormonal regimen, published in abstract form, indicate relatively few and minor morbidities that are mostly reversible with appropriate treatment (2). Unlike Moore *et al.* (1), we would no longer advise ethinylestradiol in the high dosage of 100 μg anymore because it is associated with an unacceptably high thrombotic risk (3). Moreover, we would like to argue that transdermal estrogens also can be advised under the age of 40, especially in smokers.

Moore *et al.* (1) argue that the Amsterdam group (4) reported a high incidence of depressive mood changes, hyperprolactinemia, and thromboembolic events, compared with a normal population. It is not unreasonable to assume that side effects are related to the dosage of administered hormones. Whether depression in transsexual people is due to hormonal changes is debatable. Transsexuals go through important life events during transition, both before and after sex reassignment surgery (SRS), with gains and losses. So, the question is not whether depression scores are worse in transsexual people than in a control group, but whether the score has improved after gender reassignment. Preliminary results of our follow-up study show that suicidal attempts had significantly diminished after SRS (5). Improved and consistent general well-being is one of the important reasons why we consider both hormonal treatment and SRS to be parts of a rehabilitation process wherein, gradually, bodily features are adjusted to gender identity.

Usually, after 2 yr of cross-sex hormonal treatment, SRS is performed. Moore *et al.* (5) state in their recommendation table that endometrial ultrasounds should be performed every 2 yr in female-to-male transsexuals. In Europe, female-to-male transsexuals usually undergo hysterectomy and ovariectomy after approximately 2 yr of androgen administration. Long-term androgen administration induces polycystic changes of the ovaries indistinguishable from polycystic ovaries (6). Polycystic ovaries are more at risk of malignant development.

Finally, we feel that recommendations for the initial visit should include clinical examination assessing general health, hormonal status, and complication risk and karyotyping to diagnose intersex conditions.

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Authors' Response: The Endocrine Care of Transsexual People

To the editor:

We thank Dr. Greenman (1) and Dr. T'Sjoen *et al.* (2) for their comments on our recent review article. They each add helpful additions to the recommendations presented in our review. We have a few comments to the issues raised. Both teams suggest the use of transdermal estrogen delivery for all age groups. This is reasonable and supported by the data as outlined. However, this may not offer a transition as immediate and dramatic as often desired by patients. We agree that visual field evaluation need not be done routinely because the development of a prolactinoma is rare. Certainly, freezing and storage of sperm or embryos could be recommended to individuals, although this is not a frequent request.

In regard to Greenman's (1) comment on gynecological surveillance of female-to-male (F→ M) transsexual people, vaginal ultrasound is a standard of care in following the endometrium in patients at increased risk for hyperplasia or carcinoma (3). However, a transabdominal pelvic ultrasound performed by an experienced technician may be a reasonable, although less ideal, alternative. Most importantly, a patient with new vaginal bleeding or a possible history of hyperplasia should receive prompt and thorough evaluation by endometrial biopsy and/or hysteroscopy. The recommendations from the National Institutes of Cancer and the U.S. Preventive Services Task Force (4) include routine pap smears for all sexually active women with a cervix. In postmenopausal women, the Task Force recommends ceasing cervical cancer screening after at least three negative pap smears as long as there are no abnormal pap smears in the last 10 yr. It is important to remember that individual F→ M transsexual people may be at risk for human papillomavirus and cervical cancer through current or past vaginal intercourse. Additionally, it can take years for a cervical abnormality or cancer to develop. Stopping cervical cancer surveillance can be recommended only on a case-by-case basis.

Comments made by the Amsterdam group (2) are very helpful. The prevalence of an abnormal karyotype in this population is unknown, probably small, and probably irrelevant in the decision to treat. Therefore, we do not recommend routine chromosomal analysis unless the history or physical exam is suggestive of an abnormality. We do agree with their group that in many situations, particularly in adolescents or young adults, antiandrogens might be a better first step before using feminizing hormones. However, we also want to emphasize the importance of encouraging full transition. We strongly discourage incomplete treatment, whereby individuals have a mixture of female and male external sexual characteristics.

Developing guidelines in the face of limited data can be challenging. We appreciate the input from experienced clinicians, as above, to enhance the treatment transsexual people receive.

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CHAPTER 7

GENERAL DISCUSSION AND PERSPECTIVES

GENERAL DISCUSSION AND PERSPECTIVES

The extent to which relative hypoandrogenism in the elderly contributes to clinical signs and symptoms of aging remains a largely underexplored issue that certainly deserves further attention, as many clinical features of aging in men are reminiscent of those of hypogonadism in younger subjects, and the indications for as well as the potential benefits of androgen supplementation to aging men are a subject of debate. It should however be realized that aging is accompanied by a decline of almost all physiologic functions, which in conjunction with age-associated changes in lifestyle such as retirement or relative sedentarism, may all contribute to the symptomatology of aging. It is evident that the androgen level is only one of the many factors determining the symptomatology of elderly men.

Although testosterone itself exerts androgenic and anabolic actions through binding to the nuclear androgen receptor in target cells it is essentially a prohormone, being reduced to the more active androgen DHT (with effects on the urogenital tract, hair follicles, skin and liver) whereas a fraction of testosterone can be aromatised to oestradiol. Hence, tissular action of testosterone is the complex resultant of tissue availability and locally achieved testosterone concentrations, of local intra-tissular testosterone metabolism, of expression of androgen and/or oestrogen receptors, as well as the expression of a number of co-activators and repressors of these receptors. Each of these determining factors of testosterone action can be differentially regulated in the tissues, thus offering a close to unlimited potential for differential regulation of tissular sex steroid action. The hormone blood levels are at best imperfect parameters of testosterone in the tissues. This complexity of testosterone action should be kept in mind when discussing clinical implications of declining androgen levels in aging men and possible merits and risks of androgen treatment to the elderly.

Thus, a major limitation in our ability to asses the clinical impact of the changes in androgen production in the elderly is the lack of a reliable and practical marker of tissular androgen action and our consequent relative ignorance as to physiologic androgen requirements in elderly men in general as to individualised androgen needs. In this context, diagnosis of hypogonadism in elderly men is difficult and in borderline cases always uncertain. In view of these diagnostic limitations and the inconclusive evidence that modest age-related androgen deficits really matter clinically it is advisable to reserve the diagnosis of hypogonadism, with its implication of considering testosterone administration, for those elderly men with manifest hypogonadism as established by the presence of both clear clinical symptoms and serum testosterone levels frankly below the range for young men.

With regard to diagnosis of PADAM, the AMS score was promoted as to represent a step forward in the development of a quality-of-life assessment instrument that is condition-specific for men with age-related androgen deficiency **(Chapter 3**). However, all presently available 'aging male' questionnaires have a low specificity for predicting serum testosterone in elderly men and therefore cannot substantially contribute to the diagnosis of partial androgen deficiency in the elderly. Use of such questionnaires as a substitute for the rather complex medical judgment of the treating physician in a clinical setting with confirmation of hormonal serum levels is not advisable. Furthermore, in the present state of the art their widespread use as a screening tool (even if AMS was not designed as such) to direct men for further biochemical evaluation should probably be discouraged. In view of the low specificity and the high prevalence of symptoms possibly associated with hypogonadism in the elderly population, one should avoid suggesting or solicit-

ing symptoms, as may be the case with the use of screening questionnaires. We must emphasize that our AMS study specifically addressed the situation on a healthy elderly population with mean age over 79 years. These results may not be extrapolable to younger populations. In fact, it is quite conceivable that in a younger age group symptomatology may be more specific and that the AMS scores or similar questionnaires may better predict androgen levels.

From data presently available on 'aging male' symptoms scores, it does not appear that, even if these are helpful in describing symptoms, the questionnaires contribute significantly to the diagnosis of androgen deficiency. It is presently not established whether they might serve as a pre-screening instrument to select patients for blood sampling; neither is it clear whether screening for low serum testosterone is in itself presently desirable. Taken the high prevalence in older men of non-specific symptoms loosely associated with hypoandrogenism, spontaneous active reporting of complaints may have the merit of a higher specificity, whereas soliciting complaints with screening questionnaires might lead to over-diagnosis and over-treatment.

The age-related decline in testosterone levels is associated with a number of mild, non-specific symptoms, including depressive symptoms (**Chapter 4**). The relationship between depressive symptoms and testosterone levels is also confounded by numerous factors, including medical illness, obesity, smoking, alcohol use, diet and stress, and is thus complex. Although testosterone levels decline with age, there is great interindividual variability, and the connection between serum testosterone levels and clinical psychiatric signs and symptoms is not clear-cut, since other hormonal changes are implicated as well. Studies have not consistently supported an clear role of reduced testosterone levels in major depressive disorder, although levels may often be reduced in men with treatment-refractory depression and older men with dysthymia. Low testosterone levels may also increase the risk of incident depression in older males, although this may depend upon androgen receptor genetic polymorphisms. It seems likely that the apparent disparities between studies are largely underlied by differences in study population and methodology, the homogenous age range of a population based sample being the essential feature of our study, from which the data did not support a role for testosterone in depression in elderly community-based men.

In the evaluation of a population of community-dwelling men there was a consistent correlation of AMS and GDS with the results of the non-disease specific health measures SF-36 and RDRS-2, the latter reflecting activities of daily living, suggesting that also in this relatively healthy group AMS and GDS are related to some extent to other health related variables affecting quality of life but, again, few SF-36 subscores did relate to hormonal serum levels. Testosterone has demonstrated usefulness in the treatment of a number of depressed populations, but further double-blind placebo-controlled studies are needed to fully elucidate its role in the treatment of depressive syndromes in the aging male. Clinicians treating elderly men should consider having testosterone levels measured to determine if a testosterone deficiency is a potential contributing factor for the depression. Additionally, it might be helpful to consider testosterone as an augmentation strategy for elderly men who respond partially to standard antidepressant therapy.

Nonetheless, testosterone therapy is not without potential for adverse effects, the most worrisome of which is the worsening of pre-existing prostate carcinoma. Currently, oral, short- and long-acting parenteral, and transdermal patch and gel formulations are available. The safety of testosterone administration in elderly men, especially with regard to possible effects on the prostate, lipids, and red blood cell production remains a concern despite evidence suggesting

that androgen replacement is generally quite safe in healthy aging men. Furthermore, although testosterone has been used pharmacologically since the 1940s, available androgen preparations are still not optimal. In particular, oral androgen replacement is limited by both toxicity and lack of efficacy. Current testosterone replacement methods have important limitations. Oral androgens are potentially hepatotoxic and injectable testosterone esters result in supraphysiological peaks of testosterone levels followed by hypogonadal troughs. Transdermal testosterone patches frequently cause local skin reactions and are associated with a decline in serum testosterone concentrations toward the end of the treatment period. Testosterone gels appear to cause fewer dermatological reactions but can be associated with transmission of testosterone from male patients to female partners or children. Thus, a well-tolerated orally administered agent may have unique potential as a means of androgen replacement therapy. Furthermore, gynaecomastia is a common side effect of current modes of androgen administration but does not occur with aromatase inhibition as oestrogens are reduced. Because oestradiol is a crucial mediator of hormonal feedback at the pituitary and hypothalamus in men, aromatase inhibition would be expected to enhance pituitary stimulation of testicular testosterone production in men. Thus, aromatase inhibition may be a novel means of normalizing testosterone levels in elderly men. Letrozole, an aromatase inhibitor was used in the study described in **Chapter 2**:

Subtle physiological alterations are responsible for the changes in androgen levels observed in aging. It has been suggested that the increase in gonadotropin levels in older men is a result of Leydig cell resistance to pituitary stimulation. It has also been hypothesized that the gonadotropin-suppressive activity of androgens is increased in elderly men. Finally, as aromatase activity increases with age, an alteration in the oestrogen/testosterone ratio may contribute to decreased androgen production. The effects of aromatase inhibition that we observed in our study allow rejecting the tested hypothesis that increased restraining of LH secretion by endogenous oestrogens is instrumental in the age-related decline of Leydig-cell function. We concluded that aromatase inhibition with 2.5 mg letrozole daily for 28 days produced a remarkable and comparable stimulation of gonadotropin secretion in young and elderly men, with in both groups also a marked testosterone response. Serum oestradiol levels were reduced but generally remained within the normal range for men. Longer-term studies are needed to assess the overall physiologic consequences of this combined hormonal alteration in aging men and whether this treatment might be considered as an alternative and safe approach for PADAM.

The study on the soluble transferrin receptor (**Chapter 5**) is an explorative study that paves the ground for other research that is needed to further assess the interactions of sTfR concentrations with sex steroids. Evaluating the various effects in shorter intervals, for a longer period of time, longitudinally, in different populations, or with different modes of hormonal administration are needed in order to see if the described effects do persist. The increase in Hb/Hct (10%) upon testosterone administration was associated with an increase in sTfR; the fall in Hb/Hct levels upon administration of CA combined with either oral EE or transdermal E2 had disparate effects on levels of sTfR, probably explained by the different effects on IGF-I levels. The results of the study have little practical implication for the treatment of transsexual persons, but the findings may provide insight into the origin of the gender gap in haemoglobin and haematocrit concentrations. The profound manipulation of the sex steroid milieu can only be conducted in a group of transsexual persons in a medically and ethically acceptable manner. So far, no studies are available on sTfR concentrations in partial androgen deficient elderly men. During testosterone replacement therapy, haematocrit monitoring should be standard practice and older men

should be tested periodically throughout therapy. Haematocrit and RBC volumes have been shown to rise significantly during testosterone replacement therapy and the stimulatory effects of androgens on erythropoiesis are well documented. Polycythaemia has been reported in elderly men both following intramuscular and transdermal testosterone administration. It is known that suprafysiological testosterone doses (mostly but not exclusively intramuscular administration) increase the risk for polycythaemia. Withdrawal led invariably to the normalization of the red cell volume, suggesting that the drug was responsible for the appearance of polycythaemia. Haematocrit did not increase in elderly men with initially low serum testosterone treated for 12 weeks with a daily dose of 1 mg anastrozole (an aromatase inhibitor).

Testosterone is able to increase erythropoiesis by stimulating the kidney to produce erythropoietin, but even in these circumstances serum erythropoietin levels have been found low, suggesting an erythropoietin-independent mechanism of polycythaemia induction. Therefore, more studies are needed to describe to which extent bone marrow stimulation contributes to the androgen-induced erythropoiesis. As marrow erythropoietic activity appears to be the most important determinant of sTfR levels, measurements are useful to monitor the erythropoietic response to various forms of therapy and an early therapeutic response can be predicted, even when changes in haemoglobin are not yet apparent. From our study it can be concluded that profound changing of sex steroid status has complex effects on erythropoiesis. The effects described have provided some new insights and hopefully encourage the performance of further studies in the field of sex steroid research regarding haematopoiesis.

The first aim of the study described in **Chapter 6** was to evaluate the long-term safety of the local hormonal treatment regimen. Contrary to the usual practice, whereby an inclination to maximize hormone dosage by patients as well as physicians is seen, our gender team has always treated patients by a dual-phase hormonal schedule, with lower doses compared to other centres, aiming at no or minimal side effects. Hormonal treatment should be embedded in the real-life test. The real-life experience allows the subject and the attending professional to monitor the experience in the new sex status as she/he habituates her/his responses to other people. It is our belief that a slow transition phase of usually 2 years, rather than a quick one, may be more advisable. Arguments include psychosocial reasons and, furthermore, a more gradual adaptation of the body to a changing hormonal milieu. In this regard, the proposed hormonal treatment may be recommendable. The first largely reversible phase includes antiandrogens (*e.g.* cyproterone acetate 50–100 mg daily) in male-to-female transsexuals and progestins (*e.g.* lynestrenol 5 mg daily) in female-to-male transsexuals. These drugs are prescribed at the start of the real-life test, inducing a reversible suppression of sex-specific features of the natal sex such as erections/ejaculations or menstrual bleeding. The initiation of cyproterone acetate frequently has a calming effect on male-to-female transsexual persons, in contrast with the possible induction of depression that has been described with this medication. This allows assessment of whether loss of characteristics of the natal sex alleviates the suffering of the candidate and whether induction of characteristics of the desired sex will further enhance well-being. If so, this is to be followed by administration of cross-sex hormones, with, to an extent, irreversible effects. Pharmacological ablation of endogenous sex steroid production before the initiation of exogenous cross-sex steroid treatment may reduce the need of using large dosage of cross-sex hormones due to increased sensitivity of tissues and reduce the risks of side effects and thus morbidity. Of course, these ideas need systematic clinical trials with a larger number of participants in order to find an ideal treatment, where tailoring for the individual patient will still be necessary.

Relatively few and minor morbidities were observed in our patient group that were mostly reversible with appropriate treatment. The number of complications reported in our study was markedly lower than those described by the Amsterdam group, who, on the other hand, reported on a much larger group of transsexual persons. Response bias may have influenced our results. Our treatment schedule of cross-sex hormones is acceptably safe and has some tentative management and diagnostic implications for the hormonal treatment of transsexual persons.
Little attention has been given to sexual function after sex reassignment. In general, while not all postoperative transsexuals are orgasmic, there is a much wider sexual satisfaction. It was shown in this study that male-to-female transsexual persons reported more frequently a decrease in libido. The absence of testosterone possibly explains the effect on the sexual satisfaction rate of the male-to-female group, where one out of four remained unsatisfied. Male-to-female transsexuals have to deal with the absence of testosterone. This is an additional argument in favour of the phased hormonal treatment. The adaptation to low testosterone levels may prove to be an extra diagnostic test. It was remarkable that in postoperative male-to-females only a minority reached normal free testosterone levels (reference range for women). It may be that male-to-females need a small amount of androgens to be libidinous, as is suggested for women with hypoactive sexual desire disorder. A prospective study is needed in male-to-female transsexuals to further investigate the relation between libido and (subnormal) testosterone levels.

To date, the female androgen deficiency syndrome has remained controversial because of a lack of agreement on an operational definition and because of lacking the understanding of the mechanism(s) by which testosterone may enhance libido Along this line, we started recruitment of postoperative male-to-female persons, to determine the prevalence of hypoactive sexual desire disorder in this group and to evaluate possible associations with hormonal parameters, maybe leading in future studies to pharmacological intervention.
In conclusion, it is clear that the significance and the importance of sexuality undergo an evolution during the hormonal and surgical stages of gender reassignment. Consequently, we feel that during the preoperative period more attention should be paid to sexual expectations and to possible sexual changes, in order to help the patients cope with these new sensations.

Finally, because of the low prevalence of transsexualism, the creation of a European or larger collaborative project between the specialised Gender teams (Amsterdam, Bologna, Malaga, Liège, Gent…) could be advisable. Creating a multi-centre database, monitoring (side-) effects of cross-sex hormonal treatment, developing evidence-based recommendations, conducting multicentre trials are among the possibilities in the future. Studies in transsexual persons will continue to provide important information on the effects of sex steroids in different organ systems, gender specific behaviour or diseases.

HOOFDSTUK 8

PSYCHO-ENDOCRIENE ASPECTEN BIJ OUDERE MANNEN EN TRANSSEKSUELE PERSONEN

Nederlandstalige samenvatting voor niet-medisch ingewijden

MANNELIJKE EN VROUWELIJKE HORMONEN

De zaadballen bij de man en de eierstokken bij de vrouw zorgen voor de productie van geslachtshormonen. Een kleine hoeveelheid geslachtshormonen wordt ook in de bijnieren gemaakt met als meest prominente vertegenwoordiger DHEA-S. Het basisproduct van geslachtshormonen is cholesterol, die omgezet wordt naar mannelijke en vrouwelijke hormonen. Mannen maken hoofdzakelijk androgenen, waaronder testosteron, terwijl vrouwen vooral oestrogenen, waaronder oestradiol, maken. Zowel mannelijke als vrouwelijke hormonen circuleren bij man als vrouw, maar de hoeveelheden in de bloedbaan zijn uiteraard heel verschillend volgens geslacht. Testosteron kan door het aromatase enzyme omgezet worden tot oestradiol.

De hypothalamus, een hersenstructuur, produceert GnRH (gonadotropine releasing hormoon), dat de hypofyse stimuleert tot het maken van luteinizerend hormoon (LH) en follikel stimulerend hormoon (FSH). In de teelbal staan de Leydig-cellen hoofdzakelijk onder invloed van LH en zijn ze verantwoordelijk voor testosteronproductie. De Sertoli-cellen die instaan voor de zaadcelproductie staan voornamelijk onder invloed van FSH.
Via de bloedbaan kunnen deze hormonen op afstand in het lichaam hun activiteit uitoefenen. Bepaalde doelorganen hebben receptoren die deze hormonen herkennen. Het hormoon bindt zich aan deze eiwitmoleculen en op die manier wordt de celfunctie beïnvloed. Het hormoon-receptor-complex kan zich dan binden binnen de celkern aan het DNA (deoxyribonucleic acid), dat de genetische informatie bevat. Uiteindelijk worden hierdoor specifieke eiwitten gevormd. Onder invloed hiervan wordt de ontwikkeling van interne en externe geslachtsorganen bepaald. Ook de secundaire geslachtskenmerken (zoals beharing, spiergroei, stemveranderingen bij de man en borstontwikkeling, zachtere huid en andere vetverdeling bij de vrouw) ontstaan hierdoor. Een voorbeeld van een hormoonreceptor is de androgeenreceptor (AR), waarvan de activiteit ook weer genetisch bepaald wordt. Het androgeenreceptor gen CAG repeat length polymorfisme (het aantal herhalingen van de CAG code, C = cytosine, A = adenine, G = guanine) kan bepaald worden in het laboratorium. Het aantal herhalingen van de genetische CAG-code is bepalend voor de gevoeligheid van de receptor. Meestal bevat de CAG-repeat length 6 tot 39 herhalingen van de code, met een gemiddelde van 22 herhalingen. Kortere herhalingen van de CAG-code zouden verband houden met meer androgeenreceptor transactivatie en dus een grotere gevoeligheid voor testosteron.

DE OUDERE MAN

Dankzij betere hygiëne en dankzij de huidige geneeskunde is er een toename van de levensverwachting en zijn we in staat ziekten te overleven die vroeger fataal waren. De oudere bevolking wordt ook nog ouder; ten opzichte van 1900 is de bevolkingsgroep tussen 65-74 jaar acht maal groter. Terwijl er momenteel veel aandacht besteed wordt aan de gezondheidstoestand van de vrouw rond en na de menopauze, wordt de man in deze leeftijdscategorie soms wat vergeten, ten onrechte. Net zoals bij de vrouw rond de menopauze vermindert ook bij de man de productie van geslachtshormonen bij veroudering. Bij alle vrouwen valt de productie van oestrogenen in vrij korte tijd stil, maar bij de man verloopt het proces van testosterondaling veel trager. De term andropauze is hierom geen correcte benaming, omdat er geen 'pauze' is. PADAM of 'partiële androgeen deficiëntie van de oudere man' zou een alternatieve terminologie kunnen zijn en suggereert dat de symptomen minstens gedeeltelijk afhankelijk zijn van andro-

geendeficiëntie. We spreken dus over een wat wazige entiteit, die het midden houdt tussen normale hormonale bloedspiegels en een uitgesproken tekort. De medische wereld kent al lang de klachten en de lichamelijke kenmerken van een absoluut testosteron-tekort bij jonge mannen. Mannen die bijvoorbeeld een hypofyse ingreep ondergingen of die een genetische variant hebben (Klinefelter syndroom of andere) kunnen uiterst lage testosteron-waarden vertonen. Een relatief androgeen-tekort, zoals dit bij de oudere man gezien wordt, gaat gepaard met gelijkaardige doch veel minder duidelijke symptomen en lichamelijke kenmerken. Omdat een absoluut testosteron-tekort bij de jonge man belangrijke gezondheidsimplicaties heeft, is de idee gegroeid om aandacht te geven aan het relatieve testosteron-tekort wat bij veel oudere mannen bestaat. De belangrijkheid van de diagnose van andropauze kan gestaafd worden aan de hand van de overeenkomsten met de symptomen en tekenen bij oudere mannen in vergelijking met deze van testosteron-tekort bij de jongere man, het aanduiden van een correlatie tussen symptomen en (vrije) testosteron-concentraties, en het eventuele gunstige effect van testosteron-supplementen bij oudere mannen met te lage androgeen-concentraties.

De testiculaire functie vermindert dramatisch bij veroudering. De Leydig-cellen, verantwoordelijk voor testosteronproductie verminderen sterk in aantal. Ook het aantal Sertoli -cellen die instaan voor de zaadcelproductie neemt af. Hierdoor ontstaat een andere verhouding van mannelijk en vrouwelijk hormoon bij de ouder wordende man en zien we een relatief hyperoestrogenisme. Ook is er een verandering in de dag-nachtvariabiliteit van de testosteron-concentratie in het bloed. Het actieve testosteron neemt af met de leeftijd omdat er minder aanmaak is van testosteron, de dragereiwitten in het bloed toenemen, waardoor de gebonden fractie toeneemt en de vrije fractie afneemt. Bij jonge mannen is de testosteron-concentratie duidelijk hoger `s ochtends, met een progressieve terugval in de namiddag, met de laagste concentratie `s avonds en gedurende de nacht. Op 75-jarige leeftijd is de hoeveelheid totaal testosteron in het serum ongeveer 2/3 van het gemiddelde niveau op 25- jarige leeftijd. Het vrije testosteron is slechts 40% van de gemiddelde concentratie bij jonge mannen. Belangrijke interindividuele variaties bestaan echter, waarbij 20% van de mannen ouder dan 60 jaar testosteron-waarden hebben vergelijkbaar met mannen met de hoogste testosteron-concentraties tussen 20 en 40 jaar. Androgeendeficiëntie komt voor bij 21% van de gezonde mannen tussen de 60 en 80 jaar en bij 35% van de mannen boven de 80.
Het besef van de farmaceutische industrie dat er een grote groep oudere mannen bestaat met interesse voor hormonale behandeling heeft de activiteit in dit onderzoeksgebied verhoogd. Naar analogie van de hormonale substitutiebehandeling bij gemenopauzeerde vrouwen wordt het verhogen van testosteron concentraties bij oudere mannen met te lage testosteron concentraties voorgesteld. Een belangrijke vraag blijft evenwel via welke klachten deze mannen bij de arts terechtkomen, en hoe de diagnose gesteld moet worden.

Veroudering bij de man is soms geassocieerd met een groep van symptomen zoals verminderde vitaliteit, depressieve neiging en anhedonie (minder plezier hebben in het leven), ongunstige veranderingen in het humeur en het karakter, slaapstoornissen, een verminderd concentratievermogen, verminderde spierkracht met grotere kans op vallen. Ook treden tekenen van seksuele disfunctie op de voorgrond; erectiezwakte, te kort durende erecties, verminderde libido, veranderd ejaculatiepatroon, geringer orgasmegevoel en kleiner ejaculaatvolume. Eveneens kunnen opstuivingen `hot flushes` optreden die dan mogelijks herkend worden door de echtgenote en vaak de initiatie vormen voor een verwijzing naar de arts. Deze symptomen ontwikkelen zich over een relatief lange periode, waardoor hun voorkomen nauwelijks wordt

opgemerkt. Deze klachten zijn dus weinig specifiek en uiteraard kan de oorzaak multifactorieel zijn. In het endocriene systeem van de oudere man is er niet enkel een afname van testosteron-productie, maar ook een afname van groeihormoon-, melatonine-, en DHEA-sulfaat secretie. Ook kan de verminderde fysieke activiteit voor een deel verantwoordelijk zijn voor de afname van spiermassa en botdichtheid. Het is daarom niet verrassend dat de correlatie tussen verouderingssymptomen en testosteron-concentraties in onderzoeken vaak beperkt blijkt.

De diagnose van andropauze dient gebaseerd te zijn op het voorkomen van klachten passend bij de diagnose, op de vereiste dat andere beïnvloedende aandoeningen uitgesloten werden en op de (te lage) testosteron-concentratie in het bloed. Het lichamelijk onderzoek (beharing, teelballen onderzoek, aanwezige borstontwikkeling, gezichtsveldonderzoek…) kan hierbij helpen. Geneesmiddelen inname en vroegere problemen met de testikels (trauma, infectie, bestraling…) dienen bevraagd te worden. De beste laboratoriumtest is een dosering van de vrije serum testosteron-concentratie 's morgens vroeg. In aanwezigheid van suggestieve klachten en van bij herhaling een te lage vrije testosteron-spiegel kan hormonale therapie met testosteron overwogen worden. Dit gebeurt uiteraard enkel nadat andere ziekten, die symptomen kunnen geven gelijkaardig als andropauze, zoals hypothyroidie (te traag werkende schildklier), prolactinoom, hemochromatose (ijzeropstapelingsziekte) werden uitgesloten.

Wat betreft testosteron-therapie beschikken we momenteel over intramusculaire injecties, een oraal preparaat en ook een gel. Effecten van optimale testosteron substitutie uiten zich in een snelle verbetering van geheugen, humeur en algemeen welbevinden en in een normalisatie van het slaappatroon. We zien opnieuw een afname van de vetmassa in het voordeel van de lean body (spier) massa en mogelijk is er ook een verbetering in spierkracht. Ook kan er een verbetering van de botdensiteit optreden en is er een verbetering in libido en de seksuele functie. Testosteron behandeling wordt uiteraard niet gegeven bij aanwezigheid van prostaat- of borstkanker. Voor alle andere mannen is aan deze behandeling waarschijnlijk geen ander belangrijk risico verbonden.

Deze hormonale aanpak moet een deel uitmaken van een algemene aanpak van het ouder worden, met aanpassing van voeding, matige lichaamsbeweging (weerstand training) en het vermijden of tegengaan van overgewicht. Het is immers evident dat inname van medicatie, algemene ziekte, obesiteit en andere factoren het testosteron eveneens sterk kunnen beïnvloeden. Ten slotte kunnen we stellen dat -op basis van de beschikbare gegevens- hormonale vervangtherapie bij de man de levensverwachting wellicht niet verandert, maar de levenskwaliteit kan gunstig beïnvloed worden.

KLACHTEN VAN DE OUDERE MAN

De AMS ('Aging Males' Symptoms') score.

Zoals hiervoor geïntroduceerd bestaan er argumenten dat een relatief testosteron-tekort kan bijdragen tot de klinische veranderingen bij de oudere man. Of een groep van klinische symptomen, in het bijzonder deze gerelateerd aan psychologisch welbevinden, lage androgeen concentraties kunnen reflecteren blijft echter controversieel. Het doel van de studie uit **hoofdstuk 3** was om symptomen mogelijks behorende tot de diagnose 'andropauze' te correleren met biochemische androgeen bepalingen. De bruikbaarheid van deze vragenlijsten om testosteron-tekort te detecteren bij de oudere man, is immers afhankelijk van hun mogelijkheid om

(subnormale) testosteron-spiegels te voorspellen.

In 1999 werd een vragenlijst gepubliceerd (AMS of Aging Males' Symptoms Score) die ontwikkeld werd om een meer systematische beschrijving van de ernst van de symptomen passend bij 'andropauze' te geven. De gebruiksvriendelijke vragenlijst bestaat uit 17 vragen en bestaat ook in een 'Vlaamse' vertaling. De vragenlijst beschrijft drie 'symptomen dimensies', nl. een psychologische, somatovegetatieve en een seksuele factor. De factor 'psychologische' symptomen verenigt symptomen of klachten van psychologische aard, zoals ontmoedigd zijn, depressie, irritatie, angst en nervositeit. De 'somatovegetatieve' dimensie beschrijft een complex van symptomen zoals spier- en gewrichtspijn, zweten (opstuivingen), toegenomen vermoeidheid en slaapstoornissen, verminderd algemeen welbevinden, spierkracht en/of energie. De 'seksuele' vragen peilen naar potentiestoornissen, afname van ochtenderecties, vermindering van libido en seksuele activiteit, verminderde baardgroei en het gevoel 'over de top van zijn leven te zijn'.

Wij bestudeerden de relatie van de andropauze symptomen zoals beschreven door de AMS vragenlijst met androgeen concentraties en met andere vragenlijsten die de perceptie van gezondheid en welbevinden beschrijven, zoals de goed gekende Short Form-36 (SF-36) en Rapid Disability Rating Scale-2 (RDRS-2, evaluatie van de dagelijkse activiteiten en zelfredzaamheid). De serum concentraties van seks steroid hormonen en de bindende eiwitten werden gemeten in het bloed van 161 gezonde, ambulante oudere mannen, ouder dan 79 jaar die in 2000 de AMS lijst invulden. De gemiddelde concentratie van totaal, vrij en 'bioavailable' testosteron in deze groep was 401.6, 6.8 en 151 ng/dl respectievelijk, met 24.7%, 32.4% en 52.2% van de waarden onder de normale range voor jonge mannen (< 320 ng/dl, < 6.5 ng/dl, < 152 ng/dl als referentiewaarde, respectievelijk). Het voorkomen van subnormale testosteron concentraties in onze studie groep kwam overeen met de cijfers uit publicaties van andere studiegroepen. De resultaten van de AMS scores suggereerden vooral de aanwezigheid van milde psychologische en somatovegetatieve symptomen. Seksuele symptomen werden evenwel door 88% van de mannen gerapporteerd. Nochtans toonde geen enkele van deze 3 AMS domein scores een relatie met testosteron-, vrij of bioavailable testosteronconcentraties. Wel waren er significante correlaties tussen de resultaten van de AMS scores en deze van de andere gezondheidsvragenlijsten, maar ook deze andere algemene vragenlijsten correleerden niet met androgeen-concentraties in het bloed.

De AMS vragenlijst kan helpen om de symptomen van de oudere mannen te omschrijven, maar het belang van deze informatie mag niet overschat worden. Een van de belangrijkste redenen voor het gebrek aan predictieve waarde van de klinische symptomen kan gevonden worden in het feit dat deze bij de man een multifactoriële oorzaak hebben. Bovendien kunnen levensstijlveranderingen die wel met de leeftijd geassocieerd zijn, zoals verminderde fysieke activiteit, mede een invloed hebben. Onze data ondersteunden de visie dat in deze groep van oudere mannen de symptomen gekozen voor de AMS vragenlijst de androgeen concentraties, inclusief DHEA-S, niet kunnen voorspellen. Deze resultaten beschreven de situatie van een gezonde oudere populatie met een gemiddelde leeftijd ouder dan 79 jaar. Weinig informatie was beschikbaar over dergelijke populaties, maar de resultaten kunnen mogelijks niet geëxtrapoleerd worden naar jongere of minder gezonde populaties. Gezien de correlatie van AMS met de resultaten van andere gezondheidsvragenlijsten (SF-36 en RDRS-2) wel bestond, zou dit suggereren dat AMS een specifieke levenskwaliteitschaal kan zijn bij problemen van de oudere man.

Depressieve symptomen.

In **hoofdstuk 4** wordt het voorkomen van depressie bij een cohorte van oudere mannen beschreven evenals de associatie van depressie met hormonale waarden, een androgeenreceptor polymorfisme (AR) en algemene gezondheidsperceptie. Het voorkomen van milde depressieve symptomen is niet uitzonderlijk bij oudere mannen, net als de aanwezigheid van een relatief testosteron tekort. Ook kunnen de psychiatrische symptomen van een te laag testosteron overlappen met de symptomen van depressie. De literatuur rapporteert wisselend over de relatie depressie-testosteron. Recent werd in een onderzoek - waar opnieuw geen relatie tussen beide gevonden werd - een subanalyse uitgevoerd waarbij een statistisch model met AR polymorfisme en testosteron samen depressie voorspelden.
De deelnemers van ons onderzoek waren ambulante mannen (236 in 1997 en 192 in 2000, ≥ 70 jaar bij de start van het onderzoek in 1996). Serum concentraties van testosteron, oestradiol, seks hormoon bindend globuline, dehyroepiandrosterone-sulfaat (DHEA-S), cortisol en de AR gen CAG-repeat length polymorfisme werden bepaald. Vrij testosteron en oestradiol werden berekend. De gebruikte vragenlijsten waren de Geriatrische Depressie Schaal (GDS), SF-36 en RDRS-2. De GDS vragenlijst bestaat in verschillende versies, en deze met 30 vragen werd hier gebruikt. De vragenlijst kan majeure depressie identificeren zowel in hospitaalmilieu als bij de huisarts. Een GDS score tussen 0-10 doet de afwezigheid van depressie vermoeden. Een score ≥11 kan de aanwezigheid van depressie weerspiegelen. Wij gebruikten een 'Vlaamse' vertaling van de originele Engelse vragenlijst.

De mediane leeftijd van de studiegroep was 75.3 jaar (IQR 73.5-78.5). Een positieve GDS score (≥11) werd bij 30 mannen gevonden (12.7%) in 1997. In 2000 had 17% van de deelnemers een positieve GDS score. De resultaten van 1997 en 2000 waren zwak doch significant gecorreleerd met elkaar ($r = .54$, $P = .029$). GDS scores waren hoger bij hogere leeftijd, gebruik van antidepressiva en slaapmiddelen. De resultaten van de verschillende vragenlijsten waren onderling gecorreleerd met elkaar. Er bestond een significante relatie tussen leeftijd en de scores van alle vragenlijsten. Noch in 1997, noch in 2000 bestond een relatie tussen GDS scores en (vrij) testosteron of het AR polymorfisme. In 1997 waren hogere GDS scores gerelateerd aan hoger oestradiol, vrij oestradiol en DHEA-S concentraties. Met een kwartiel analyse was de kans op een positieve GDS score 3.67 (1.07-12.64) maal hoger voor die mannen met het hoogste kwartiel van vrij oestradiol in vergelijking met die mannen met het laagste kwartiel van vrij oestradiol. Deze associatie werd echter niet langer bevestigd in 2000. GDS scores waren eveneens niet gecorreleerd met body mass index en aromatase activiteit (gemeten door de verhouding testosteron/oestradiol). Dit onderzoek ondersteunde niet de idee dat testosteron een belangrijke rol speelt bij het voorkomen van majeure depressie (gemeten met de GDS) bij oudere mannen.

GONADOTROPINE RESPONS OP AROMATASE-INHIBITIE

De oorzaken van de afname van testosteron productie op hogere leeftijd van de man zijn zowel testiculaire veranderingen als een gewijzigde neuro-endocriene regeling van de LH secretie, met in vergelijking met jonge mannen toegenomen gevoeligheid voor seks hormoon feedback. Het is gekend dat oestradiol een belangrijke rol speelt in de regeling van de feedback op de hypofyse. Ondanks een toegenomen vetmassa en een toegenomen aromatase activiteit

bij stijgende leeftijd (testosteron wordt in het vetweefsel door het aromatase enzym omgezet tot oestradiol, een 'vrouwelijk' hormoon) zien we een beperkt effect op de serum concentraties van oestradiol. Een farmacologische inhibitie van de aromatase activiteit geeft zowel bij jonge als oudere mannen aanleiding tot hogere gonadotropine en testosteron concentraties.

In de klinische studie beschreven in **hoofdstuk 2**, werd de hypothese getest dat een relatief lager testosteron bij oudere mannen zou kunnen het gevolg zijn van toegenomen oestradiol feedback. Letrozole, een specifieke en krachtige aromatase inhibitor, die momenteel toegediend wordt bij vrouwen met gemetastaseerde borstkanker, vermindert de oestradiol concentratie met 30-50 % en doet testosteron en LH secretie toenemen bij gezonde mannen. De hypothalame-hypofysaire-gonadale wijzigingen geïnduceerd door een gedeeltelijke afname van de oestradiol feedback werden bestudeerd bij een groep van 10 gezonde jonge en 10 oudere mannen. Letrozole 2,5 mg of placebo werd door hen dagelijks ingenomen gedurende 28 dagen. Bij de start van de studie hadden de oudere mannen vergelijkbare concentraties van testosteron, oestradiol, LH en FSH, maar hogere concentraties van SHBG (een seks hormoon bindend eiwit) en een lager vrij testosterone (vrij = niet eiwit gebonden) t.o.v. de groep jonge mannen. Letrozole verminderde de oestradiol concentraties met 46% bij de jonge mannen, die significant verschillend was t.o.v. de vermindering bij oudere mannen (- 62%). Onder inname van letrozole, maar niet bij placebo, stegen LH en testosteron concentraties vergelijkbaar in beide groepen, met een gemiddelde toename van testosteron van + 146 % in de jonge vs. + 99 % bij de oudere mannen, wat tussen de groepen niet significant verschillend was. Bij de groep behandeld met letrozole was de maximale LH respons na GnRH stimulatie (na 20 minuten) + 152 % en + 52 % t.o.v. de basiswaarde, respectievelijk bij jonge en oudere mannen. Hieruit kan geconcludeerd worden dat aromatase inhibitie op een krachtige manier spontane LH secretie, LH secretie na GnRH stimulatie en testosteron secretie stimuleert zowel bij jonge als oudere mannen. De gelijkaardige tot grotere antwoorden bij de jonge mannen in vergelijking met de ouderen, ondersteunen dus niet de hypothese, dat toegenomen oestradiol feedback op de LH secretie bij de ouderen verantwoordelijk is voor de leeftijdsgebonden afname van de testosterone concentraties in het bloed.

CONCLUDEREND

In de toekomst moet het mogelijk worden te identificeren wiens klachten en symptomen precies passen bij de diagnose andropauze en wie het meeste baat heeft bij een behandeling. De geteste vragenlijsten tonen aan dat het niet eenvoudig is om 'andropauze' klachten of depressieve klachten eenvoudig te koppelen aan hormonale cijfers. Klachten waarvan gedacht wordt dat zij bij andropauze horen, waaronder depressieve klachten, hebben ongetwijfeld een multifactoriële oorzaak. Ouder worden bij de man wordt begeleid door een afname in veel fysiologische functies, en op endocrien gebied door een afname androgeen secretie (uit geslachtsklieren en bijnieren) maar ook door onder meer een afname van groeihormoon secretie. Levensstijl veranderingen kunnen eveneens belangrijk zijn, zoals aangeduid door de impact van afname van fysieke activiteit of de afname van spiermassa. De AMS of ADAM (Androgen Deficiency of the Aging Male) vragenlijsten kennen nu al een uitgebreide verspreiding en gehoopt wordt vanuit commercieel oogpunt dat veel oudere mannen deze invullen, wat impliceert dat ook velen uiteraard positief zullen scoren. Wij beschrijven het gebrek aan relatie met hormonale cijfers, maar vinden de vragenlijsten nuttig om in een gesprek met de patiënt een gedetailleerde symptoombeschrijving te verkrijgen. Daarom blijft het ook noodzakelijk een diagnose

van andropauze niet enkel te baseren op deze vragenlijsten (waarop men snel positief scoort) maar de diagnose voor te behouden aan die mannen mét klachten én duidelijk te lage testosteron spiegels. Pas dan kan hormonale therapie samen met adequate beweging en een gezondere levensstijl het lichamelijke ouder worden wellicht uitstellen en vooral levenskwaliteit beïnvloeden. Aangezien de beschikbare testosterone supplementen niet vrij zijn van bijwerkingen wordt voortdurend gezocht naar alternatieven. Uit experimenten lijkt het dat aromatase inhibitie een verder te onderzoeken behandelingsvorm is.

STUDIES BIJ TRANSSEKSUELE PERSONEN

Er is sprake van genderidentiteitsstoornis wanneer een persoon met normale lichamelijke seksuele kenmerken overtuigd is dat hij/zij tot de andere sekse behoort. Dergelijke tegenstelling veroorzaakt ernstige problemen voornamelijk van psychologische aard, die men genderdysforie noemt. Betreffende de oorzaken van transseksualiteit bestaat nog geen duidelijkheid. Transseksualiteit kan niet uitgelegd worden door variaties in chromosomen patroon of door genitale, genetische, gonadale of hormonale afwijkingen. Vroeger werd gedacht dat het fenomeen louter een waanbeeld was. Dierexperimenteel onderzoek heeft duidelijk gemaakt dat ook het centrale zenuwstelsel een geslachtsdifferentiatie ondergaat. Postmortaal hersenonderzoek heeft de eerste indicatie gegeven dat transseksualiteit mogelijks een substraat heeft in het centrale zenuwstelsel. De seksuele differentiatie van de hersenkernen komt niet steeds overeen met de chromosomale structuur.
Transseksualiteit komt vaker voor bij mannen dan bij vrouwen. Volgens de meest recente (Nederlandse) cijfers is de prevalentie 1 op 11900 mannen en 1 op 30400 vrouwen. Het vermoeden bestaat dat het transgender fenomeen over heel de wereld voorkomt.
Volgens de zorgenstandaard, uitgewerkt door de HBIGDA (Harry Benjamin International Gender Dysphoria Association)- een wereldwijde organisatie van professionelen in transseksualiteit- zijn drie criteria vereist om van 'Gender Identity Disorder' te kunnen spreken: 1) de betrokkene moet verlangen aanvaard te worden als behorende tot de andere sekse, meestal begeleid door de wens zijn of haar lichaam zoveel mogelijk aan te passen aan de gewenste sekse door middel van hormonale behandeling en van chirurgie, 2) deze wens moet gedurende een langere periode aanwezig zijn, 3) de vraag mag geen symptoom zijn van een andere mentale kwaal of van een interseks-aandoening.

Het Genderteam van het Universitair Ziekenhuis van Gent werd in 1985 opgericht. Momenteel bestaat er een multidisciplinair team dat tot 9 verschillende disciplines omvat, zoals psychiatrie, psychologie, endocrinologie, urologie, plastische chirurgie, gynaecologie, hoofd- en hals chirurgie, dermatologie en logopedie. Ook in ons centrum wordt een procedure van geslachtsaanpassende behandeling (hormonale therapie en nadien chirurgische ingrepen) gevolgd die geënt is op de procedure die men in de meeste genderklinieken hanteert.
In de behandeling van transseksuelen zijn vier fasen te onderkennen: de diagnostische fase, de real life test, waarin de hormonale behandeling plaatsvindt, de chirurgische behandeling, en de nazorg.

De diagnostische fase

Tijdens het eerste jaar wordt van de psychiater verwacht dat hij/zij nagaat of de patiënt wel voldoet aan de formele diagnostische criteria van genderidentiteits-stoornis. De psychiater heeft minimaal een aantal gesprekken met de patiënt waarin systematisch en uitvoerig een aan-

tal onderwerpen aan de orde komen, zoals symptomatologie en ernst van de genderdysforie nu en in het verleden. In deze fase vindt ook lichamelijk en endocrinologisch onderzoek plaats bij de endocrinoloog in voorbereiding van eventuele hormonale therapie. Al tijdens de diagnostische fase gebeurt een algemeen lichamelijk nazicht, inclusief bloedonderzoek met hormonale bepalingen en karyotypering. Ook wordt het onderwerp (verlies van) fertiliteit besproken.

Real life test + hormonale behandeling

In deze fase van de behandeling krijgt de patiënt hormonale therapie maar er wordt ook contact gehouden met de behandelende psychiater. Een zo goed mogelijke lichamelijke aanpassing van het lichaam naar dat van het gewenste geslacht wordt beoogd. Tevens begint hij/zij de real life test, voor zover dit nog niet gebeurde. Met deze test wordt bedoeld dat de patiënt als voltijds lid van het gewenste geslacht gaat leven. Deze real life ervaring staat centraal in de behandeling. Verloopt deze test succesvol gedurende anderhalf à twee jaar dan volgt de chirurgische behandeling. Waar meestal patiënten en artsen de neiging hebben om de hormonale dosering snel te maximaliseren, heeft het Genderteam UZ Gent altijd geopteerd om zijn transseksuele personen te behandelen volgens een mild gedoseerd, maar effectief bifasisch hormonaal schema, met als bedoeling geen of minimale bijwerkingen te induceren. Cyproterone acetaat 50-100 mg, een anti-androgeen bij man-vrouw transseksuelen en lynestrenol 5 mg, een progestageen bij vrouw-man transseksuelen worden initieel, gedurende ongeveer 1 jaar, voorgeschreven. Dit is een reversibele hormonale behandeling die desgewenst nog kan gestopt worden. De bedoeling is om geslachtspecifieke kenmerken zoals erectie of menstruatie te onderdrukken. In deze fase wordt een daling van het libido bij man-vrouw transseksuelen al ervaren. Dit kan een extra diagnostische test betekenen. Oestrogenen (in hun verschillende toedieningsvormen) bij man-vrouw transseksuelen en testosteron bij vrouw-man transseksuelen vervolledigen de hormonale behandeling, en worden verder gezet als unieke therapie na de geslachtsaanpassende chirurgie. Met dit behandelingsschema werden bij het Gentse follow-up onderzoek relatief weinig en mineure complicaties gezien, meestal omkeerbaar door aangepaste behandeling, zoals beschreven in hoofdstuk 6.

De chirurgische behandeling

Alvorens hiertoe over te gaan wordt de patiënt uitvoerig besproken in het volledige genderteam. Een voorbereidend gesprek met de plastisch chirurg vindt plaats. Pas na 2 jaar hormonale behandeling volgen de chirurgische interventies. Bij man-vrouw transseksuelen wordt een vagina, inclusief clitoris, gecreëerd. Borstvergroting kan uitgevoerd worden als de behandeling met vrouwelijke hormonen ontoereikend was. Bij vrouw-man transseksuelen wordt een mammectomie verricht in het tweede jaar van de hormonale behandeling. De falloplastie (het maken van een penis) gebeurt via vrije flap techniek. Testiculaire prothesen en een erectieprothese kunnen aanvullend worden voorgesteld.

De nazorg

De patiënt wordt enkele malen teruggezien bij de plastisch chirurg en de uroloog. De hormonale behandeling vindt in principe levenslang plaats. Aanvankelijk zijn er de halfjaarlijkse, later de jaarlijkse consulten bij de endocrinoloog, voor oppuntstelling van de hormonale behandeling, bloedcontroles en botdichtheidmetingen. Niet zelden zijn er vervolgconsulten bij de behandelende psychiater gewenst en dit wordt zelfs aangeraden.

De hormonale veranderingen die hier teweeg gebracht worden zijn uiteraard veel meer uitgesproken dan de relatieve tekorten die in de hoofdstukken over de oudere man beschreven werden.

HET FOLLOW-UP ONDERZOEK

Een hormonale behandeling en geslachtsaanpassende chirurgie worden beide gezien als eerste keuze behandeling voor transseksuele personen. Evaluatie van deze behandelingen blijft onverminderd nodig. Een uitgebreid onderzoek van postoperatieve transseksuele mensen behandeld in ons centrum werd in 2003 uitgevoerd **(hoofdstuk 6)**. Algemene gezondheid en seksuele tevredenheid werden bevraagd. Specifieke logopedische, chirurgische en urologische topics horend bij deze studie werden beschreven in diverse andere publicaties. Honderd en zeven Nederlandstalige potentiële deelnemers die een geslachtsaanpassende behandeling ondergingen tussen 1986 en 2001 werden gecontacteerd om aan deze studie deel te nemen. Bij de 55 uiteindelijke deelnemers werden relatief weinig en slechts mineure gezondheidsproblemen gemeld, waarvan de meeste reversibel waren met aangepaste behandeling. Er bestond wel een trend naar meer algemene gezondheidsproblemen bij man-vrouw transseksuele personen, mogelijks uitgelegd door de hogere leeftijd van deze deelneemsters en hun rookgedrag. Depressie en hoge bloeddruk werden bij respectievelijk 25% en 21% van de dames gerapporteerd.

Zoals logisch verwacht werd een significant verschil tussen androgeen concentraties tussen man-vrouw en vrouw-man transseksuele personen gevonden en dit werd ook weerspiegeld in de hematocriet waarden. De mediane testosteron-dosering op het moment van onderzoek, onafhankelijk van het moment van de laatste toediening, bij de vrouw-man transseksuele personen toonde een laag cijfer, nl. 285 ng/dl en slechts 25% van de mannen had op het moment van onderzoek een voldoende hoge testosteron concentratie. Deze gegevens stimuleerden ons om de androgeen onderdosering van sommige mannen aan te passen. Daar waar alle man-vrouw transseksuelen, met continue oestrogeeninname, normale vrouwelijke testosteron concentraties in het bloed hadden, had slechts 32.1 % normale vrije testosteron cijfers. Verder onderzoek zal uitwijzen of dit te lage vrije testosteron verantwoordelijk is voor gebrek aan libido dat door sommige man-vrouw transseksuele personen gerapporteerd wordt.

Na geslachtsaanpassende behandeling waren de verwachtingen op emotioneel en sociaal gebied vervuld, maar op fysiek en seksueel gebied was dit minder duidelijk. Nochtans rapporteerde 80% van de deelnemers een verbetering van hun seksuele activiteit. De vrouw-man transseksuele personen masturbeerden meer frequent dan de man-vrouw transseksuele personen, rapporteerden meer seksuele tevredenheid, opgewondenheid en ze beschreven het makkelijker bereiken van een orgasme. De meerderheid van de deelnemers vermeldde een verandering in orgasmegevoel naar krachtiger en korter bij vrouw-man transseksuele personen en naar meer intens, zachter en langerdurend bij man-vrouw transseksuele personen. Meer dan 2/3 van de man-vrouw transseksuele personen beschreven de secretie van vaginale vloeistof tijdens opwinding, komende van de Cowper klieren, die bij de chirurgie ter plaatse blijven. Bij vrouw-man transseksuele personen met een erectieprothese waren de seksuele verwachtingen meer vervuld, in vergelijking met deze mannen zonder erectieprothese, maar pijn bij seksueel contact werd dan weer vaker gemeld.

Wij vonden dat seksualiteit een belangrijke evolutie ondergaat tijdens en na de transitiefase, deels tegengevolge van de hormonale therapie. Daarom zou in de diagnostische en

hormonale fase meer aandacht moeten besteed worden aan seksuele verwachtingen en mogelijke seksuele veranderingen die zullen optreden, met als bedoeling de transseksuele persoon te informeren over deze nieuwe ervaringen.

DE TRANSFERRINE RECEPTOR.

Bij de hormonale en chirurgische behandeling van transseksuele personen worden de geslachtshormonen zeer grondig beïnvloed. Dit laat ons toe de rol van geslachtshormonen op verschillende parameters bij de mens te ontdekken. In de studie beschreven in **hoofdstuk 5** wordt het effect van hormonale behandeling op de vrije transferrine receptor beschreven. Het is algemeen gekend dat mannen hogere hematocriet en hemoglobine concentraties hebben in vergelijking met vrouwen. Dit is vermoedelijk te wijten aan het sekseverschil in serum testosteron-concentratie. Mannen met een testosteron-tekort hebben lagere hemoglobine en hematocriet concentraties en behandeling met testosteron doet deze variabelen toenemen tot normale waarden. De mechanismen achter het effect van testosteron op aanmaak van rode bloedcellen is niet helemaal gekend. Recent onderzoek heeft aangetoond dat bij androgeen-geïnduceerde aanmaak van rode bloedcellen er geen toename is van de erythropoietine concentraties. Anderzijds werd ook beschreven dat inductie van androgeen tekort met een LHRH- (luteinizing hormone-releasing factor) agonist eveneens geen veranderingen van serum erythropoietine concentraties veroorzaakte. Het is interessant te vermelden dat er tussen mannen en vrouwen geen verschillende erythropoietine concentraties bestaan, ondanks een significant verschil in testosteron en hemoglobine concentraties. Daarom lijkt het dat de toename van hemoglobine en hematocriet concentraties niet enkel door erythropoietine bepaald wordt en dat testosteron een rechtstreeks effect op het beenmerg heeft. Onze studie beschreef de effecten van behandeling met geslachtshormonen op hemoglobine en hematocriet bij transseksuele personen door middel van een kwantitatieve parameter die aanmaak van rode bloedcellen ter hoogte van het beenmerg weerspiegelt, de vrije (soluble) transferrine receptor. IJzer wordt in het plasma getransporteerd door transferrine. Transferrine geeft ijzer vrij aan de cel door de interactie met een specifieke membraan receptor, de transferrine receptor (TfR). Een vrije vorm van de TfR (sTfR) werd bij dier en mens beschreven. De meest belangrijke determinant van sTfR concentraties is beenmerg activiteit voor aanmaak van rode bloedcellen.

In onze studie kregen 19 man-vrouw transseksuele personen ofwel oraal ethinyl oestradiol (EE) (n=12) of transdermaal 17ß-oestradiol (E2) (n=7); beide behandelingen werden gegeven inclusief een anti-androgeen, cyproterone acetaat (CA). Zes mannen werden met CA alleen behandeld. Vijftien vrouw-man transseksuele personen werden behandeld met intramusculaire testosteron esters. Een evaluatie van de aanmaak van rode bloedcellen en variabelen die hiermee een verband hebben werd geanalyseerd, voor en 4 maand na hormonale behandeling. De androgeen behandeling zorgde voor een toename van de aanmaak van rode bloedcellen ter hoogte van het beenmerg, indirect geëvalueerd door een toename van sTfR. Zowel CA met oraal EE als met transdermaal E2 reduceerden plasma testosteron concentraties tot uiterst lage waarden, begeleid door een afname van de parameters van rode bloedcel aanmaak. De CA + orale EE combinatiebehandeling induceerde een afname van sTfR, wat niet zo was bij CA + transdermaal E2 behandeling. Dit kon niet worden uitgelegd door de duidelijke afname van testosteron die gelijkaardig was in beide groepen. Het verschil kon mogelijks verklaard worden door verschillende effecten op IGF-I, een groeihormoonfactor.

CONCLUDEREND

Het follow-up onderzoek binnen ons Genderteam heeft aangetoond dat een bifasische hormonale therapie een effectieve en voldoende veilige transitie kan verzorgen. De eerste reversibele fase is een belangrijke fase van de real-life ervaring, waarbij seks-specifieke karakteristieken onderdrukt worden. Als het lijden van de transseksuele persoon hiermee vermindert, kan dit eventueel als extra diagnostische test voor de diagnose transseksualiteit gezien worden. Of de inductie van de gewenste geslachtskenmerken het welzijn van deze persoon zal bevorderen, kan hieruit mogelijks afgeleid worden. Een farmacologische ablatie van endogene productie van geslachtshormonen zou kunnen een lagere dosis van cross-seks hormonen toelaten, om zo het risico op bijwerkingen te verminderen. Dit is een onderwerp dat vergelijkend onderzoek vraagt, bij grotere groepen mensen.

Gezien de zeldzaamheid van de diagnose van transseksualiteit moeten Europese of nog bredere samenwerkingsverbanden gelegd worden tussen de verschillende centra die een goed functionerend Genderteam hebben (Amsterdam, Bologna, Malaga, Liège en Gent voor Europa). Onderzoek bij transseksuele personen kan ons immers helpen in het verdere onderzoek van de complexe effecten van geslachtshormonen op verschillende orgaansystemen en aandoeningen. Zoals blijkt uit het onderzoek over aanmaak van rode bloedcellen geëvalueerd door de sTfR bepaling bij transseksuele personen, kunnen hormoonafhankelijke geslachtsverschillen verder gedetailleerd beschreven worden.

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