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Review

Androgen deficiency in women; role of accurate testosterone measurements

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ABSTRACT

Androgen deficiency in women has been recognized as a distinct clinical syndrome that affects thousands of women particularly women in the postmenopausal period of their life. This syndrome has been described by several names including female androgen deficiency syndrome as well as hypoactive, sexual desire disorder. A recent large survey concerning sexual problems in women also adds personal distress as a potential contributor to the low sexual desire found in some women with sexual dysfunction. Recognition of an androgen deficiency syndrome however, has been controversial and limited to a clinical diagnosis due to the lack of accurate and sensitive methods for measuring androgens in women. Up until now, available methods for measuring the sex steroids have been dependent on antibody based assays that employ a range of different detection systems including the use of isotopes such as tritium and I-125 or chemical signalling molecules that produce chemiluminescence. These assays have become increasingly more sensitive for the measurement of testosterone but are still incapable of providing the proper low-end sensitivity for analyzing testosterone in female blood specimens. Assays for testosterone performed either manually or with highly automated immunoassay instruments have been used to measure testosterone in women but with varying degrees of success. Existing immunoassay-based methods are quite adequate for measuring testosterone levels in males but lack sufficient sensitivity to accurately and reproducibly measure testosterone in females and pre-pubertal children. Recent advances with the use of ultrasensitive methods such as mass spectrometry coupled to either gas or liquid chromatography have improved the technology for measuring testosterone and other low concentration sex steroids like estradiol to the degree that mass spectrometry based methods are now capable of measuring the testosterone levels found in normal women and in women with extremely low levels of testosterone as observed in a true androgen deficiency disorder. This application of mass spectrometry for measuring testosterone should allow clinicians to better define female androgen deficiency and facilitate further investigation in the diagnosis and optimal management of androgen deficiency in women.

Contents

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1. Introduction

Female androgen insufficiency is a recently described syndrome in women characterized by the presence of reduced androgen levels in the circulation that leads to an impairment in sexual drive, reduced libido, a depressed mood and signs and symptoms of limited androgen exposure such as decreased muscle mass, reduced bone density and a decreased sense of well being. At an international consensus conference on androgen deficiency in women held in 2001, a consensus statement was developed that coined the term "female androgen insufficiency disorder" with specific guidelines established for both the assessment and diagnosis of this disorder [1]. Since that time other reports on female sexual dysfunction have appeared that incorporate personal distress as a major contributing factor to diminished female sexual function with or without androgen deficiency [2]. Overt signs and symptoms of diminished androgen secretion in women are quite often difficult to ascertain clinically and symptoms can be slow and subtle in presentation and yet involve complex mechanisms and expression that develop over time. The expression of androgen deficiency can range from mild degrees of hypoandrogenism to a significant and marked deficiency in androgens that can trigger abnormal physiologic and reproductive events that are either stopped or reversed with androgen supplementation [3].

Androgen deficiency can occur in women at any age, either during their reproductive period of life or following menopause although it is in the postmenopausal period when clinical signs and symptoms of low androgen exposure seem to be the most pronounced. Because of the dominating effects of estrogen in women, particularly their effects on mood and sense of well being that can mask the impact of decreased androgen exposure, the diagnosis of androgen insufficiency can only be made in women who have had relatively normal exposure to estrogen over time either through the occurrence of regular menstrual cycles indicative of normal endogenous production or as a result of estrogen replacement therapy such as the case in many postmenopausal women and others with hypogonadism [4]. The clinical manifestations of chronic and reduced androgen exposure can be prolonged in onset over several years and thus can easily be confused with general signs and symptoms of normal aging or from other metabolic events known to reduce androgen production. This includes women with hypothalamic–pituitary disease, ovarian disorders, adrenal disease, a medication history known to affect steroid biosynthesis and/or metabolism or from idiopathic causes [3,5]. Once true hypoandrogenism is considered, the diagnosis can only be made by the appropriate and accurate measurement of low but detectable circulating levels of testosterone using ultrasensitive methods. Androgen replacement therapy appears to be the cornerstone of the therapeutic interventions used to resolve or minimize the symptoms that result from androgen deprivation although controversy still exists regarding the therapeutic use of androgens in women [6]. A guideline for androgen therapy in women was published in 2006 by The Endocrine Society [7]. The Endocrine Society at the time recommended against making a diagnosis of androgen deficiency in women due to a lack of accurate testosterone data from women and because an androgen deficiency syndrome in women is still not well defined.

2. Androgens in women

Androgens have long been recognized as important to the normal physiology of women and do play a key role in the physical, sexual and emotional well being of females [8]. The biological requirement for androgens in women is supported by considerable clinical evidence emanating from a number of successful androgen replacement therapy trials that document their short-term benefits [9]. The normal circulating level of testosterone in both reproductive age women and the postmenopausal female however still needs proper validation since most methods for measuring testosterone have until now been inadequate for determining the true normal reference interval in women. With that said, the only way to establish the presence of true androgen deficiency in women is by the accurate measurement of total and/or free testosterone using assay methods capable of detecting the low levels that exist with this disorder in order to properly treat this condition and not expose normal women to inappropriate amounts of exogenous androgens. Androgen therapy by itself can have untoward negative side effects in some women so it is important to ascertain with certainty that true androgen deficiency exists through the measurement of circulating testosterone levels with accurate and sensitive methods [10].

Table 1
Causes for low androgen production in women [3].

- Dysfunction of hypothalamic–pituitary axis
  - Turners syndrome, Kallman's syndrome, prolactinoma
- Surgical or medical oophorectomy
- Surgical or medical adrenalectomy
- Premature ovarian failure
- Chronic use of birth control medications
- Old age
- Cushings syndrome
- Estrogen administration
- Radiation and/or chemotherapy
- Thyroid disease
- Select medications (anti-hypertensives, opiates, dopamine antagonists)

Understanding the biosynthesis and metabolism of androgens is important to appreciate the underlying physiologic and metabolic events that can and do occur with an androgen deficiency state [8]. In reproductive age women, the daily production of testosterone is shared equally between the ovaries and the adrenal and accounts for approximately one-third of the testosterone found in the circulation (Fig. 1). Peripheral conversion of androgen precursor steroids to testosterone in non-steroid producing tissues accounts for the remaining two-thirds of the testosterone found in the circulation of women. These ratios change after menopause when the ovaries are in senescence and thus contribute much less to the androgen pool. The peripheral conversion of adrenal precursor steroids like androstenedione and dehydroepiandrosterone takes on a larger role in the postmenopausal women.

Although excess androgen production disorders are by far the most common abnormalities found with androgen metabolism in women, androgen deficiency as stated earlier has been recognized as a multi-factorial clinical disorder that can produce a variety of symptoms that can be reversed in some women with proper diagnosis and treatment. The most common clinical symptoms reported that result from an androgen deficiency state is oriented around a pronounced reduction in a women's libido [6]. Symptoms' include reduced sexual motivation and fantasizing about sex, reduced sexual arousal and a general lack of enjoyment in sex. Physical and psychological evidence of a chronic androgen deprivation state includes a significant reduction in both muscle mass and bone mineral density, a perceived overall negative attitude towards life including alterations in mood, affect and energy level, and an increase in vasomotor symptoms. Depression, insomnia, and headache are other symptoms that have been associated with the female androgen deficiency syndrome. The organic causes for a low testosterone level in women as stated can be numerous and varied (Table 1) and thus looking for a root cause for low androgen production in women is important to investigate because proper treatment can improve the quality of life in many women. Other sources for an abnormality in testosterone production can occur

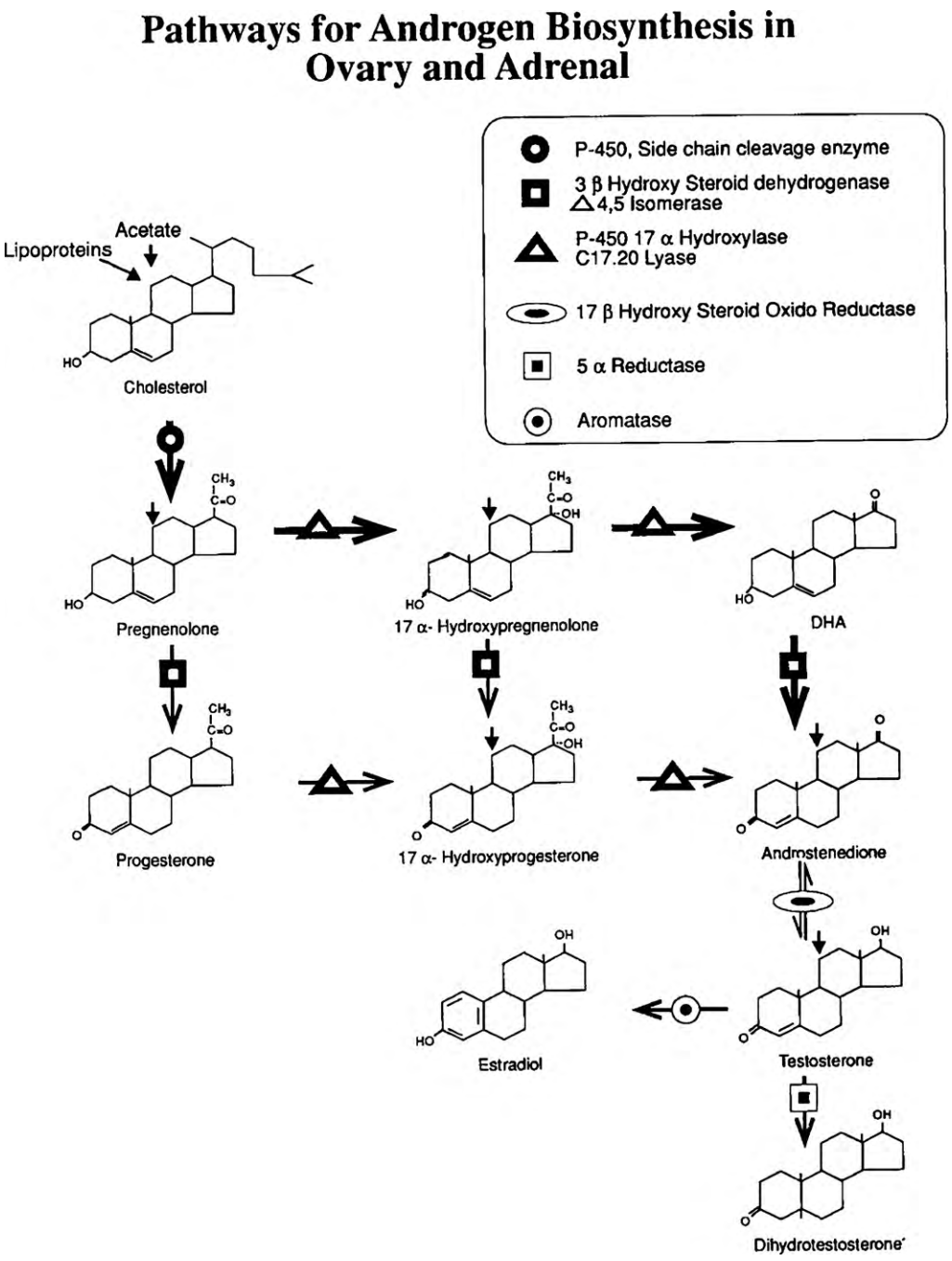

Fig. 1. Pathways for androgen biosynthesis in ovary and adrenal.

with diseases and/or dysfunction that affect steroid biosynthesis and availability including disorders of the hypothalamic–pituitary axis, ovarian disease conditions, adrenal insufficiency, the use of chronic glucocorticoid therapy, and the use of exogenous estrogen administration.

3. Androgen metabolism in women

To appreciate the availability and action of androgens in women, it is important to understand and delineate the many different steroid forms found in the circulation that possess androgenic properties [11]. Some steroids like androstenedione and dehydroepiandrosterone (DHA) function as circulating androgen precursor substrates and are readily converted to more potent androgen products in peripheral, non-steroid producing tissues [11]. Both androstenedione and dehydroepiandrosterone are synthesized and released in significant quantities from the ovary and adrenal respectively, as part of normal steroid physiology and metabolism in these organs (Fig. 1). The primary function of these precursor steroids as we currently understand their biological role, is to serve as a circulating reservoir of steroid substrate that can easily be converted to the sex steroids by steroid metabolizing tissues on an as needed basis. Both of these steroids by themselves have only mild to weak androgenic properties but are readily metabolized to the more potent androgen, testosterone as well as a number of other steroid metabolites in steroid metabolizing tissues like the liver and adipose tissue (Fig. 2). We still do not have an appreciation of whether any or all of the steroid metabolites stemming

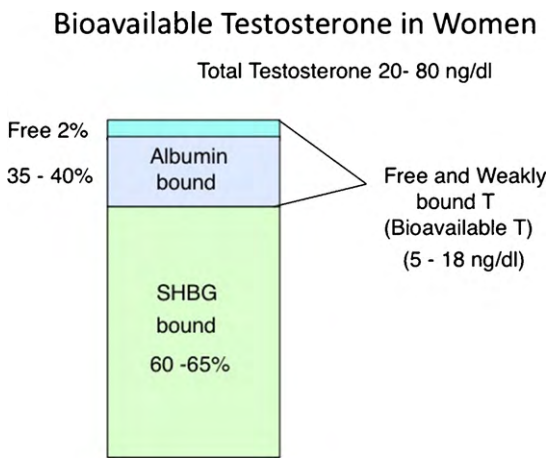

Fig. 2. Metabolism of androgenic steroids.

from dehydroepiandrosterone or androstenedione metabolism are biologically active and/or possess androgenic properties but suffice it to say testosterone itself is the principal androgen moiety that carries out the major effects of androgens in target tissues. Testosterone interacts with and binds to high affinity cytoplasmic testosterone receptors in androgen sensitive tissues to elicit its androgen effect at the genome level either directly or through the intracellular conversion of testosterone to dihydro-testosterone (DHT) involving an alpha reductase enzyme system. Approximately 6% of testosterone is converted to DHT in the cell although we still do not fully understand why this step exists. DHT can elicit the same androgen message at the nuclear level at the same regulatory region as testosterone itself. DHT is also measurable in the circulation in appreciable concentrations but the levels do not accurately reflect the androgenic state of the individual. DHT in the circulation appears to be simply a by-product of testosterone metabolism in tissues. To have a biological effect in androgen sensitive tissues, DHT must be produced intracellularly from testosterone. In some tissues like the skin, DHT is further metabolized to a terminal metabolic product named 3α-androstanediol-glucuronide. The biological reason behind this unique metabolism in the pilosebaceous unit of the skin is not fully appreciated however increased metabolism of DHT to 3α-androstanediol-glucuronide is associated with the increased presence of acne and is sometimes used as a biomarker for androgen mediated acne vulgaris. In females, an important metabolic step for androgenic steroids is their aromatization to estradiol in both sex steroid sensitive tissues and non-sex steroid organs and tissues like the liver and adipose tissue. Approximately 50% of the testosterone-produced on a daily basis is metabolized and conjugated to glucuronide in the liver via specific hydroxylase and glucuronide enzymes and destined for metabolic disposal.

4. Assessing free testosterone in women

To properly assess normal androgen production in women requires the ability to accurately measure testosterone itself either as total testosterone or as free testosterone. Free testosterone is measured to compensate for the effects of the binding proteins that carry testosterone in the circulation. Most of the testosterone that circulates in the blood is bound in one way or another to carrier proteins that have different degrees of affinity for testosterone. From 60% to 65% of testosterone in women circulates bound to a high affinity Sex Hormone Binding Globulin (SHBG). SHBG bound testosterone essentially functions as a circulating reservoir of this potent androgen (Fig. 3). Testosterone bound to SHBG is generally not available for tissue receptor uptake by sex steroid sensitive tissues or for interaction with steroid metabolizing tissues like the liver due to its high affinity binding to SHBG that exceeds the binding affinity for cellular testosterone receptors. Testosterone also circulates in appreciable amounts bound to albumin (35–40%) and also minimally to corticosteroid binding globulin (CBG) (<5%). The binding to these plasma proteins is relatively weak however and thus testosterone can easily disassociate from these proteins to interact with the testosterone receptor in sex steroid responsive tissues. The term bioavailable testosterone has been used to describe the 35–40% of testosterone that is loosely bound to the carrier proteins albumin and CBG. Less than 2% of testosterone circulates in an absolute free state in the blood at any one time and this percentage appears to change very little on a day to day basis even in states of low to moderate hyper or hypoandrogenism. To appreciate the presence and impact of free testosterone in assessing the availability of testosterone in the circulation, investigators over the years have turned to a variety of analytical methods to estimate the amount of testosterone that is present in a free state. Except for the measurement of free T by equilibrium dialysis, most of the in vitro methods developed that purport to assess free T only provide an estimate of the circulating free T concentration [12].

One approach that has been used is to accurately measure both total T and SHBG independently and then calculate a free T index from the results. This approach is fast and simple but is dependent on the accuracy of the total testosterone method and on the specific measurement of the SHBG level itself that is prone to fluctuate depending on a women's overall hormonal and nutrient status. A number of factors can cause significant fluctuations in circulating SHBG levels including estrogen itself, estrogen containing oral contraceptives, adrenal gland hormones (cortisol), dietary habits and certain disease states such as obesity, thyroid disease, diabetes, liver disease, renal disease and androgen excess states. Women on other medications that affect the liver can also demonstrate significant alterations in the production rate of SHBG. Thus it is easy to expect that extreme levels of either a suppressed or elevated SHBG can compromise the accuracy and predictive value of the calculated free T index.

Another more popular approach used by a number of laboratories including our own is to determine the free and weakly bound or bioavailable testosterone level using an isotope equilibrium technique that measures the testosterone that is weakly bound to albumin and CBG as well as free T itself [13]. This method is based on an ammonium sulfate precipitation technique that follows the incubation of serum with tritium labelled T. The tritium labelled T binds to all available protein moieties and can be traced to reflect the partitioning of T between the different binding components in the blood. Ammonium sulfate at a specific concentration selectively precipitates only the SHBG bound T fraction allowing for calculation of the percent free T that includes not only free T but T bound to albumin and CBG as well. The percentage free T is then applied to the total T measurement to obtain the mass free T concentration. This method, is called the free and bioavailable T method and correlates well clinically with results from the equilibrium dialysis method and appears to reflect clinically the total bioavailable T pool and corresponding T binding protein effects better than the measurement of only free testosterone by itself. We have successfully used the bioavailable T assay for a number of clinical studies of androgen excess and showed excellent correlation to clinical findings in women in various stages of hyperandrogenism [14]. Rigorous analysis of the bioavailable T method on patients who have very low to deficient levels of free testosterone is however quite limited and needs to be validated further to assess the ability of this method to accurately reflect the low free T levels found in women with true androgen deficiency.

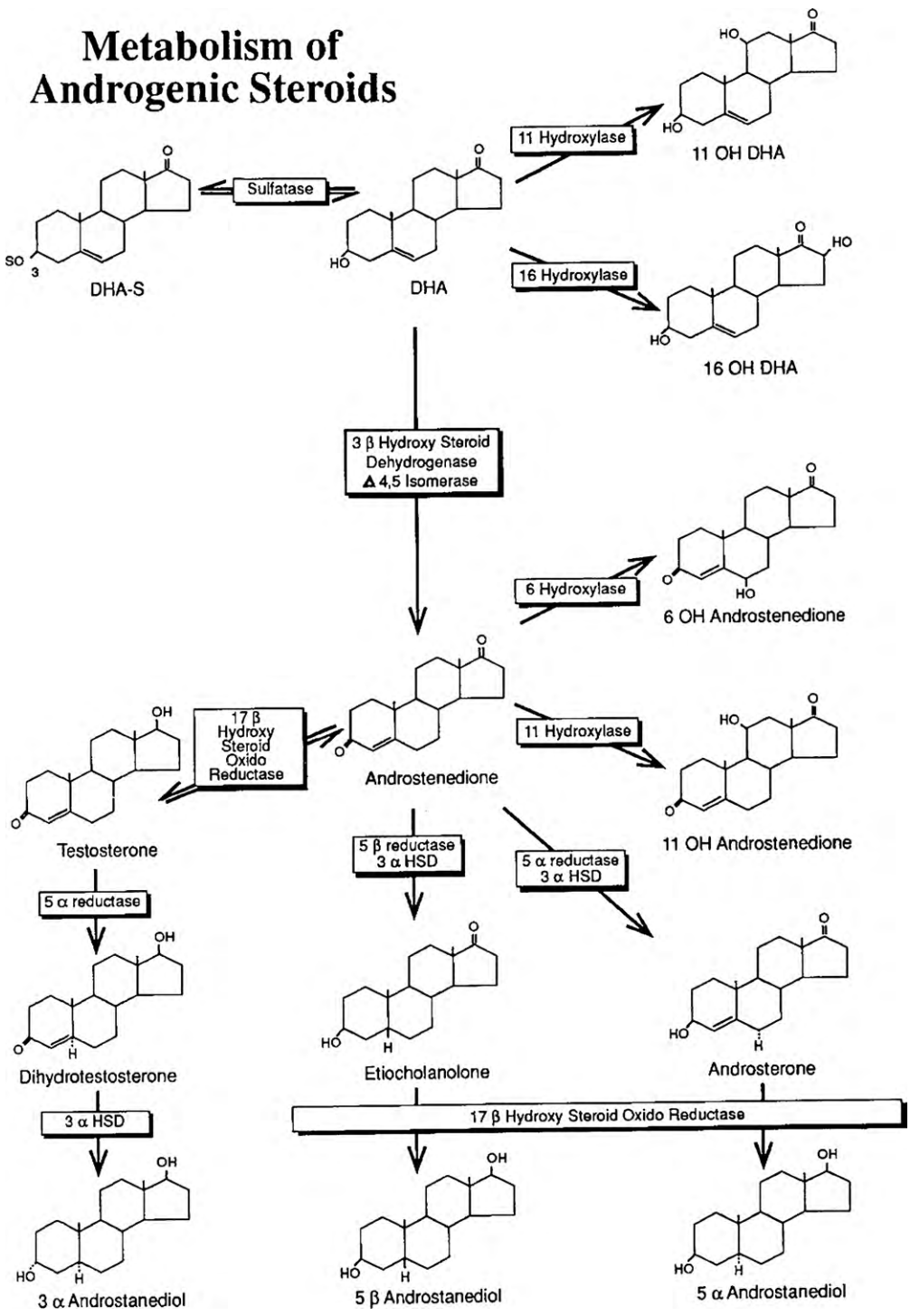

Fig. 3. Bioavailable testosterone in women.

5. Measuring total testosterone in women

As mentioned previously, the ability to measure either total or free serum testosterone levels accurately and precisely is essential to establishing the diagnosis of true androgen deficiency. Unfortunately most of the methods for measuring total testosterone that utilize immunoassay principles have been optimized for male testosterone measurements where the concentrations of T are usually at the high end of the spectrum. Current immunoassay methods for T available in both hospital laboratories and reference laboratories appear to be adequate for determining androgen deficiency in males however as shown in a number of published clinical studies, most if not all immunoassay methods for total testosterone when applied to females are marginal at best even when used to establish the diagnosis of an androgen excess disorder [15]. The use for example of a cutoff T level of 60 ng/dl to make the diagnosis of polycystic ovarian syndrome is right at the cusp of method accuracy and precision when employing immunoassay methods for testosterone [14]. From studies in the literature, there is no question that most current immunoassay methods for T when used for women are relatively inaccurate and do not have the low-end sensitivity requirements for measuring the low levels of testos-

terone usually found in the circulation of normal women and are certainly of little to no practical value when used to test women for androgen deficiency. This dilemma has come to light at several recent consensus conferences that have focused on the overall inaccuracy of most current methods used for measuring sex steroid hormones in women whether performed manually or on an automated immunoassay instrument [16]. To advance the technology needed to measure both testosterone and estradiol in women with acceptable accuracy and precision at all the concentrations encountered, investigators have recently turned to ultrasensitive methods that employ mass spectrometry coupled to either gas chromatography or high performance liquid chromatography. This approach appears to resolve the sensitivity and accuracy needs for detecting the low levels of sex steroids found in females as well as prepubertal children [17].

A few seminal studies exposed the need for improved ultrasensitive methods for measuring the sex steroids. In a report that evaluated several different immunoassay methods for testosterone comparing results to those obtained with a mass spectrometry based method, it was noted that all of the testosterone methods performed either manually or on the type of automated instruments typically found in most routine hospital and reference laboratories lacked both accuracy and acceptable sensitivity for the measurement of testosterone in women [18]. From this study it was concluded that most immunoassay-based methods for testosterone at their lowest limit of detection were unable to reproducibly and accurately measure testosterone in normal women or children. It was also quite apparent from these studies that none of the immunoassay-based methods would be capable of measuring the extremely low levels of T observed in women with the androgen deficiency syndrome.

One particular study by Wang et al. [15]. compared total testosterone measurements in males on four commonly used automated immunoassay analyzers along with two well established radioimmunoassay methods with results compared to a liquid chromatography–tandem mass spectrometry method (LC/MSMS) on specimens submitted to several different laboratories in a blind sample fashion. The mass spectrometry testing carried out on these samples was performed in a highly reputable laboratory used to screen Olympic athletes for exogenous testosterone use [19]. The results of this tightly controlled comparison study clearly showed that although there was acceptable inter-method correlation between the different automated methods and the two RIA methods with LC/MSMS, only the results obtained for adult males showed acceptable accuracy and precision. In addition it was shown through bias plots that the automated platform methods were unable to accurately and reproducibly detect levels of testosterone below 100 ng/dl (3.47 nM). With the upper limit of the normal reference range for testosterone in women well below this threshold, the results clearly suggested that most methods whether automated or manual if based on immunoassay principles irrespective of the strength of the detection signal, were not capable of measuring testosterone accurately in blood from women or children. Thus the only sure way that testosterone can be accurately measured in women with androgen deficiency is with a mass spectrometry based method.

Other studies have appeared since Wang et al. [15] report that further document the relative inaccuracy of immunoassay-based methods for testosterone and confirm the enhanced accuracy and precision of mass spectrometry based methods for the measurement of testosterone in women. Several investigators have demonstrated that the sensitivity of mass spectrometry based methods for testosterone can easily reach down to the low levels of testosterone encountered in normal women and are indeed quite capable of detecting the extremely low levels observed in women with an androgen insufficiency disorder [20–23]. Advances in mass spectrometry instruments and methods for measuring steroids continue to be made that further improve upon the desired specificity and sensitivity with this methodology [24–29]. The use of isotope dilution techniques and the derivatization of specimens with dansyl chloride along with the use of multiple mass transitions have improved and further enhanced the low-end sensitivity of T assays performed by LC/MSMS. These modifications have also improved the periodic but annoying effects of interfering substances that have been a long time nemesis of mass spectrometry methods that can compromise the true low-end sensitivity of these methods. The next step in moving LC/MSMS along as an accepted routine method in both hospital clinical laboratories and reference laboratories is the development of true inter-method standardization in the different mass spectrometry laboratories. The availability of purified standards from organizations like the National Institute of Standards (NIST) to allow for inter-laboratory quality assurance practices needs to be empowered and made widely available to bring all laboratories to the same point with their technology. There is work along these lines taking place both in the United States and in Europe that should allow for worldwide uniformity of testosterone measurements and traceability of methods to the International System of Units (SI). Unfortunately, the United States is still heavily entrenched in using mass units (ng/dl) to report testosterone levels while most of the rest of the world uses SI units (nM) to report results for testosterone. The target globally is to have everyone embrace SI units. This will improve the information exchange for test results from study to study, irrespective of the country of origin.

Using mass spectrometry coupled to liquid chromatography, representative normative values for females have been reported as listed in Tables 2 and 3 [30]. Achieving routine measurements of steroids using mass spectrometry based methods to include both estradiol and testosterone will only expand the utility of this analytical approach for determining disorders of both androgens and estrogens in males and females.

Table 2
Reference ranges for total testosterone by LC/MSMS in females [30].

Age (years)	ng/dl	nM (SI units)
<8	<8	<0.28
Tanner stage 1	<8	<0.28
Tanner stage 2	<24	<0.83
Tanner stage 3	<28	<0.97
Tanner stage 4	<31	<1.1
Tanner stage 5	<33	<1.4
18–49	4–44	0.13–1.5
50–60	2–48	0.07–1.7
60–80	2–42	0.07–1.5

6. Treating women for the female androgen deficiency disorder

Treatment strategies – once the diagnosis of female androgen deficiency is made, for many women the option of exogenous testosterone therapy is available [9,10]. From an androgen treatment perspective, there has been considerable progress in recent years regarding the development of different modalities of testos-

Table 3
Reference ranges for free testosterone by LC/MSMS in females [30].

Age (years)	pg/ml	pM (SI units)
<8	0.2–5.0	0.7–17.4
10–17	0.2–6.3	0.7–21.9
18–50	0.2–6.3	0.7–21.9
50–70	0.2–6.6	0.7–22.9
>70	0.1–3.7	0.3–12.8

terone therapy available to women. With the development and use of testosterone containing transdermal patches as well as testosterone creams and gels that are easily applied to the skin, many of the barriers associated with the periodic administration of testosterone by injection in women have been overcome. However because of the lack of long-term safety studies, the FDA in the United States several years ago voted against the use of the testosterone patch for women. Considerable controversy still remains regarding the use of testosterone therapy in women including the use of transdermal androgen therapy. When an endocrinologists does consider testosterone as a viable treatment option in select women, the current recommendation for androgen treatment is that therapy should be restricted to the short-term until long-term safety issues have been resolved. Reported risks and side effects from androgen therapy include the development of hirsutism, acne, alopecia, liver dysfunction, deepening of the voice, abnormal lipid changes, and virilization of a female fetus if pregnant. Since androgens can be aromatized to estrogens, risks associated with estrogen therapy need also be considered. In addition, testosterone therapy should not be considered in women of reproductive potential who are not on adequate contraception. Further, pregnant or nursing women or other types of patients not yet included in androgen therapy clinical trials should be avoided. The number of reported adverse events associated with testosterone replacement treatment in women has been quite limited when replacement protocols are optimized to achieve physiologic levels of circulating testosterone [9]. There are of course other reasons for considering androgen replacement therapy in women besides those with true androgen deficiency syndrome. Androgen therapy is a consideration in women who have a known physiologic or endocrine cause for reduced androgen production, including patients with hypopituitarism, oophorectomy, or adrenal insufficiency.

In conclusion, the syndrome of female androgen deficiency in women appears to be a legitimate disorder that is coming to light as advances in the sensitive and accurate measurement of testosterone are made. Although treatment strategies to cope with this disorder are still a matter of controversy, when diagnosed properly using accurate and ultrasensitive methods for either total or free testosterone, there are indeed off-label treatment options that can be considered to correct the deficiency and improve the quality of life in some women. One must be mindful however about the lack of and need for long-term safety data from clinical trials testing prolonged androgen use in women. Advances in technology however with ultrasensitive methods like mass spectrometry for measuring both total and free testosterone appear to be maturing along with improved and easier treatment options for testosterone replacement therapy.

Contributors

Written solely by author.

Competing interest

None declared.

Provenance and peer review

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