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Androgen insufficiency in women: diagnostic and therapeutic implications

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The proposed key symptoms of the female androgen insufficiency syndrome (FAIS) include reduced libido, diminished well being and lowered mood. The diagnosis of FAIS is made on the basis of these symptoms in the setting of a low serum free testosterone level. However, there is currently no readily available inexpensive assay which reliably measures free testosterone levels in the female range. The diagnosis of FAIS is further complicated by the lack of data demonstrating a minimum serum free testosterone level which, if below this, correlates with the symptoms of FAIS. Despite the complexities involved with defining FAIS, the symptoms have been reported to respond well to testosterone replacement. There is a need for formulations of testosterone therapy specifically designed for use in women, along with clear guidelines regarding optimal therapeutic doses and long-term safety data.

Key words: androgens/female androgen insufficiency syndrome/testosterone

Introduction

At a recent consensus conference, a group of clinical investigators from a wide variety of fields related to women's health proposed a set of symptoms and signs describing the female androgen insufficiency syndrome (FAIS). The designated constellation of symptoms includes reduced libido, diminished well being and lowered mood accompanied by a low serum free testosterone concentration. However, the measuring of testosterone levels is limited by the lack of routinely available sensitive assays for both total and free testosterone. Most available testosterone assays show poor reliability at the lower end of the circulating female concentration range. The normal reference range for women is not well defined, as there is a lack of a large normative database of androgen levels for women. This is an area of ongoing research. Results of testosterone levels can only be interpreted in light of these limitations. The present evidence for the existence of a FAIS is based primarily on clinical experience, limited observational studies and few randomized, placebo-controlled trials (RPCTs). It is important to consider this a working definition, on which to base future research.

Although the FAIS remains formally undefined, there is evidence of potentially therapeutic effects of androgen replacement on overall well being, mood, sexual function and bone health in women with low testosterone levels.

Physiology of circulating androgens in women

Androgens have important physiological roles in women, acting both as precursors for estrogen biosynthesis and directly via the androgen receptor. Circulating androgen levels decline in the years preceding menopause. This may be attributed to the gradual reduction in adrenal androgen production with age and to the loss of cyclical ovarian androgen production in the late reproductive years. Those who experience surgical menopause, suffer adrenal insufficiency or pituitary insufficiency, or who have experienced premature ovarian failure also have reduced androgen production.

Biosynthesis of androgens takes place both in the adrenal and in the ovary. Adrenal androgen secretion is stimulated by adrenocorticotropic hormone (ACTH) while ovarian androgen secretion is stimulated by LH. A physiological negative feedback system that regulates androgen homeostasis has not been demonstrated in the female.

In women, androgens circulate in the nanomolar to micromolar concentration range, in contrast with estrogens, which circulate in concentrations in the picomolar range. The major androgens found in women in descending order of their serum concentrations include; dehydroepiandrosterone sulphate (DHEAS), dehydroepiandrosterone (DHEA), androstenedione, testosterone (T) and dihydrotestosterone (DHT). Of these androgens, testosterone is the most potent (Burger, 2002).

DHEAS is secreted solely by the adrenals. Its serum concentrations increase from about age 7 to 8 (the adrenarche), peak in the third and fourth decades and then decline steadily with age. There are no significant changes in DHEAS during the menstrual cycle nor in relation to the menopause.

DHEA is produced by the adrenals (50%) and the ovaries (20%) from pregnenolone and from peripheral conversion of DHEAS (30%). The decline in DHEA with age parallels that of DHEAS.

Androstenedione is produced in equal amounts by the adrenals and the ovaries from cholesterol via progesterone. It shows diurnal variation and a midcycle elevation in concentration, which parallels the midcycle peak of estradiol.

The most significant biologically active androgen in males and females is testosterone. Testosterone is secreted by the adrenals (25%), the ovaries (25%) and by peripheral conversion of circulating androgens, predominantly androstenedione (50%). Testosterone levels fluctuate during the normal menstrual cycle with a midcycle peak, dropping off in the luteal phase and are lowest in the early follicular phase. Testosterone levels also show diurnal variation with an early morning peak.

DHT is derived almost entirely from peripheral conversion of testosterone by the enzyme 5α-reductase. This occurs predominantly in androgen-sensitive tissues such as the skin. A small amount of DHT is produced by the adrenals. Once synthesized, DHT is metabolized intracellularly and is therefore not a reliable measure of total DHT production.

Androgens are obligatory precursors in the biosynthesis of estrogens via the aromatization of androstenedione to estrone and testosterone to estradiol.

Sex steroid-hormone binding globulin (SHBG) is a major determinant of the bioavailability of sex steroids. Variations in the plasma levels of SHBG impact significantly on the amount of free or bioavailable testosterone and other bound sex steroids. SHBG has a differing binding affinity for sex steroids in the following order; DHT > testosterone > androstenedione > estradiol > estrone. SHBG also weakly binds DHEA, but not DHEAS. Testosterone circulates in peripheral blood with ~66% bound to SHBG and ~33% loosely bound to albumin. Only 1–2% of total circulating testosterone is in the unbound form (free T). Bioavailable testosterone consists of the unbound fraction of testosterone (free T) in addition to albumin-bound testosterone. As such, when investigating for androgen insufficiency, measures of total testosterone along with SHBG are required.

Levels of circulating androgens in relation to age and menopause

Unlike estrogen levels, testosterone levels do not change significantly in relation to the menopausal transition but rather decrease gradually as a function of age in early adulthood. Testosterone levels decline with age prior to menopause (Zumoff *et al.*, 1995; Davis *et al.*, 2002). There is an ~50% fall in both total and free testosterone between the ages of 20 and 40–45, with only a very slight fall in circulating concentrations thereafter. In addition, circulating SHBG levels fall across (Zumoff *et al.*, 1995) the menopausal transition with a subsequent rise in free androgen levels (Burger *et al.*, 2000).

Most recently a study of 149 healthy premenopausal women with regular cycles, no exogenous hormone therapy and no complaint of low libido showed a statistically significant decline with age for each of free testosterone, DHEAS, androstenedione and DHT, measured after organic solvent extraction by validated methodology (Davis *et al.*, 2002). In the late reproductive years there is failure of the midcycle rise in free testosterone which characterizes the menstrual cycle in young ovulating women (Mushayandebvu *et al.*, 1996). This occurs despite preservation of normal free testosterone levels at other phases of the cycle. The mean plasma concentrations of testosterone in women transitioning through the menopause are also significantly lower than younger ovulating women sampled in the early follicular phase (Longcope *et al.*, 1986).

There is considerable controversy as to whether the postmenopausal ovary is a significant source of androgen production. In postmenopausal women, concentrations of testosterone have been shown to be higher in the ovarian vein than in systemic venous blood, suggesting that the postmenopausal ovary continues to secrete androgens (Judd *et al.*, 1974). Moreover, testosterone levels decrease in postmenopausal women following oophorectomy (Judd *et al.*, 1994). In a cross-sectional study of 684 women aged 50 to 89 years, total, but not bioavailable, testosterone levels increased with age, reaching premenopausal levels in the 70–79 decade with relatively stable levels thereafter (Laughlin *et al.*, 2000). On the other hand, Couzinet *et al.* (2001) found similarly low levels of total and bioavailable testosterone in postmenopausal women with intact ovaries ($n = 15$) to oophorectomized postmenopausal women ($n = 15$). These findings suggest that the postmenopausal ovary is not a major source of androgens. Likewise, Davis *et al.* (2002) found no difference in levels of total and free testosterone, DHEAS, DHT, androstenedione and SHBG in 309 surgically menopausal women with low libido on hormone therapy (HT) compared with naturally menopausal women with normal libido also on HT.

In summary, androgen production of the postmenopausal ovary is variable and requires further study. It appears that total and free testosterone levels decline with age in premenopausal women from the early to mid reproductive years (Zumoff *et al.*, 1995), remain stable across the menopausal transition (Burger *et al.*, 2000) and then either remain stable, continue to decline or increase with increasing age.

Levels of circulating androgens in relation to ethnicity

In the SWAN study, changes in hormone levels across the menopausal transition were reported in 3029 women aged between 42 and 54 years from five ethnic groups (Lasley *et al.*, 2002). These women were of Caucasian, Japanese, Hispanic, African American or Chinese descent and were followed over a 2 year period. DHEAS concentrations were highest among Chinese and Japanese women and lowest among African American and Hispanic women. Across all ethnic groups, DHEAS levels decreased between the ages of 45 and 50 years, after which mean DHEAS levels increased, particularly in relation to the number of women in the late perimenopause. The age-related decline was largest in Caucasian women, whereas the subsequent increase in DHEAS tended to be larger in Chinese, Hispanic and Japanese women with a smaller increase in African American and Caucasian women. Total testosterone levels as measured by chemiluminescent immunoassay showed parallel changes with those

of DHEAS, with an age-related decline but a less distinct rise from age 51. Mean testosterone levels were lowest in Hispanic women.

The HERITAGE study provides further evidence that the plasma levels of steroid hormones, including androgens, are under significant genetic influence as demonstrated by linkage analysis, which varied with ethnicity (Ukkola *et al.*, 2002). In particular, genes coding for testosterone and DHT were located in the vicinity of different genomic regions depending on whether the person was of black or white origin.

Defining the female androgen insufficiency syndrome

A recent consensus conference recommended that the female androgen insufficiency syndrome (FAIS) is defined by a pattern of clinical symptoms and signs in the presence of decreased bioavailable testosterone and normal estrogen status (Bachmann *et al.*, 2002). Clinical symptoms of the proposed deficiency state (see Table I) may include decreased libido, sexual receptivity and pleasure; a diminished sense of well being; dysphoric mood and/or blunted motivation; and persistent unexplained fatigue. Clinical signs might include bone loss, decreased muscle mass and strength, adipose tissue redistribution, decreased sexual hair and changes in cognition or memory, however, there are currently insufficient data to support the latter.

Many of these symptoms are non-specific and are common to other disorders such as depression, and are affected by multiple variables including socio-economic, environmental and life circumstances. Furthermore, whether depression can be a consequence of, or a cause of, androgen insufficiency remains unclear and requires further study for clarification.

It is well known that the effects of estrogens are strongly linked to well being, mood and sexual functioning, and thus a diagnosis of androgen insufficiency should be made only in women who are well estrogenized. In premenopausal women, adequate estrogenization is likely in the presence of regular cycles (periods every 21 to 35 days) and the absence of hot flushes or vaginal dryness. Postmenopausal women should be receiving estrogen replacement, preferably parenterally, to avoid the increases in SHBG caused by oral estrogen.

To validate the diagnosis, the free T concentration should be at or below the lowest 25th percentile of the normal range for 20–40-year-old women of reproductive age.

Table I. Criteria for the diagnosis of female androgen insufficiency sydrome

Criteria for the diagnosis of female androgen insufficiency sydrome	
Symptoms and Signs	Poor sense of well being Dysphoric mood Impaired sexual function (low libido, decreased sexual receptivity and pleasure) Persistent unexplained fatigue Vasomotor instability* Vaginal dryness* Decreased muscle strength* Poor memory* Bone loss
Adequate estrogenization	no hot flushes and regular menstrual cycles or receiving estrogen replacement therapy
Low free testosterone level	Measured by equilibrium dialysis or low calculated free T (derived from the laws of mass action) at or below the 25th percentile of the normal range

*Insufficient data

Measuring androgen levels in women

Biochemical verification of FAIS requires either a morning free T and SHBG, total T and SHBG (for calculation of a free T index) or free T and total T. Blood should be drawn between 8:00 and 10:00 a.m. and after day 8 of the cycle, and preferably before day 20, due to the fluctuation of testosterone, resulting in higher levels at this time (Vierhapper *et al.*, 1997). A serum sample is preferred over plasma.

No rapid, simple, assay of total testosterone has been shown to produce reliable results in women with low testosterone levels. Free or bioavailable (non-SHBG-bound) testosterone measures are the most reliable indicators of tissue testosterone exposure. Ideally, free T should be measured by the gold standard, equilibrium dialysis; however, this test is expensive and not readily available. The free T assays which are more readily available and less expensive are neither sensitive nor reliable enough in the low female range. The free androgen index (total T nmol/l ÷ SHBG nmol/l × 100) provides a clinical approximation of free T. However, it is unreliable when SHBG levels are low (Davis *et al.*, 2003). The measurement of SHBG is relatively simple to perform with good reproducibility. There is considerable agreement that if total T can be reliably measured and SHBG and albumin are known, then free T can be calculated using the laws of mass action and used reliably as an accurate measure of free T (Sodergard *et al.*, 1982; Vermeulen *et al.*, 1999; Klee and Heser, 2000). In a recent study of 147 women, calculated free T was found to correlate well with free T measured by equilibrium dialysis ($r = 0.99$; $P < 0.0001$). On the other hand, free T measured by analogue direct radioimmunoassay (RIA) did not correlate as well with equilibrium dialysis ($r = 0.81$; $P < 0.0001$) (Miller *et al.*, 2004). It follows that in clinical practice calculated free T is an accurate, less expensive and readily available measure of T levels in women.

In a recent study Taieb *et al.* (2003) compared 10 commercially available testosterone immunoassays with isotope-dilution gas chromatography–mass spectrometry (ID-GC/MS) and concluded that testosterone immunoassay results for specimens from females are inaccurate by an average factor of 2 and in some cases by a factor of almost 5. Furthermore, Stanczyk *et al.* (2003) compared four different commercial direct testosterone immunoassays with extraction chromatography RIA and also concluded that commercial immunoassays are unreliable for measuring testosterone levels in female serum samples. As to whether ID-GC/MS measures of T levels are comparable with extraction chromatography RIA remains to be determined. Clearly there is a need for a reliable, commercially available assay.

Measurements of free testosterone by analogue assays are notoriously unreliable, particularly at the lower end of the normal female range and are not recommended for use (Klee and Heser, 2000). Salivary testosterone has been used reliably in studies of women with hyperandrogenism, however, the normal

reference range is excessively large and there is questionable accuracy in the lower ranges (Baxendale *et al.*, 1982). Moreover, salivary testosterone levels should not be equated to levels of free testosterone in serum.

Interpreting serum T levels is further complicated by the intracrine physiological action of T at the cellular level. Testosterone can be converted to either DHT or estradiol by the enzymes 5α-reductase and aromatase, respectively, in the same cells on which they act. Thus, tissue sensitivity to androgens will vary according to the amount and activity of these enzymes, which may vary considerably between individuals and does not correlate with serum T levels. The androgen receptor (AR) also differs between individuals, which may result in variability in end-organ response to absolute circulating levels of androgens. This is an area of androgen physiology that requires further investigation. Nonetheless, peripheral androgen levels (non-SHBG-bound testosterone and its precursors) may be indicative of the hormonal milieu of the brain, which may influence sexual behaviour either directly or via aromatization to estrogen within the brain.

DHEA and DHEA-S have also been recognized to have important intracrine actions. Labrie *et al.* (2003) have reported that most of the androgens in women, especially after menopause, are synthesized in peripheral intracrine tissues from DHEA and DHEA-S. Most of the enzymes required for the synthesis of androgens from these precursors are present in the same cells of peripheral target tissues on which they exert their action. Subsequently, there is minimal spillage of these locally synthesized androgens into the peripheral circulation, further highlighting the complexities associated with the use of serum T levels as an indication of biologically available androgens.

Thus, even with highly sensitive assays for total and free T, measurement of testosterone will provide only an indication of androgen deficiency or excess, but not an absolute measure of tissue exposure or tissue sensitivity and responsiveness, and the clinical features will be the mainstay of diagnosis. This unfortunately is a limitation pertaining to much of the data regarding sex steroids and female sexual function.

To date, a relationship between a specific level of free testosterone and sexual symptoms has not been established (Dennerstein *et al.*, 2002). Studies examining the relationships between circulating endogenous testosterone levels and sexual activity have produced varying results. This is likely due to testosterone levels in the circulation being of limited value as an indicator of tissue androgen exposure and responsiveness. The diagnosis of diminished sexual function due to low testosterone is a diagnosis of exclusion. In the absence of a reliable free/total testosterone assay, the measurement of testosterone is used to exclude women in whom testosterone therapy may lead to adverse effects.

Other androgens

DHEAS is usually measured, rather than DHEA, because the half-life is much longer, resulting in more stable levels. The immunoassay for DHEAS is relatively reliable and simple to perform. There is a consensus that DHEAS does not vary in concentration within the various phases of the menstrual cycle, and that it is not bound to SHBG. It also does not seem to be affected by estrogen therapy at standard doses. A number of authors have shown normal, age-related decline curves for DHEAS, which are all quite compatible. If low levels are found, a morning cortisol level should be checked to investigate for adrenal insufficiency.

Other parameters

Women presenting with low libido and fatigue should have routinely measured: iron stores (which might be low despite normal hemoglobin); thyroid stimulating hormone (TSH) to exclude subclinical thyroid disease, and on clinical suspicion, a screen for autoimmune disease causing chronic fatigue (ANA).

Measurement of estradiol and FSH is indicated to diagnose premature ovarian failure in amenorrheic young women or to evaluate menopausal status in hysterectomized women. However, in the latter a full symptom history is often more useful. Amenorrhea with low FSH and low estradiol is suggestive of hypothalamic amenorrhea, hyperprolactinemia or other rare pituitary disease. Prolactin should be measured in premenopausal women with oligomenorrhea, amenorrhea and/or galactorrhea.

Differential diagnosis

The need to formally exclude depression may be assessed clinically by careful history taking, or the clinician may employ the use of a validated questionnaire such as the Hamilton Depression Scale or the Beck Depression Index, when there is uncertainty. Antidepressant therapy may improve sexual dysfunction by treating depression. Alternatively, antidepressants may worsen sexual dysfunction as a side effect of treatment and a change of antidepressant therapy may be required to clarify the predominant factor. In some women combination therapy with androgens and antidepressants may be required to improve sexual dysfunction.

Causes of androgen insufficiency in women

Female androgen insufficiency (FAI) can be part of the natural aging process in some women, whereas other women will have a clear underlying aetiology as a cause for FAI (see Table II). However, not all women will experience a decline in androgen levels that translates into the clinical symptoms of FAI. The reason for this is unclear, it may be that in women with a lower T level to begin with, a more rapid rate of decline in T levels or a reduced sensitivity to T at the receptor level are more likely to manifest symptoms of the FAIS.

FAI can be secondary to a number of medical conditions including; hypopituitarism (Miller *et al.*, 2001), premature ovarian failure (Doldi *et al.*, 1998), bilateral oophorectomy, ovarian damage as a result of chemotherapy or radiotherapy, Turner's syndrome, primary and secondary adrenal insufficiency or bilateral adrenalectomy (Abraham, 1974).

Changes in SHBG concentration will affect the levels of bioavailable testosterone. Elevations in estradiol (as occurs during pregnancy), hyperthyroidism and liver disease cause a marked increase in SHBG levels with a subsequent decrease in serum free T levels, whereas hypothyroidism, obesity, and hyperinsulinemia are associated with decreased SHBG levels.

Various drugs have also been associated with a decrease in circulating androgen levels. Exogenous corticosteroid use,

Table II. Aetiology of Female Androgen Insufficiency Syndrome

Aetiology of Female Androgen Insufficiency Syndrome	
Ovarian causes	Surgical and natural menopause
	Premature menopause
	Ovarian failure secondary to chemotherapy and radiotherapy
Endocrine causes	Adrenal Insufficiency
	Panhypopituitarism
	Hyperthyroidism
Disease States	HIV wasting syndrome
	SLE
	Rheumatoid arthritis
	Anorexia Nervosa
Drugs	Exogenous estrogen therapy (OCP, HRT)
	Excessive thyroxine replacement
	Glucocorticoid therapy

thyroxine replacement and oral estrogen therapy (either as hormone therapy or contraception) can lower free tesosterone levels indirectly due to a rise in SHBG with a subsequent decrease in bioavailable testosterone. Glucocorticoid therapy also leads to ACTH suppression and consequently reduced adrenal androgen production (Abraham, 1974).The use of exogenous estrogens additionally suppresses gonadotrophin release, with a subsequent decline in gonadal sex steroid production. Standard transdermal estradiol patch therapy has little or no effect on SHBG levels (Vehkavaara *et al.*, 2000; Mazer, 2002). On the other hand, when very high levels of estradiol are achieved following several weeks to months as seen with estradiol implants, SHBG will increase (S.Davis, Australia, clinical observation in women treated with estradiol implants; personal communication). SHBG also increases markedly from baseline with the new contraceptive patch delivering norelgestromin and ethinyl estradiol (Ortho-McNeil Pharmaceutical, Inc.; data on file). Intranasal estradiol does not increase SHBG significantly (Mattson *et al.*, 2000).

The ability of progestins to alter endogenous free testosterone levels is primarily related to their effects on SHBG and secondarily to their suppressive effect on LH (Bellantoni *et al.*, 1991; Onobrakpeya *et al.*, 2001; Gill *et al.*, 2002). The androgenic progestins [levonorgestrel (LNG), norethisterone acetate (NETA)] partially attenuate the increase in SHBG associated with oral estrogen (Lobo, 1988; Darney, 1995), whereas oral medroxyprogesterone acetate (MPA) has a smaller attenuating effect and oral micronized progesterone (MP) has virtually no influence on SHBG (Ottosson, 1984). Similarly, the transdermal administration of progestins would be expected to have a minimal impact on SHBG (Shulman *et al.*, 2002).

Other conditions that have been associated with AI include anorexia nervosa, rheumatoid arthritis, systemic lupus erythematosus and human immunodeficiency virus infection.

Indications for androgen replacement

Androgen replacement may be indicated in the presence of the clinical signs and symptoms of FAIS which are accompanied by a free T concentration in the lowest quartile of the relevant female reference range and normal estrogen status (Bachmann *et al.*, 2002). Prior to commencing therapy, depression and other social and environmental causes for her symptomatology must be excluded.

Moreover, women on oral estrogen with a low total T and high SHBG with a low free T, should be switched to a non-oral form of estrogen therapy for a period of 12 weeks. The subsequent fall in SHBG coupled with a rise in free T may obviate the need for T therapy.

Evidence for beneficial effects of testosterone therapy

A systematic review, which analyses a broad range of outcomes of testosterone in peri- and postmenopausal women, is to be published in The Cochrane Library which will provide an evidence-based review of the beneficial and adverse effects of testosterone therapy (Somboonporn *et al.*, 2004b).

Effects on mood and well being

There is evidence of a relationship between androgen deficiency and lowered mood and sense of well being which improves with testosterone therapy in pre- and postmenopausal women. The benefits have been noted in naturally and surgically menopausal women.

Sherwin *et al.* (1988) reported that the addition of intramuscular testosterone to estrogen replacement in surgically menopausal women resulted in improved well being and increased energy levels compared to those who received estrogen alone. The use of transdermal testosterone patches (300 μg daily) in surgically menopausal women over a 12 week period resulted in significant improvement in 'Psychological General Well Being Index' scores when compared to placebo (Shifren *et al.*, 2000).

In naturally postmenopausal women, therapy with testosterone has positive effects on mood and well being (Brincat *et al.*, 1984; Montgomery *et al.*, 1987). Treatment with testosterone implants (50 mg) in addition to estrogen therapy in postmenopausal women was found to also improve general well being (Burger *et al.*, 1987).

In a randomized placebo-controlled cross-over trial of healthy premenopausal women complaining of loss of libido, testosterone cream resulted in not only an improvement in libido, but also in mood and general well being (Goldstat *et al.*, 2003).

The evidence for other androgens is not as strong. DHEA given orally (50 mg daily) or transdermally (10% cream) has been claimed to improve well being when compared to placebo (Labrie *et al.*, 1997; Morales *et al.*, 1997), however neither study included formal well being questionnaires. Oral DHEA has demonstrated positive effects in women with adrenal insufficiency, through improvement in mood as well as depression and anxiety scores (Arlt *et al.*, 1999). Most recently, a study of DHEA in women with hypopituitarism demonstrated no benefit in a quality of life questionnaire during the randomized phase of the study (Johannsson *et al.*, 2002) and DHEA was not found to be beneficial in a study of elderly men and women (Flynn *et al.*, 1999). Evidence that the use of DHEA in women with even low normal levels is beneficial or safe is lacking.

Androgens and sexual function

Female sexuality is influenced by many factors. With increasing age there tends to be a reduction in libido, with a reported

decline immediately following oophorectomy (Nathorst-Boos *et al.*, 1992). There is also a significant decline in sexual interest in naturally menopausal women at the time of the menopausal transition (Dennerstein *et al.*, 1994). The influence of testosterone on this change has not yet been determined, as published studies have compared early follicular phase testosterone levels, which are usually low, with postmenopausal levels and have not utilized sensitive assays (Dennerstein *et al.*, 1997). Coital frequency in women also declines with increasing age and there is a decrease in coital frequency at the time of the menopausal transition independent of age (Frock and Money, 1992). Low testosterone levels have been found to be closely correlated with reduced coital frequency and loss of sexual desire (McCoy and Davidson, 1985).

Following bilateral oophorectomy there is an ~50% reduction in the levels of circulating testosterone (Judd *et al.*, 1994). Observational studies have shown that oophorectomized women more frequently report a worsening of their sexual life postoperatively than those who have undergone a hysterectomy alone or even a natural menopause (Nathorst-Boos *et al.*, 1992). However, some women after hysterectomy with or without oophorectomy, experience an improvement in sexual function possibly due to a reduction in dyspareunia or menorrhagia.

The role of steroids and their influence cortically on libido is yet to be determined. There are androgen and estrogen receptors in the female brain and estrogen and testosterone have been found post-mortem to be concentrated in the human female hypothalamus and preoptic area, with testosterone levels being 10-fold greater than those of estradiol (Bixo *et al.*, 1995). What remains unclear is whether testosterone acts predominantly directly via the androgen receptor or whether aromatization to estrogen locally within the human brain is required for the effects on libido and mood. Mouse studies suggest that aromatization of testosterone to estrogen within the brain is responsible for sexual activity in rodents (Ogawa *et al.*, 2000; Simpson *et al.*, 2000).

Estrogen therapy in menopausal women improves vasomotor symptoms, vaginal dryness and general well being, but has little or no benefit for low libido (Utian, 1972; Campbell *et al.*, 1977). However, in women suffering from dyspareunia secondary to atrophic vaginitis, the use of estrogen replacement may improve libido following the relief of vaginal symptoms (Studd *et al.*, 1977a). There is little evidence that any of the added progestins improve or worsen sexual problems when given with estrogen (Walling *et al.*, 1990; Shulman *et al.*, 2002).

Testosterone has been found to restore libido in postmenopausal women. Sherwin *et al.* (1987) observed that the use of intramuscular estrogen with high dose testosterone in oophorectomized women resulted in improvements in sexual motivational behaviours, namely desire, fantasy and arousal as well as increases in coital frequency and orgasm, with improvements greater than those seen with estrogen alone. Sarrel *et al.* (1998) compared esterified estrogen alone with esterified estrogen plus methyltestosterone (MT) in postmenopausal women. Those taking combined therapy reported improved sexual desire, satisfaction and coital frequency. No such improvement was noted in the estrogen alone group (Sarrel *et al.*, 1998). Subcutaneous testosterone implants improve sexual activity, satisfaction, pleasure and orgasm, above that of estrogen therapy alone (Studd *et al.*, 1977b; Burger *et al.*, 1984; Davis *et al.*, 1995). A testosterone patch releasing 300 μg daily of testosterone in conjunction with conjugated equine estrogen (CEE) versus placebo plus CEE resulted in improved sexual function as determined by increased sexual fantasies, masturbation and coital frequency (Shifren *et al.*, 2000). The use of testosterone therapy in these studies was not associated with adverse effects on lipids nor serious virilization effects (Davis *et al.*, 2000).

In a single blind controlled study of 20 postmenopausal women complaining of loss of libido, unresponsive to adequate estrogen replacement, the use of a 50 mg testosterone implant in combination with an estradiol implant was compared to estradiol alone (Burger *et al.*, 1987). Total testosterone concentrations were just above the upper limit of normal (3.5–3.7 nmol/l). Those receiving combined implants showed a marked improvement in the various sexual measures recorded. The dose of testosterone used in this study could be considered to be in the high physiological or low pharmacological range. No significant changes were seen in total serum cholesterol, triglyceride or cholesterol subfractions. Davis *et al.* (1995) conducted a single blind randomized trial of 34 postmenopausal women identified as *not* having sexual dysfunction over a 2 year period. Women received either estradiol implants 50 mg alone or estradiol 50 mg with testosterone 50 mg. The combined treatment increased serum testosterone concentrations to high in the normal range and improved all parameters of sexual function measured using the Sabbatsberg Sexual Self-rating Scale as compared with estradiol alone (Davis *et al.*, 1995).

On the other hand, Myers *et al.* (1980) conducted a 10 week double blind study of 40 naturally menopausal women randomized to one of four treatment groups: conjugated equine estrogen (CEE) 0.625 mg, CEE 0.625 mg plus medroxyprogesterone acetate (MPA) 5 mg, CEE 0.625 mg plus methyltestosterone (MT) 5 mg or placebo. The CEE plus MT group reported increased pleasure from masturbation versus the other three groups, but there were no differences in mood ratings, sexual behavior or sexual arousal (Myers *et al.*, 1980). This outcome may reflect inadequate treatment time, too small study numbers, inability of MT to be aromatized, the effects of CEE on raising SHBG levels with a subsequent decline in free T levels or no true difference of therapy.

Shifren *et al.* (2000) conducted a 12 week study in estrogen-replaced, surgically menopausal women with decreased libido that demonstrated significant improvements in sexual function with physiological testosterone therapy. These women were receiving estrogen therapy and were treated with transdermal testosterone matrix patches (150 or 300 μg/day) or placebo, each for 12 weeks, in an RCT cross-over trial. Although there was a considerable placebo response, women receiving the higher testosterone dose experienced significant increases in the frequency of sexual activity and pleasure-orgasm (Shifren *et al.*, 2000). At the higher dose the percentages of women who had sexual fantasies, masturbated or engaged in sexual intercourse at least once a week increased 2- to 3-fold over baseline. A post-hoc analysis showed that in contrast to the younger subjects in the study (under the median age of 48 years), the 'older half' of the population had a much smaller placebo response and experienced significant improvements in sexual function parameters at both doses of testosterone (150 or 300 μg/day). Positive well being and depressed mood (measured by the Psychological

General Well Being Index) also improved at the higher testosterone dose.

In another RPCT with a cross-over design of 45 premenopausal women presenting with low libido, treatment with transdermal testosterone significantly improved sexual motivation, fantasy, frequency of sexual activity, pleasure, orgasm and satisfaction (Goldstat *et al.*, 2003). The women receiving testosterone also showed a significant improvement in the total score and all subscale scores of well being as measured by the Personal General Wellbeing Index (Goldstat *et al.*, 2003). The mean free androgen index was just above the proposed upper limit for young women, although no true range has been formally established for this estimate of free testosterone.

No clinical human studies to date have differentiated whether any of these effects are AR or ER mediated, or both. The effect of aromatase inhibition on sexual function in women treated with transdermal testosterone is currently under investigation and this may help resolve this issue.

Other beneficial systemic effects of androgen therapy in women

Vasomotor symptoms

Another important role for androgens may be in the control of vasomotor symptoms. Burger *et al.* (1984) reported that in postmenopausal women with poorly controlled hot flushes despite standard estrogen therapy, the addition of a testosterone implant improved the control of the hot flushes.

Vaginal blood flow

Androgens also play a role in the arousal phase of the female sexual response by inducing vaginal vaso-congestion during sexual arousal (Tuiten *et al.*, 1996; Sarrel *et al.*, 1998; Tuiten *et al.*, 2000). Testosterone appears to be important for its vasomotor effects (Worboys *et al.*, 2001), enhancing vaginal blood flow and lubrication (Leiblum *et al.*, 1983; Tuiten *et al.*, 2000). These effects may be due to direct androgen actions or be due in part to estradiol biosynthesis from testosterone in the vascular bed (Harada *et al.*, 1999).

Bone health

Androgens also play an important role in bone physiology. Androgen receptors are found within all three types of bone cells, osteoblasts, osteoclasts and osteocytes. They are most abundant within the osteoblast (Abu *et al.*, 1997). The aromatization of androgens within bone to estrogen is also important for maintenance of bone mineralization (Morishima *et al.*, 1995). In premenopausal women, free testosterone has been found to correlate with the bone mineral density (BMD) in the lumbar spine, hip and distal radius (Buchanan *et al.*, 1988). In postmenopausal women, androgen levels are positively correlated with BMD (Davidson *et al.*, 1982) and lower bioavailable testosterone is associated with loss of vertebral height, which is a known surrogate marker for vertebral osteoporosis (Jassal *et al.*, 1995). In a study of older postmenopausal women by Cummings *et al.* (1998) the relative risk of hip fracture increased with increasing SHBG and decreasing free testosterone.

Interventional studies support the beneficial effects of the addition of androgens to hormone therapy for the management of postmenopausal bone loss. Surgically menopausal women treated with either oral esterified estrogens (EE) daily or EE plus methyltestosterone for 2 years had a greater increase in vertebral BMD with the addition of testosterone (Watts *et al.*, 1995). Barrett-Connor *et al.* (1999) in a similar study of 2 years' duration in surgically menopausal women compared four regimes; CEE (0.625 mg), CEE (1.25 mg), CEE (0.625 mg) plus methyltestosterone (1.25 mg) and CEE (1.25 mg) plus methyltestosterone (2.5 mg). All treatment groups had an increase in BMD at the hip and spine, but with the higher dose combination of estrogen and testosterone having the greatest effect (Barrett-Connor *et al.*, 1999). In a small study of 20 postmenopausal women on long-term estrogen therapy, treatment with 50 mg testosterone implants plus 50 mg estrogen implants resulted in a 5.7% increase in BMD at the spine and 5.2% increase at the hip versus no change in BMD from baseline for the women who remained on oral estrogen alone (Savvas *et al.*, 1992). In a single blinded 2 year study Davis *et al.* (1995) randomized postmenopausal women to receive either 50 mg estradiol implants or 50 mg estradiol implants plus 50 mg testosterone implants. There were increases in BMD for both treatment groups at the hip, lumbar spine and total body, with an earlier and more significant increase in BMD in the group treated with combined androgen and estrogen therapy (Davis *et al.*, 1995).

Cardiovascular effects

Oral androgen therapy has been associated with an adverse lipid profile, with a reduction in high density lipid (HDL) levels, and is not an ideal form of androgen replacement. This effect on lipid profile does not appear to occur with parenteral T therapy. Following treatment with combined subcutaneous implants with estradiol 40 mg and T 100 mg, no significant changes were seen in total serum cholesterol, triglyceride or cholesterol subfractions (Burger *et al.*, 1984). There is no evidence that parenteral testosterone therapy has adverse cardiovascular effects (Goodman-Gruen and Barrett-Connor, 1995; Bernini *et al.*, 1999; Davis *et al.*, 2000; Worboys *et al.*, 2001). Conversely, it has been suggested that parenteral T therapy may be associated with improved cardiovascular risk (Yue *et al.*, 1995; Worboys *et al.*, 2001). There is evidence also that endogenous androgen levels are inversely related to carotid artery wall thickness, a marker of atherosclerosis (Bernini *et al.*, 1999). Exogenous parenteral testosterone does not adversely effect body composition and may increase lean mass (Davis *et al.*, 2000).

Contraindications to androgen replacement

Contraindications to testosterone therapy include pregnancy and lactation, androgen-dependent neoplasia, severe acne and/or hirsutism, androgenic alopecia, a history of polycystic ovary syndrome and situations where increased libido is an undesirable consequence.

Potential risks of testosterone replacement in women

The safety issues associated with testosterone treatment depend on the dose and hormone levels attained, the duration of treatment, the type of androgenic agent and its route of administration.

Androgenization

It is essential that women be well-estrogenized prior to androgen replacement in order to avoid the unwanted side effects of

unopposed androgens. Therefore, a woman must be premenopausal and cycling regularly or receiving estrogen replacement if postmenopausal prior to commencing T therapy. Providing that circulating levels of free T are kept within, or close to, the upper limit of the normal physiological range, masculinizing effects are extremely unlikely (Slayden, 1998). Maintenance of supraphysiological levels, in contrast, will lead to clitoral hypertrophy. Long-term use of injectable T-esters (in combination with injectable estrogens) at doses of 150 mg every 2–4 weeks has been shown to produce virilization in women (including temporal balding, voice deepening and clitoral enlargement) (Urman *et al.*, 1991). In contrast, transdermal administration of testosterone at doses comparable to premenopausal hormone production has not been associated with virilization (Shifren *et al.*, 2000; Goldstat *et al.*, 2003). More careful monitoring of free T levels should be undertaken in women with increasing hirsutism, acne, temporal balding and/or deepening of the voice.

Hepatic dysfunction

Hepatic side effects of androgen therapy in women are predominantly associated with the use of oral methyltestosterone (MT) and other 17-alkylated derivatives in supraphysiological doses. High doses of orally administered androgens such as MT may be associated with hepatoxicity (peliosis hepatis, hepatic neoplasms and cholestatic jaundice) but this has not been a problem for lower dose therapy (Barrett-Connor *et al.*, 1999). The use of low dose testosterone replacement as recommended in women has not been associated with any adverse hepatic events. In addition, subcutaneous and intramuscular modes of testosterone replacement have not been associated with serious hepatic side effects (Slayden, 1998).

Breast cancer

The relationship of testosterone administration and breast cancer is not established, however, preliminary research indicates testosterone may negate the unfavourable effects of estrogen on the postmenopausal breast (Zhou *et al.*, 2000; Dimitrakakis *et al.*, 2002). In a recent literature review (Somboonporn and Davis, 2004a) it was concluded that experimental studies in cell lines exposed to androgens and various animal studies, involving rats, mice and rhesus monkeys exposed to combined estrogen and androgens showed an overall protective effect. In addition, the risk of breast cancer is not increased in women with PCOS defined by a hyperandrogenic state. On the basis of various epidemiological studies, the review also concluded that the risk of breast cancer was not associated with endogenous total T levels in premenopausal women. The risk of breast cancer in premenopausal women in relation to endogenous free T levels and exposure to exogenous testosterone has not been assessed to date. A positive association was noted between breast cancer risk and endogenous total T as well as free T levels in postmenopausal women, however, this was no longer significant after adjusting for estradiol levels. This observation may be explained by increased aromatase activity in the setting of estrogen depletion after menopause, and thus increased capacity to convert testosterone to estradiol in adipose tissue with age, suggesting that women be well estrogenized prior to the administration of testosterone to minimize the risk. However, the risk associated with exposure to exogenous testosterone in postmenopausal women is unclear, as these studies had various methodological limitations. In summary, the effects of androgens on the breast are unclear and require further study.

Venous-thromboembolic (VTE) disease

The risks of testosterone therapy in those with a history of VTE are currently unclear and testosterone therapy is best avoided in this group of women.

Pregnancy

In treating premenopausal women the risk of exogenous androgen to a fetus is a separate and genuine concern. However, virilization of a female fetus does not appear to be tightly correlated with maternal testosterone levels, with the occurrence limited to women exhibiting masculinization (McClamrock and Adashi, 1992; Sarlis *et al.*, 1999). Nonetheless, treatment of any women who have the potential to become pregnant should involve reliable contraception and extremely cautious monitoring.

In summary, androgen therapy is relatively contraindicated in women with hyperlipidemia or liver dysfunction. The safety of androgen therapy in women with, or at high risk for, cardiovascular disease (CVD), VTE or breast cancer is uncertain.

Options for androgen replacement

There are currently no approved androgen therapies available for treatment of sexual dysfunction in women. Although there are several androgen therapies being designed for use in women, these remain at an investigational level. Currently available androgens are only approved for use in men.

Testosterone

In postmenopausal women, these preparations are typically given concomitantly with an estrogen and progestin or estrogen only in hysterectomized women.

The currently available options of T therapy intended for specific use in women are very limited. In the USA the only available therapy is an oral preparation of a small dose of methyltestosterone. Oral androgen therapy is associated with suppressed HDL levels and is not ideal for long-term administration.

In Australia, T therapy is commonly administered with a 50 mg T implant inserted subcutaneously under local anaesthetic. This is achieved by halving a 100 mg implant, which is specifically made for males. These implants remain effective for a period of 4–6 months at which time a low free T or total T with SHBG should be confirmed prior to repeat implantation. If the diagnosis is unclear, a therapeutic trial of one to three T-ester injections may be given at 3–4 weekly intervals. If there is a positive response, long-term therapy with a T implant is warranted. T-ester injections are associated with peak supraphysiological levels and are not suitable for long-term use. Alternatively, a trial of a 1% testosterone cream may be given for a 4–6 week period prior to insertion of a T implant.

Testosterone undecanoate is a 40 mg capsule, which is taken orally and is absorbed via the intestinal lymphatics and thereby avoids a first-pass effect in the liver. This formulation is specifically designed for men and the safety and efficacy in women are unknown. Following administration, serum concentrations are

very variable and short-lived, and its use is not recommended in women (Buckler *et al.*, 1998).

T is also available in the form of troches for buccal administration but there are no published pharmacokinetic, safety or efficacy data available to validate this formulation and its use cannot be advocated.

There are currently various testosterone transdermal patches (Shifren *et al.*, 2000), a 1% testosterone cream (Goldstat *et al.*, 2003), transdermal sprays, patches, creams and gels along with intranasal sprays in development in large-scale clinical trials.

In summary, based on available data no specific testosterone therapy or dose can yet be recommended. However, achieving physiological free testosterone levels by transdermal delivery appears to be the best approach for minimizing the adverse effects of androgens.

Androgen precursors

The evidence for the effectiveness of androgen precursors is inconclusive. In the USA, oral formulations of the androgen precursors DHEA and androstenedione are both available without prescription as 'dietary supplements'. DHEA replacement is specifically indicated in adrenal insufficiency; however, its role in other causes of FAI is still unclear.

There are extensive data on DHEA in studies involving a small number of subjects, which demonstrate an increase in plasma levels of DHEA, DHEAS, androstenedione, T, DHT and following 1 month of oral administration of DHEAS (50 mg per day) in postmenopausal women (Stomati *et al.*, 2000). It is important to note that androgen precursors are also estrogen precursors and thus may raise both testosterone and estradiol/estrone levels. As DHEA is converted to both testosterone and estradiol, any study that demonstrates positive effects of oral DHEA on sexual function cannot distinguish between the role of DHEA alone or as a precursor of testosterone and/or estradiol.

In summary there are no strong data to support beneficial effects of exogenous DHEA on sexual function in health or in adrenal insufficiency. The use of DHEA is further complicated by the lack of well standardized preparations for oral use and the suppression in HDL associated with its use. Safety issues for oral DHEA include acne, hirsutism and a reduction in HDL–cholesterol and other hepatic proteins (including SHBG) (Arlt *et al.*, 1999; Barnhart *et al.*, 1999). Studies are still too small to exclude with certainty other risks such as hepatotoxicity in humans. If treatment with DHEA is considered, DHEAS should be below the normal female range.

Androstenedione appears to be more likely to cause virilizing side effects than DHEA because supraphysiological testosterone levels are attained with the administration of androstenedione (Kicman *et al.*, 2003). To date, there have been no RCTs evaluating sexual function with androstenedione in women.

There are currently insufficient data to support the use of androgen precursors for the purpose of managing female sexual dysfunction.

Future options

New approaches to androgen therapy now include the use of tissue-selective sex steroids such as tibolone; and in the future, potentially selective androgen receptor modulators now called SARMs. However, their role in androgen insufficiency has not been specifically studied and the extent to which such therapies should be seen as alternatives to androgen therapy is yet to be established.

Evidence for the effectiveness and safety of all of these preparations in women is limited. It remains to be established whether improvement in libido with short-term therapy will result in a sustained improvement or whether long-term therapy is required. If the latter is the case, long-term safety data are required before long-term therapy can be recommended.

Monitoring androgen therapy

There is no established level of free testosterone below which a woman can be said to be deficient, nor any level to which a woman should be restored that determines that she is replete. Such absolute levels are not likely to be established because of the large inter-individual variability and the intracrinology of androgens in women. Clinical symptoms along with testosterone levels are used to monitor therapy (Table III).

Once the administration of testosterone is commenced, continuation for >6 months should be dependent on a definite improvement in sexual function and satisfaction.

Women treated with hormonal therapy require follow-up and ongoing monitoring with regular breast and pelvic examination, mammography and, in the presence of abnormal bleeding, endometrial biopsy. Physical examination should include assessment of the skin and hair for seborrhea, acne, hirsutism and androgenic alopecia. These virilizing side effects may appear very gradually, even after a year or more of treatment.

Free/bioavailable T levels or total T with SHBG and calculated free T should be monitored regularly with the aim of maintaining these values at least within the normal range for premenopausal women, to reduce the likelihood of side effects. The target levels for T in older women require further clarification.

As SHBG levels may fall somewhat with increased circulating testosterone, baseline SHBG may be a useful predictor of risk of

Table III. Basic biochemical investigations for women presenting with low libido

General	Haemoglobin Iron studies TSH
Premenopausal and amenorrhea	Estradiol + FSH (e.g. hypothalamic amenorrhoea/premature ovarian failure) Prolactin
Androgen profile	SHBG Free testosterone by equilibrium dialysis (gold standard) or; Total testosterone after organic solvent extraction and calculation of free testosterone (laws of mass action equations) or; Total testosterone by RIA (with awareness of limitations) and calculation of free testosterone or; Total testosterone and calculation of free androgen index: total testosterone nM/SHBG nM × 100 (if SHBG in normal range) DHEA-S Early morning cortisol (if adrenal insufficiency is suspected)

excess androgenization with testosterone treatment, and should be measured in all women prior to such therapy.

Although no adverse effects on lipids have been found with short-term parenteral therapies, a lipid profile, and, in the presence of a family history of diabetes or significant obesity, fasting insulin and glucose levels should be considered.

Additional biochemical investigations such as liver function tests should be based on clinical judgement.

Conclusions

The diagnosis of female androgen insufficiency syndrome is made on the basis of a constellation of symptoms, along with a low serum free testosterone after excluding several important medical conditions including depression. The diagnosis is complicated by the lack of readily available and inexpensive yet reliable assays of serum free testosterone. Moreover, there are insufficient data in women to define a free testosterone level below which it equates to symptoms of the FAIS. Given the intracrinology of androgen action, clinical outcome is difficult to predict from blood levels alone, even if superior assays are available. Ultimately, the decision to institute any hormonal therapy must be individualized and the patient adequately informed about risks and benefits.

Recommendations

The characteristics of female androgen insufficiency syndrome require further validation. That is, normal ranges for the various androgens in women by decade and ethnic background and how these levels correlate with symptoms of the FAIS need to be established. This is currently being addressed in a large cross-sectional study being conducted in Australia. This study will assess the relationships between sexual parameters and free and total testosterone and other sex steroids in a randomly recruited sample of women from the community, aged 18 to 75 years (Davison *et al.*, 2004).

Improved assays for total and free testosterone measurement in the female range that are reliable, inexpensive and readily available to clinicians need to be developed.

The efficacy and long-term safety of testosterone therapy in female sexual function requires further validation with the use of RPCTs. There are currently a number of studies being undertaken in this area.

Preparations of testosterone specifically designed for use in women are required.

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