FERTILITY AND STERILITY®
VOL. 77, NO. 4, SUPPL 4, APRIL 2002
Copyright ©2002 American Society for Reproductive Medicine
Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Screening for androgen deficiency in women: methodological and interpretive issues

André T. Guay, M.D.

Center For Sexual Function, Lahey Clinic Northshore, Peabody, Massachusetts

Objective: To appreciate the problems in obtaining and interpreting androgen levels in women.

Design: Review of the literature to compare various laboratories and methods of analysis of serum androgens.

Patient(s): Normal control populations culled from the literature to compare with patients previously reported on by us; data from our laboratory quality control compared with data from the literature.

Result(s): Investigators and laboratories disagree as to the best methods of measuring testosterone (T) levels in women. Measuring DHEAS levels is much less controversial.

Conclusion(s): Serum T levels should be measured in the morning hours and during the middle third of the menstrual cycle in premenopausal women. DHEAS levels may be measured at any time. Androgens decrease with age, but age-related levels are not accurate because current control populations have never been screened for sexual dysfunction. Such control populations need to be obtained. (Fertil Steril® 2002;77(Suppl 4):S83–8. ©2002 by American Society for Reproductive Medicine.)

Key Words: Total testosterone, free testosterone, free androgen index, DHEAS, female sexual dysfunction

Free testosterone (T) serum levels correlate better with physiological activity than do total T levels; equilibrium dialysis is the most sensitive assay but very difficult for most routine laboratories to perform. The calculated free T may become the alternative method of choice in the short term.

ANDROGENS IN WOMEN

In women, the main androgen of clinical concern is T, which is the blood test most frequently obtained for screening of androgen deficiency. Testosterone and its precursors are produced in the adrenal glands and in the ovaries, but over half of the circulating T comes from conversion in peripheral tissues (1). Destruction or suppression of the adrenal glands and/or ovaries will lead to a decrease in androgen secretion. Symptoms of decreased libido, decreased energy, and possibly decreased arousal may signal the need to check serum T.

The main precursor hormone in the adrenal gland is DHEA, most of which is excreted in the sulfated form, DHEAS. The chief precursor of T from the premenopausal ovary is androstenedione (A), which is also the chief source of estradiol (E_2). The postmenopausal ovary secretes a significant amount of T even though the production of E_2 is decreased. In the peripheral target tissues, T is 5-hydroxylated to its active metabolite dihydrotestosterone (DHT) (2, 3).

DHT is evaluated mostly in clinical research but is rarely measured clinically. Androstenedione is measured occasionally, especially in younger women, to evaluate ovarian steroid production. The assays for these hormones are fairly accurate and reproducible and, therefore, not controversial. The assay for DHEA is likewise uncomplicated but not measured often because of its short half-life and variable serum levels. DHEAS has a much longer half-life and more stable serum levels, making interpretation less problematic.

A major problem in assessing T is the inaccuracy of the measurements by current assays. These assays were developed for men who have higher levels of circulating T. All assays lose accuracy when trying to measure levels in the lower ranges. The total T assay has been used for decades in women but is generally

Received October 16, 2001; revised and accepted January 8, 2002.
Reprint requests: André T. Guay, M.D., Center For Sexual Function, Lahey Clinic Northshore, One Essex Center Drive, Peabody, Massachusetts 01960 (FAX: 978-538-4712; E-mail: andre.t.guay@lahey.org).

0015-0282/02/$22.00
PII S0015-0282(02)02965-5

TABLE 1

Clinical conditions that affect the production of SHBG.

Increased SHBG	Decreased SHBG
Estrogen therapy	Androgen therapy
Birth control pills	Obesity
Hormone replacement therapy	Insulin
Pregnancy	Prolactin
Thyrotoxicosis	Hypothyroidism
Endogenous production	Endogenous deficiency
Exogenous therapy	Antithyroid drugs
Cirrhosis	Acromegaly
Hypogonadism	
Antiepileptic drugs	

Guay. Laboratory tests in androgen deficiency. Fertil Steril 2002.

believed to be inaccurate because 66% of the T is bound to sex hormone–binding globulin (SHBG), and the levels of SHBG may shift very rapidly during various clinical conditions. Table 1 outlines some of the more common clinical conditions that affect the production of SHBG. As SHBG rises, so does the total T, but the free or active T will decrease, possibly giving false information about the actual level of T that is biologically active, as it is uncertain whether tissue androgen levels are decreased. Even the assays for free T suffer from inaccuracy and/or difficulty in laboratory execution, especially in commercial laboratories.

Clinically, it is important to know that birth control pills will not only increase SHBG but will also decrease the ovary's production of hormones, especially androgens. The same is true for the postmenopausal woman receiving estrogen replacement therapy (4).

INADEQUATE NORMAL CONTROLS

Women selected for control groups that establish normal levels for T and DHEAS have never been screened for sexual dysfunction. According to Laumann et al. (5), sexual dysfunction may occur in >40% of women between the ages of 18 and 59 years in the United States. Laumann and colleagues state that 32% of women they studied had decreased sexual desire. Because there are an estimated 75 million women in this age group, this would mean that 24 million women suffer from libido problems. We do not know what percentage of these women have androgen deficiency, but there may be enough to invalidate present normal control populations. New normal curves adjusted for age need to be established, with elimination of women with sexual difficulties by detailed interview and/or adequate questionnaires.

NORMAL AGING CHANGES

It is well accepted that DHEA and T production decrease with age in men as well as in women (6–8). As mentioned above, these aging curves need to be revisited with control populations screened for sexual dysfunction. The normal curve published by Zumoff et al. (6), often quoted to show the decline in T and DHEAS in women between the ages of 20 and 50 years, probably should be adjusted upward. A number of points of T on the scattergram shown in the article may actually be abnormally low. The main criteria for inclusion of the women as normal controls were regular menstrual cycles and the avoidance of birth control pills. However, some women with androgen deficiency may have regular menses. Weight may also be a factor; Maccario et al. (9) published a curve of decreasing DHEAS with age but found that under the age of 40 years, obese women had higher levels than thin women. The reason for this was uncertain, and those investigators postulated excessive pituitary production of ACTH. Part of the controversy in age-related decline in androgens, especially with T, may reside in the variations in their control populations. Many of the studies also had few women in their control groups; Zumoff et al. (6) looked at a decline in T over 30 years in only 33 women.

ANDROGEN DEFICIENCY STATES

Because androgens are produced in the adrenal glands and the ovaries, any condition that affects them may produce a deficiency of these hormones. Pituitary insufficiency, from adenomas, hypophysitis, or other forms of destruction, will produce a deficiency from both organs. Drugs that block the function of the ovary or the adrenal will effectively decrease androgen production. This would be true for cortisone treatment affecting the adrenal gland or for the use of birth control pills affecting the ovary. Premature menopause, chemotherapy, or hysterectomy in the younger female and natural menopause in the older female will decrease T output. Numerous general medical conditions that cause hypothalamic amenorrhea in menstruating women, including serious debilitating illnesses like HIV infection, also decrease T output. The effect would be a decreased ovarian production of androgens. Addison's disease will decrease not only cortisone production from the adrenal gland but also T precursors. There are numerous enzyme deficiencies in the adrenal gland, called adrenal hyperplasia, that might also decrease T precursors.

Along with T, the level of DHEAS will be useful in the clinical evaluation of the patient when insufficiency of the adrenal gland is suspected. Deficiency may be seen in hypopituitarism, hypoadrenalism, and in some variations of congenital (or acquired) adrenal hyperplasia. During evaluation of women for androgen excess, a DHEAS assessment is frequently ordered because DHEAS is often elevated, especially in polycystic ovary syndrome. Both T and DHEAS decrease with age, especially between the ages of 20 and 40 years. The curves showing decline with age are less controversial for DHEAS, probably because there is less

data for the decline of T with age. DHEAS continues to decline after menopause (9), but the production of T remains fairly stable, whereas estrogen levels may rise in later years (10).

TIMING OF ANDROGEN SAMPLING

It does not appear that DHEA or DHEAS levels vary much during the course of a 24-hour cycle. This is probably because DHEA production is not under the control of pituitary ACTH, as is cortisol production. The controlling enzyme is P450 C117, which has two actions. The 17-hydroxylase portion regulates cortisol metabolism, and the 17-20 lyase portion regulates the production of DHEA. Only the 17-hydroxylase portion is controlled by ACTH. This is verified by the small variation in cortisol production during a person's lifespan, whereas the DHEA production up-regulates at adrenarche and down-regulates gradually after the age of 20 years, with a further major decrease after menopause (11).

Some investigators feel that T production in women is not diurnal, as it is in men, but other investigators disagree, saying that there is a slight variation during the day, with the higher production in the morning. With this in mind, it would be better to draw blood for androgen levels, especially T, during the morning hours, between 8 AM and noon.

The production of T, and also of A does vary during the menstrual cycle. There was a controversy on this point in the past, but the majority of investigators and clinicians now feel that androgens and ovarian precursors peak toward midcycle. Massafra et al. (12) performed detailed measurements during three separate parts of the follicular as well as the luteal phases, highlighting the fact that the highest levels are in the middle third of the cycle (Fig. 1). It would be prudent, therefore, to measure androgens from day 8 to 18 of the menstrual cycle.

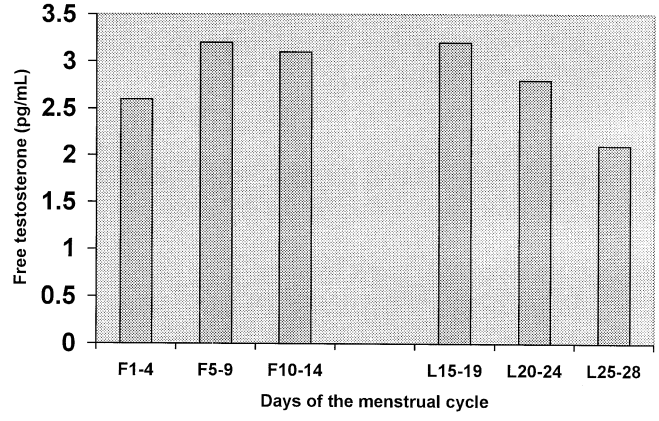
POPULATIONS MUST BE IDENTIFIED

Confusion between normal and abnormal levels of T in women may be related to ill-defined populations. It is agreed that T levels decrease with age in women as they do in men, but the normal aging curves in women were formulated with no screening of the candidates for sexual dysfunction, neither by investigators nor by companies producing kits for laboratory determination of T. Some normal controls may have had low T levels, with the result that the normal ranges for T should be adjusted upward. Women considered for a normal control population should be screened for sexual dysfunction by interview and/or questionnaires.

In the investigation of women with presumed T deficiency or T excess, the population studied must be defined carefully to avoid faulty conclusions. Table 2 outlines a population of postmenopausal women, previously identified

FIGURE 1

Free T levels during the various stages of the follicular (F) and luteal (L) phases of a normal menstrual cycle. (Adapted from Massafra et al. [(12)].)



Guay. Laboratory tests in androgen deficiency. Fertil Steril 2002.

by us (13), who complained of decreased libido and who had decreased T levels. It is fairly well agreed that estrogen therapy will decrease free T levels because it will increase SHBG, causing more binding of T, resulting in an increase in total T and a decrease in the free fraction. Table 2 differentiates those women who were on estrogen therapy from those who were not. There was no difference in the total or the free T levels whether the women were or were not on estrogen therapy. One explanation might be that estrogen therapy made no difference in free T levels. The more reasonable explanation is that all of these women were T deficient, having such low levels that a further decrease was unable to be determined by the current assays, which are extremely imprecise at the lower levels. An alternative explanation might be that the major cause of the low T levels is adrenal gland production of androgens, and not the ovary.

TABLE 2

Possible effect of estrogen therapy on T levels in a group of postmenopausal women with decreased T levels and with complaints of decreased libido.

Parameter	On hormone replacement therapy	No hormone replacement therapy	*P* value
n	16	22	—
Age (yr)	58.6	54.5	.6
Total T (ng/dL)	18.1	16.3	.2
Free T (pg/mL)	0.41	0.47	.3

From Guay and Jacobson (13). Reprinted by permission of the publisher.

Guay. Laboratory tests in androgen deficiency. Fertil Steril 2002.

TESTOSTERONE ASSAYS

The main area of controversy in the subject of androgen deficiency in women has been, and still is, the laboratory analysis of T. For decades, the total T assay has been performed, which measures both freely circulating T and that portion bound to protein. More recently, free T, the fraction found circulating freely in blood unbound to protein, has also been measured. Even more recently, measurement of bioavailable T has been obtainable, and this measures both free T and that fraction loosely bound to albumin. Women have a higher fraction of T bound to SHBG than men. An easier method of T measurement has been reported, using saliva, which was initially held to be better for mass screening. Very recently, a more practical bioassay has been developed. We shall look at these assays in more detail, although they all share the limitation that they were developed to measure the higher levels of T found in men. There is a need to develop an assay specifically for the lower levels found in women.

Salivary T measurement has never gained wide support, even for mass screening (14), because the normal range is quite large, with questionable accuracy in the lower ranges. Salivary T measurement has some correlation with the free-T assay, which is quite inaccurate in the lower ranges. The salivary T assay is not felt to be useful for mass screening but may have some use in following T in individual patients. A newer *T bioassay* is being developed and may be accurate but is early in development. It uses a portion of a yeast cell in combination with components of viral protein and functions as an androgen receptor (15).

As mentioned above, the *total T* assay has been the standard for decades, but the present consensus is that it is inaccurate in the lower ranges of serum T, which is where the values reside in women. Another valid observation is that it measures not only the bioactive T but all fractions, including that fraction bound to SHBG, which is not immediately available for tissue activity (16). Table 1 shows some of the clinical conditions that can affect SHBG levels and will give falsely low or falsely elevated values. SHBG rises in men as they age, which makes this assay more inaccurate with advancing age. It is not felt that SHBG changes with age in women, but definitive determinations in large numbers have yet to be done.

Bioavailable T assay measures the free fraction of T and that fraction loosely bound to albumin, both fractions of which are thought to be readily available for use by peripheral tissues (17). The assay is felt to be difficult and time consuming, but the accuracy is fairly good, especially in men (16). The SHBG is precipitated with ammonium sulfate. In one version, the supernatant is measured for total T. In another, radioactive tracer is added before the precipitation, then the ratio between the precipitate and the supernatant can be calculated to render the percentage of bioavailable T. This assay would be difficult to do in most commercial or medical center laboratories.

The most accurate theoretical way to look at T is to measure the fraction that is circulating freely in blood and is immediately available for use by the tissues. It is agreed that this might be accomplished using a *free T* assay. The most accurate method and the current gold standard in measuring free T is the *equilibrium dialysis assay* (16, 18). It purports to measure the 1–2% of the free T circulating in the blood. Some argue that this is difficult to achieve because the levels in women are so low to start with. A recent modification enables the assay to be more sensitive in the lower ranges, making it more suitable for female patients (19). The test is still difficult and time consuming and not suitable to many clinical facilities and commercial laboratories. It involves equilibrating radioactive T in a sample, followed by separation of the nonprotein T by dialysis or by ultra filtration.

Recently, Vermeulen et al. (16) have evaluated a simpler method that correlates best with the equilibrium dialysis method. It is called the *calculated free T* method, which yields what has been called the *free androgen index* or, more accurately, the *free testosterone index* (FTI) (20). It entails doing two assays, one for total T and the other for SHBG. The results are calculated into the following formula: (total T $\times$ 100)/SHBG. Normal controls have not been established, and the few studies conducted have yielded levels in special populations of small numbers of women. This formula also makes assumptions, especially about albumin levels. Vermeulen et al. (16) reported that variations of 25% above and below the assumed 4.5 g/dL albumin levels yielded very little change in the calculation. Unfortunately, many patients with chronic illness will have larger shifts of albumin than have been tested for. Doing two separate assays will also be more costly and time consuming.

The most common assay performed in the United States is the *analog free testosterone* assay. It is quite reproducible and is less labor intensive (21). However, it is considered by many to be the least accurate assay (22), though the data supporting this are sparse; these objections are found mainly in a few letters to the editor (23). One objection is that the analog T used by the developer as a standard is a trade secret and cannot be analyzed by others. A noteworthy observation is that the midpoint of the normal range is only 30% of that reported by the equilibrium dialysis method, or reflecting a 0.5–0.75% free T concentration in female blood. Despite this, Vermeulen et al. (16) reported a high correlation between the analog method and the equilibrium method (r = .937). In this method, radioactive analog tracer is bound to the anti-T antibody but not to SHBG or to albumin. Some critics contend that the assay does actually vary as a function of SHBG and that this invalidates the results.

Table 3 notes a paper supporting the analog free-T assay and one criticizing it, along with some data at the Lahey Clinic Central Laboratory (personal communication; Ronald Swanson, M.D.). It bears reiterating that the numbers of women studied in all reports are small, but less so in the

TABLE 3

Comparison between various laboratories of analog free T assay parameters in women.

Parameter	Investigators (reference no.)			
	Wilke and Uttey (21)	Winters et al. (22)	Vermeulen et al. (16)	Lahey Clinic (personal communication)
n	37	28	32	76
Age range (y)	20–50	25–45	50–70	16–73
FT to TT	0.51			0.79
FT to SHBG	−.09	−0.30		−0.31
aFT to edFT			0.937	

FT, free T; TT, total T; aFT, analog free T; edFT, equilibrium dialysis free T.

Guay. Laboratory tests in androgen deficiency. Fertil Steril 2002.

Lahey Clinic data. In the Lahey laboratory, the free T correlates better with total T, as opposed to the results of Wilke and Utley (21). In agreement with the Lahey clinical data, Winters et al. (22) have shown a negative correlation of free T and SHBG, as opposed to the noncorrelation shown by Wilke and Utley (21), supporting that estrogen therapy will raise SHBG while decreasing free T. Besides the small number of patients studied, another reason for the discrepancy between reports might be the lack of familiarity of some laboratories with the analog free-T assay. In addition, comparisons between methods should be done with adequate numbers of patients from defined populations.

CONCLUSIONS

Most of the controversies concerning androgen deficiency in women revolve around T rather than the other androgens. Testosterone measurements should be performed during the morning hours. They should also be measured during the middle third of the menstrual cycle, preferably during days 8 to 18 because the T, as well as the androstenedione, will show the highest values during these days. The lowest levels will occur in the late luteal and early follicular phases. Populations studied should be precisely defined or erroneous conclusions may result. Testosterone and DHEA levels decrease normally with age; however, precise levels of T have not been accurately collected, as adequate screening of presumed normal female populations have not been done. Women considered for inclusion as normal controls should be screened for sexual difficulties by detailed interview and appropriate questionnaires.

Most will agree that the free T level will accurately reflect androgen action or deficiency. All of the current assays were developed for male ranges of T, and an assay specific for the low levels found in women must be developed. The measurement of free T by equilibrium dialysis is regarded by most as the gold standard and recently has been modified to be more sensitive at lower blood levels, but it is difficult to perform and not suitable for most laboratories. Other assays for free T are generally felt to be lacking, although the evidence to suggest this is controversial and based on very little data. Adequate comparative trials should be carried out.

The calculated free-T assay seems to be the most acceptable approach at this time, but again data are minimal, and it needs to be tested in larger populations. Most reference laboratories can easily do the total T and SHBG assays needed to calculate the FTI.

References

1. Guay AT. Advances in the management of androgen deficiency in women. Med Asp Hum Sex 2001;1:32–8.
2. Lobo RA. Androgens in postmenopausal women: production, possible role, and replacement options. Obstet Gynecol Surv 2001;56: 361–76.
3. Roger M, Nahoul K, Scholler R, Bagrel D. Evolution with ageing of four plasma androgens in postmenopausal women. Maturitas 1980;2: 171–7.
4. Davis SR, Tran J. Testosterone influences libido and well being in women. Trends Endocrinol Metab 2001;12:33–7.
5. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence and predictors. JAMA 1999;281:537–44.
6. Zumoff B, Strain GW, Miller LK, Rosner W. Twenty-four-hour mean plasma testosterone concentration declines with age in normal premenopausal women. J Clin Endocrinol Metab 1995;80:1429–30.
7. Vermeulen A. Androgen replacement in the aging male—a critical evaluation. J Clin Endocrinol Metab 2001;86:2380–90.
8. Harman SM, Metter EJ, Tobon JD, Pearson J, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. J Clin Endocrinol Metab 2001;82:724–31.
9. Maccario M, Mazza E, Ramunni J, Oleandri SE, Savio P, Grottoli S, et al. Relationships between dehydroepiandrosterone-sulphate and anthropometric, metabolic and hormonal variables in a large cohort of obese women. Clin Endocrinol 1999;50:595–600.
10. Labrie F, Belanger A, Cusan L, Gomez JL, Candas B. Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. J Clin Endocrinol Metab 1997;82:2396–402.
11. Liu CH, Laughlin GA, Fischer UG, Yen SSC. Marked attenuation of ultradian and circadian rhythms of dehydroepiandrosterone inpostmenopausal women: evidence for a reduced 17,20-desmolase enzymatic activity. J Clin Endocrinol Metab 1990;71:900–6.
12. Massafra C, De Felice C, Agnusdei DP, Gioia D, Bagnoli F. Androgens and Osteocalcin during the menstrual cycle. J Clin Endocrinol Metab 1999;84:971–4.
13. Guay AT, Jacobson J. Decreased free testosterone and dehydroepiandrosterone-sulfate (DHEAS) levels in women with decreased libido. J Sex Marital Ther 2002;28 Suppl:129–42.
14. Orentreich N, Brind JL, Rizer RL, Vogelman JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab 1984;54:551–5.
15. Raivo T, Palvimo JJ, Dunkel L, Wickman S, Janne OA. Novel assay for

determination of androgen bioactivity in human serum. J Clin Endocrinol Metab 2001;86:1539–44.
16. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666–72.
17. Cumming DC, Wall SR. Non sex hormone binding globulin bound testosterone as a marker of hyperandrogenism. J Clin Endocrinol Metab 1985;61:873–6.
18. Bhasin S, Storer W, Berman M, Callegari C, Clevenger B, Phillips J, et al. The effects of supraphysiological doses of testosterone on muscle size and strength in normal men. N Engl J Med 1996;335:1–7.
19. Sinha-Hikim I, Arver S, Beall G, Shen R, Guerrero M, Sattler F, et al. The use of a sensitive equilibrium dialysis method for the measurement of free testosterone levels in healthy, cycling women and in human immunodeficiency virus-infected women. J Clin Endocrinol Metab 1998;83:1312–8.
20. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. J Clin Endocrinol Metab 2001;86:724–31.
21. Wilke TJ, Utley DJ. Total testosterone, free-androgen index, calculated free testosterone, and free testosterone by analog RIA compared in hirsute women and in otherwise-normal women with altered binding of sex-hormone-binding globulin. Clin Chem 1987;33:1372–5.
22. Winters SJ, Kelley DE, Goodpaster B. The analog free testosterone assay: are the results in men clinically useful? Clin Chem 1998;44: 2178–82.
23. Rosner W. Errors in the measurement of plasma free testosterone [letter]. J Clin Endocrinol Metab 1997;82:2014–5.