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Reviews

Prostate-Specific Antigen as a Marker of Hyperandrogenism in Women and Its Implications for Antidoping

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BACKGROUND: Since its discovery in the 1970s, prostate-specific antigen (PSA) has become widely known as a biomarker of prostate cancer in males but has often been overlooked in female malignancies. Although the serum concentration of PSA differs between men and women by about 1000-fold, studies have suggested that PSA concentrations drastically differ among healthy females and those who exhibit increased androgen production.

CONTENT: There have been reports of increased PSA expression in women exhibiting hyperandrogenic states, including polycystic ovary syndrome and hirsutism, as well as marked increases in a subset of breast cancer patients. These findings have not only revealed the remarkable diagnostic potential of PSA in a diverse range of clinical conditions but also point to its potential of becoming a useful biomarker of steroid hormone doping among female athletes. Recently, highly sensitive assays that can measure PSA at low limits of detection have been developed, which will aid in the discrimination of PSA between these different conditions.

SUMMARY: The overall aim of this review is to revisit the expression of PSA in hormonally-regulated tissues and in female malignancies, and to demonstrate how the regulation of PSA permits its use in antidoping initiatives.

Hyperandrogenism, defined as excess of androgen production, is a common endocrine disorder that affects approximately 5%–10% of young women *(1)*. Females exhibiting a hyperandrogenic state can often be distinguished by various features of their clinical presentation, including acne, hirsutism, and androgenic alopecia *(1)*. Along with the examination of physical clinical attributes, the measurement of hormones can provide insight on the status of a hyperandrogenic state in females. Consequently, given the overproduction of androgens during hyperandrogenism, androgen-regulated genes, such as prostate-specific antigen (PSA),[4] are often overexpressed in these conditions, and can serve as surrogate diagnostic markers of increased androgenic activity.

PSA has become historically known for its association with prostate cancer in males; however, few studies have highlighted its utility in other clinical applications. PSA is a 33-kDa glycoprotein that belongs to the family of kallikrein-like serine proteases but has relatively low enzymatic activity in comparison to other family members *(2)*. It is directly regulated by the androgen receptor, which promotes the transcription of PSA through its interaction with androgen response elements on the PSA gene. Although widely recognized to be exclusively produced by epithelial cells of the prostate gland, extraprostatic production of PSA has been reported in various types of female tissues and conditions. Over the years, several groups have examined PSA expression in androgenic and other conditions, and have demonstrated that PSA concentrations in serum and urine are increased in a subset of breast cancer patients, hirsute females, and those with polycystic ovary syndrome (PCOS) (Fig. 1; Table 1). For instance, women with PCOS have been reported to have urinary PSA concentrations almost 200 times higher than females without PCOS *(3)*. In addition, other studies have documented increased PSA expression in female-to-male transsexuals after testosterone treatment, thereby further demonstrating the androgen regulation of PSA *(4)*.

Recently, there has been an emergence of ultrasensitive PSA assays that are superior in performance compared to commercially available assays, and are capable of quantifying PSA at low linear ranges, in addition to detecting PSA at femtomolar concentrations *(5, 6)*. The ability to detect near zero PSA concentrations will not only have implications for monitoring men who have

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[4] Nonstandard abbreviations: PSA, prostate-specific antigen; PCOS, polycystic ovary syndrome; LOD, limit of detection; IRMS, isotope-ratio mass spectrometry.

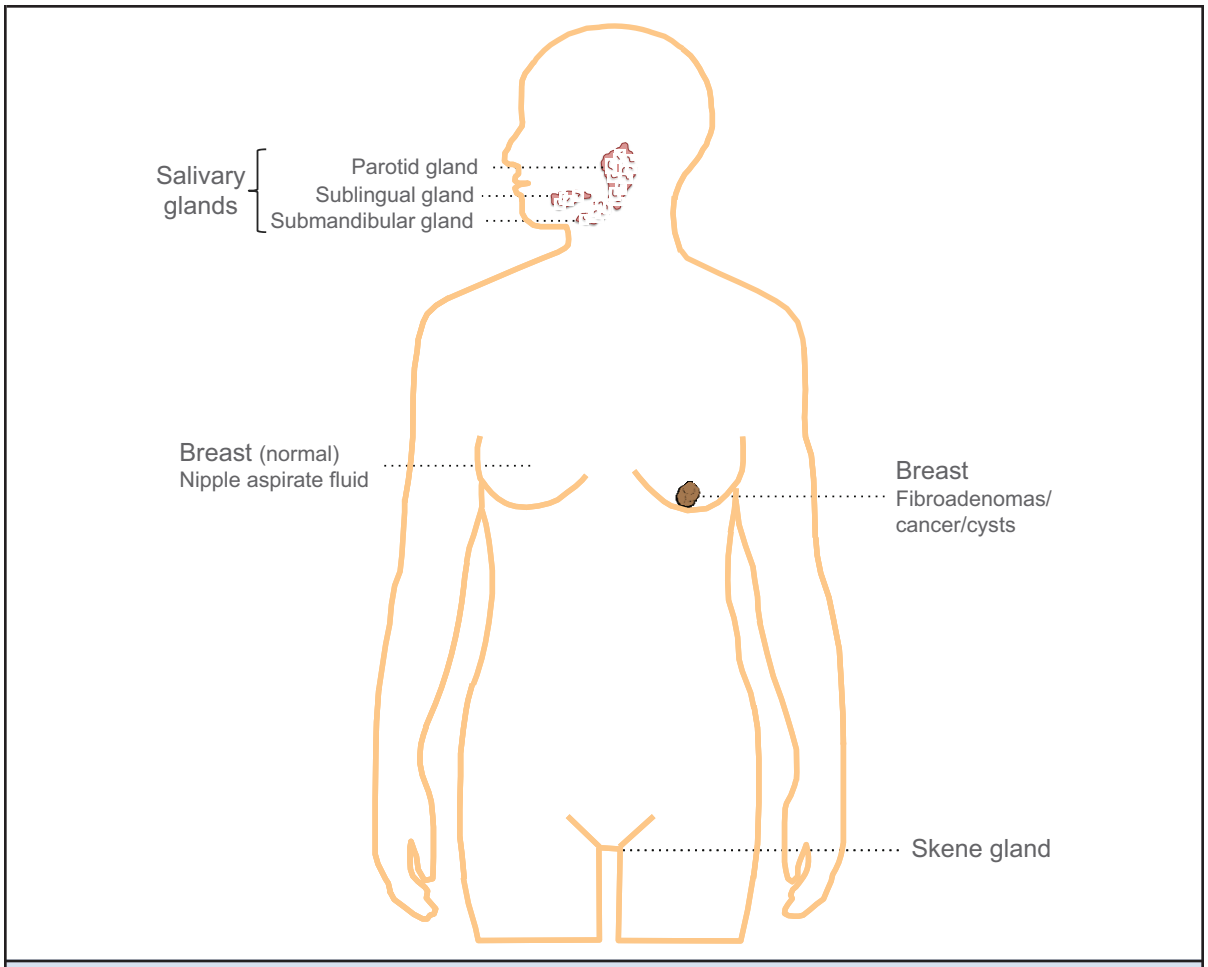

Fig. 1. Anatomical sites of PSA production in females.
PSA is produced in healthy breast tissue (increased in nipple aspirate fluid), fibroadenomas, breast cancer, and salivary and Skene glands.

undergone radical prostatectomy but will enable the detection of PSA in females, which is much lower than in males.

There is no question that PSA is an excellent diagnostic marker of hyperandrogenic conditions in women, including hirsutism and PCOS, and it has also exhibited discriminatory power in breast cancer diagnosis and prognosis. Given that PSA is regulated by androgens, there has been recent recognition that the diagnostic utility of PSA could be further extended as a biomarker of sex identification and of steroid hormone doping by female athletes *(7)*. This realization could have major implications for world antidoping agencies that are continuously searching for new methods of detecting and deterring the use of illegal androgenic steroids in sports. In this review, we will highlight studies that have documented the expression of PSA in hyperandrogenic syndromes, discuss the development of novel PSA ultrasensitive assays, and provide insight on how this existing knowledge and new technology can be applied to antidoping initiatives.

PSA Production and Regulation by Androgens

PSA is well known for its use as a diagnostic and monitoring biomarker for prostate adenocarcinoma in males *(8)*. For many years, it was believed that PSA was exclusively produced by the male prostate, however, since its discovery, it has been increasingly recognized that PSA is not prostate-specific, but it is also produced in various hormonally-regulated female tissues. In fact, numerous studies, including our own which dates back to 1994, have shown beyonda doubt that PSA is produced at relatively low levels in the female breast, the periurethral glands (also known as paraurethral or Skene glands), and some tumors such as breast, lung, ovarian, salivary, etc. *(9–14)*. Similar to the situation in males, small amounts of PSA diffuse from the site of production and storage

Table 1. Increases of serum and urine PSA in hyperandrogenic syndromes and other conditions.

Condition	PSA increase in	Fold-difference	Application	Reference
Normal males and females	Serum of males vs females	1000	Sex identification	Diamandis *(7)*
PCOS	Urine	200[a]	Diagnosis of PCOS	Obiezu et al. *(3)*
	Serum	3–5		Burelli et al. *(26)*; Ukinc et al. *(27)*; Mardanian et al. *(28)*
Hirsutism	Serum	2[a]	Diagnosis	Melegos et al. *(30)*; Negri et al. *(31)*; Escobar-Morreale et al. *(32)*
Female-to-male transsexuals (after testosterone administration)	Serum	10–20	Effectiveness of therapy	Obiezu et al. *(4)*; Slagter et al. *(34)*
	Urine	80		Obiezu et al. *(4)*; Slagter et al. *(34)*
Fibroadenomas and breast cysts	Serum	1000[a]	Diagnosis	Borchert et al. *(33)*
Breast cancer	Tissue	ND[b]	Prognosis	Monne et al. *(35)*
	Serum	ND	Diagnosis	Black et al. *(15)*; Mashkoor et al. *(42)*
	Nipple aspirate	20[a,d]	Prognosis/diagnosis	Sauter et al. *(40)*
Breast cancer + megestrol acetate	Serum	2–20[c]	Prognosis (unfavorable)	Diamandis et al. *(41)*
Normal breast + Brevicon	Tissue	50[a]	ND	Yu et al. *(22)*

[a] Compared to apparently normal females.
[b] ND, not determined.
[c] Before and after treatment.
[d] Lower in cancer.

(e.g., from the breast ducts in females) into the general circulation. If sufficiently sensitive analytical methods are used, PSA can be detected in serum, urine, nipple aspirate fluid, ascites fluid, milk of lactating women and cerebrospinal fluid of both males and females, which has been demonstrated repeatedly *(15–17)*.

The regulation of the PSA gene has been very well studied, because it has been shown that PSA is upregulated by androgens and androgenic progestins in prostate, breast, and other tissues *(18, 19)*. The PSA gene promoter on chromosome 19q13.4 contains 3 classical androgen response elements, which facilitate its regulation by the androgen receptor *(20, 21)*. Steroid hormones act by first penetrating the cell membrane from serum (by diffusion), and subsequently bind to specific receptors in the cytoplasm (androgen receptor for PSA), before entering the cell nucleus where they bind to specific DNA sequences called "androgen response elements" to initiate transcription of groups of genes. With this mechanism, after androgen stimulation, the levels of PSA mRNA and protein can increase by many 1000-fold, thus amplifying the signal. Because the PSA protein is secreted, it is often present in serum or urine and could be easily measured by highly sensitive ELISA assays.

Although only males have a prostate gland, there is a prostate equivalent in females called "Skene gland" or the "female prostate" *(10)*. The Skene gland is located outside the vaginal wall toward the lower end of the urethra. This gland is able to produce PSA and secrete it into the urine, especially after androgen stimulation. The female breast can also produce PSA and secrete it into the systemic circulation *(17, 22)*. When a woman is exposed to an androgenic steroid (endogenous or exogenous), the androgens stimulate androgen receptor-positive cells to produce and secrete PSA. In the female breast, it has been shown that PSA is secreted and stored in nipple aspirate fluid *(23)* and diffuses into the general circulation where it can increase the serum PSA concentrations *(17, 22)*. After androgens stimulate PSA production in the Skene gland of women, PSA enters the urine where it can be measured by ELISA. Thus, serum and urinary PSA are both markers of androgenic activity in females.

Hyperandrogenism and the Overproduction of PSA in Females

Hyperandrogenism is characterized by an excess level of androgens in the body, and results in several clinical manifestations, with the 2 most common being PCOS and hirsutism. Below, we highlight several studies that have examined the expression of PSA in these female conditions.

POLYCYSTIC OVARY SYNDROME

PCOS is a common endocrine disorder that can affect 15%–20% of women of reproductive age, depending on the diagnostic criteria used *(24)*. The disease is often characterized by chronic anovulation and excessive androgen production, in addition to the accumulation of ovarian cysts, although deviations from these features have been reported *(3)*. Although the etiology of PCOS remains unclear, high insulin production and genetic predisposition have been identified as potential causes *(25)*. Given the high level of androgen production in PCOS females, it is logical to assume that PSA would be increased as well.

In a previous study examining PSA expression in PCOS, our group discovered that urinary PSA concentrations were indeed significantly increased in PCOS patients [mean (SE) of 820 (344) ng/L] in comparison to age-matched controls [mean (SE) = 4.3 (1.8) ng/L] *(3)*. Moreover, a weak correlation was also observed between urinary PSA and serum 3-α-androstanediol glucuronide (another marker of hyperandrogenism) and between urinary PSA and serum testosterone. Meanwhile, another group reported higher serum concentrations of PSA in PCOS, thereby corroborating the aforementioned findings, but did not observe significant differences in PSA concentrations during various stages of the menstrual cycle, or between pre- and postmenopausal women *(26)*. This finding suggests that PSA production is not affected by hormonal changes during the menstrual cycle and that the source of its production lies outside of the female reproductive system. In more recent reports, serum PSA and free PSA concentrations have been found to be increased in both ovulatory and anovulatory PCOS patients, and correlated with hirsutism and other hyperandrogenic states *(27, 28)*. Interestingly, unlike the situation in prostate cancer, total PSA, and total PSA to free PSA ratios have been shown to have comparable diagnostic performance in PCOS *(29)*.

HIRSUTISM

Hirsutism is defined as excessive terminal hair growth in patterns often observed in males and is present in 80% of females with excess androgen production *(1)*. The condition can also result from increased sensitivity to androgenic compounds. In a previous study, serum PSA concentrations were compared between 22 hirsute women and 50 controls, and were found to be markedly increased among women with the condition *(30)*. Moreover, concentrations of PSA and 3-α-androstanediol glucuronide, a specific metabolite of androgen action, showed a significant positive correlation, whereas PSA and 3-α-androstanediol glucuronide showed a significant negative correlation with patient age in hirsute women *(30)*. Interestingly, women with hirsutism receiving a 6-month antiandrogen treatment regimen displayed a reduction of PSA concentration at the end of the trial *(31)*. Another study investigating possible tissue sources of PSA production revealed that the adrenal and ovary did not contribute to PSA upregulation in hirsute women *(32)*. As a result, these findings further demonstrate the regulation of PSA by androgens, and its potential as a marker of androgenic activity.

Fibroadenomas and Breast Cysts

In a small study of 2 female patients with fibroadenomas and 2 female patients with breast cysts, we have demonstrated that these 4 patients had serum PSA in the range of males (approximately 1000-fold higher than in normal women) *(33)*. If these data are reproduced in a larger series, they could suggest that serum PSA could be a diagnostic test for this condition.

Female-to-Male Transsexuals

Sex transition involves a series of medical interventions, whereby the physical transition often commences through treatment with sex hormones. To determine whether PSA expression varies during the hormonally-induced transition, a previous study conducted by our group measured serum and urinary PSA concentrations before, and at 4 and 12 months posttestosterone treatment of female-to-male transsexuals, who were treated intramuscularly with 250 mg testosterone every 2 weeks *(4)*. Interestingly, serum PSA concentrations increased from a mean of 1.1 ng/L to 11.1 ng/L and then to 22 ng/L by 4 and 12 months posttreatment, respectively. The corresponding mean values in urine were 17 ng/L, 1420 ng/L and 18 139 ng/L, respectively *(4)*, thereby showing that in young women who receive testosterone, serum concentrations of PSA are increased by more than 10-fold and urine concentrations by about 80-fold.

This original report was later followed by another study in 2006 with 28 female-to-male transsexuals who received 250 mg of testosterone intramuscularly every 2 weeks *(34)*. As expected, after testosterone administration, serum testosterone concentrations increased by about 15-fold. Mean serum PSA increased from 2.3 ng/L before treatment, to 20 ng/L after treatment (P value <0.001). Median PSA was 1 ng/L (before treatment)

and 16 ng/L after 12 months of treatment. Urinary concentrations of PSA increased from 5.4 ng/L before treatment to 1604 ng/L after treatment, suggesting that this parameter is even more sensitive than serum PSA as a marker of hyperandrogenism. Increases of urinary and serum PSA in these patients were similar after 4 and 12 months of treatment *(34)*. These preliminary data, generated exclusively by our group over the last 20 years, suggest that *a*) serum and urinary PSA is a highly sensitive marker of integrated endogenous androgenic activity in females and *b*) serum and urinary PSA is a highly sensitive marker of exogenous androgen administration in females.

PSA in Breast Cancer

Besides its presence in hyperandrogenic conditions, numerous studies have also documented increased concentrations of total and free PSA in breast cancer patients, which is not surprising given that many breast cancer tumors are hormonally dependent. In an early study, PSA messenger RNA was detected in 30% of breast cancer tissues examined *(35)*. This seminal finding prompted others to analyze whether PSA expression in breast cancers has diagnostic or prognostic potential. Subsequent studies determined that the predominant form of PSA in serum of healthy women is bound to α1-antichymotrypsin, in contrast to that in serum of breast cancer patients *(36–39)*. Because total and free PSA concentrations display different discriminatory power in prostate cancer patients, a previous study examined the utility of using total and free PSA a diagnostic marker for breast cancer patients. Using an immunoassay with a detection limit of 0.001 μg/L (1 ng/L), the authors found that the percentage of breast cancer patients with free PSA in serum was 5 times higher than that of healthy women and women with benign conditions *(15)*. In addition, PSA concentrations have been found to be lower in nipple aspirate fluid of breast cancer patients than women within the reference interval and were a good predictor of breast cancer in conjunction with menopausal status *(40)*.

Another study examining the link between PSA and hormone receptor expression revealed that immunoreactive PSA is associated with the presence of progesterone receptors *(16)*. For instance, the steroid hormone receptor-positive breast cancer cell lines, T47D and BT474, produced PSA upon stimulation with steroid hormones and tamoxifen *(19)*. The prognostic value of plasma PSA was also evaluated in patients with metastatic breast cancer treated with the progestin, megestrol acetate MA *(41)*. Roughly 50% of patients displayed increased concentrations of plasma PSA while receiving megestrol acetate, and had a 3-fold increase in relative risk of death *(41)*. Interestingly, PSA was also determined to be increased in healthy women receiving the progestin-containing oral contraceptive, Brevicon, thereby demonstrating the ability of female tissues to produce PSA upon stimulation with androgenic or progestational steroids *(22)*.

In a recent study, PSA concentrations were significantly increased in preoperative women with breast cancer compared to healthy controls, which declined after tumor resection *(42)*. Approximately 84% of breast cancer patients had free PSA as the prevalent molecular form in serum compared to controls (0%) and cancer patients after surgery (<2%), suggesting that PSA may be useful for monitoring therapy response *(42)*. In addition, others have suggested that total and free PSA values could be used for differentiating benign and malignant breast cancer lesions *(43)*.

Emergence of New Technologies and Ultrasensitive Assays to Measure PSA

Throughout the past 2 decades, it has become increasingly recognized that ultrasensitive PSA assays with low detection limits are useful for detecting biochemical recurrence after radical prostatectomy of prostate cancer patients. However, the accurate measurement of PSA has long been hindered by the lack of sensitive assays to detect PSA after removal of the prostate in these patients, which would provide valuable insight into patient prognosis. This has led to a series of generations of ultrasensitive PSA assays, for which each successive generation has displayed a 10-fold improvement in the limit of detection (LOD) of PSA. For example, the very first generation of PSA assays had detection limits of 100–500 ng/L, which were later superseded by ultrasensitive assays (third generation) that are capable of measuring PSA concentrations of 10 ng/L. These assays display superior performance to conventional PSA immunoassays, which carry LODs of 100 ng/L, and are currently used for cancer screening (Table 2) *(39)*.

Since then, as the development of more sensitive assays has accelerated, the ability to detect biochemical recurrence of PSA earlier in men who have undergone radical prostatectomy has become useful for monitoring patient relapse. An immuno-PCR-based method with nucleic acid detection was developed that could quantify a PSA concentration as low as 0.65 ng/L, with a LOD of 0.27 ng/L *(44)*. Meanwhile, a gold nanoparticle biobarcode assay was developed with a detection limit of 330 fg/mL, which demonstrated the ability to detect measurable PSA in a cohort of patients after prostate removal *(45)*.

This was followed by the development of higher generation assays, as Wilson et al. developed a fifth-generation digital immunoassay for PSA using a bead-based ELISA, with a sensitivity of <0.05 ng/L and a limit

Table 2. Detection limits of ultrasensitive PSA assays.

PSA assay	Detection limit, ng/L	Reference
Conventional*	100–500	Ferguson et al. *(39)*
Third generation	1–10	Ferguson et al. *(39)*
Immune-PCR	0.6	McDermed et al. *(44)*
Gold nanoparticle	0.3	Thaxton et al. *(45)*
Digital/bead-base	0.05	Wilson et al. *(46)*
Single molecule	0.014	Rissin et al. *(47)*
Silver nanoprism plasmonic biosensor	0.004–0.01	Liang et al. *(5)*
Electroluminescence	0.002 (complexed PSA), 0.02 (free PSA)	Nikolenko et al. *(6)*

of quantification that is 2 orders of magnitude lower than third-generation PSA assays *(46)*. In addition, another group has developed single-molecule ELISAs that are capable of detecting serum PSA concentrations in patients in the subfemtomolar concentration, and can be as low as 14 fg/mL *(47)*.

Recently, Liang et al. developed a silver nanoprism plasmonic biosensor capable of detecting PSA concentrations in the range of 10 fg/mL to 100 ng/L, and a LOD of 4.1 fg/mL *(5)*. The latest and most sensitive assay reported to date has been based on electrochemiluminscence technology and displays detection limits of 2 fg/mL for complexed PSA and 20 fg/mL for free PSA *(6)*, which are low enough to measure serum PSA concentrations in females. As a result, it may be worthwhile to use these new assays to measure androgenic activity in females, particularly those suspicious of drug doping.

The reference intervals for females, based on the above assay *(6)* are 0.2–1.5 ng/L (complexed) and 0.08–0.26 ng/L (free PSA). These authors found no difference in either total, complexed, free, or % free PSA between healthy women and women with breast cancer (ER+, HER2+, or triple negative) *(6)*.

Implications for Antidoping

The use of appearance- and performance-enhancing drugs among elite athletes is a prevailing problem in competitive sports, because it compromises the authenticity of an athlete's performance during competitions. It is estimated that 14%–39% of athletes have purposely doped *(48)*, whereas only 2% of samples examined by the World Anti-Doping Agency are identified as being positive *(49)*. The use of drugs to enhance performance raises ethical concerns within international sports organizations, because most officials agree that it constitutes a form of cheating *(50)*. Having an advantage over nondoping competitors disrupts a level-playing field, explaining why one of the main objectives of antidoping programs is to deter athletes from doping.

Among performance-enhancing drugs, anabolic androgenic steroids (particularly testosterone) are the most commonly used *(51)*, which can improve athletic performance in both sexes by affecting muscle and bone tissue, erythropoiesis, the immune system and behavioral patterns *(52–54)*. Circulating concentrations of testosterone positively correlate to muscle mass, muscle strength, and bone mass *(53)*. It is clear that a gain in such attributes can greatly enhance strength, endurance, and overall athletic ability.

Androgens may also contribute to decreased risk of injuries and to improved health status in athletes. The synthetic machinery of testosterone, the prototypical anabolic steroid, is mainly located in the testes of men and in the ovaries of women. In both sexes, weaker androgens such as androstenedione, dehydroepiandrosterone, and its sulfate are produced by the adrenal cortex and can be converted to testosterone in peripheral tissues or act directly on the androgen receptor *(52)*. Testosterone is biologically active in its free form, although it predominately is strongly bound to SHBG (sex-hormone-binding globulin).

Steroid hormone profiling of urine and serum is a common practice in clinical endocrinology and is frequently used for diagnostic purposes. Doping control agencies use the same routine techniques as a way to longitudinally measure and monitor the steroid composition of athletes *(50)*. The urinary steroid profile is composed of concentrations and ratios of various endogenously produced steroidal hormones, their precursors, and metabolites, including testosterone, epitestosterone, dihydrotestosterone, and androsterone. Any deviation of steroid concentrations in comparison to an individual's predetermined normal reference intervals will raise suspicion *(55)*. Parent steroids and metabolites are usually measured by gas chromatography/combustion/isotope-ratio mass spectrometry (IRMS) and associated techniques. These assays are best performed in dedicated laboratories, accredited by international antidoping agencies *(56, 57)*. Steroid profiles, as determined routinely in

doping control, provide essential information for several purposes because they are recorded and stored incrementally, with each collected drug-testing sample. Hence, it is of utmost importance that laboratory standards are comparable within the group of World Anti-Doping Agency-accredited drug testing sites, to enable correlation of data from one athlete on different occasions. Several studies have demonstrated only small intraindividual variation of steroid profile parameters, especially within the ratios used for doping control purposes (i.e., testosterone/epitestosterone ratio). However, the issue regarding the lack of sensitivity of IRMS remains a prevalent problem because several steroid preparations may produce IRMS ratios similar to endogenous levels, making it impossible to discern performance-enhancing drug users.

Longitudinal long-term stability within the individual's steroid profiles supports the more sensitive detection of doping and provides one of the most important parameters of sample individualization in sports drug testing. More recently, it has been shown that screening tests are superior in detecting values outside the reference interval in longitudinal biomarkers. These tests compare sequential measurements of a biomarker against previous tests performed on the same individual. The importance of such a project was stressed by comprehensive studies concerning testosterone-gel administrations. In these studies, the discriminating power of individual reference intervals was significantly superior to conventional population-based reference intervals, especially from volunteers showing basal values of testosterone and epitestosterone ratios of <1. These longitudinal measurements are included in the recently introduced athletic passport, which is an individual, electronic record for professional athletes in which profiles of biological markers of doping and results of doping tests are collated over a period of time *(58–60)*. Doping violations can then be detected by noting variances from an athlete's established levels, outside permissible limits.

Common challenges of current antidoping programs often include the ability to detect new designer substances and the analytic capacity to detect low concentrations of prohibited substances in athletes who have doped. It is well known that there is a current need for newer and more sensitive analytical detection methods. As previously mentioned, the advent of ultrasensitive technologies will revolutionize the way illegal drug doping is detected as small changes in androgen-regulated genes can be detected when various tissues are stimulated by hormones.

Given the increase in PSA concentrations during states of hyperandrogenism in women, it is reasonable to speculate that PSA measurements in women may be a practicable alternative for examining exogenous androgenic steroid use in female athletes. This assay would serve as a valuable supplement for current steroid profile testing and has future potential to be incorporated in the athlete's biological passport. It is important to note that this method would be useful only if PSA concentrations stay increased for a longer duration in comparison to current markers. Nonetheless, the advantage of this method is that it is capable of detecting both old (known) and new (unknown) androgenic substances, because the principle of this method is based on the activation of the androgen receptor, irrespective of the structural nature of the steroid ligand. In spite of this benefit, there are limitations to incorporating the PSA test into the athlete's passport, such as cost-efficiency. The use of specialized immunoassays required to measure traceable amounts of PSA would not be economically practical compared to multiplexed MS assays. Another caveat of using PSA to detect steroid use is the potential for contamination from male partners. To avoid such sources of error, precautions must be taken to wash the area thoroughly before sample collection (in the case of urine sample collection). Meanwhile, the higher prevalence of PCOS among athletes in comparison to the overall female population may be another limitation in discerning female athletes who have doped *(61)*. In light of this, it can be assumed that only transient increases in PSA concentrations would provide adequate specificity to be used within the athlete's biological passport. Rather, PSA as an indicator of hyperandrogenism among elite female athletes may be more applicable for regulatory purposes, as highlighted by the recent decision made by the Court of Arbitration for Sport to suspend the International Association of Athletics Federations regulations on hyperandrogenism, based on a lack of evidence regarding the link between testosterone concentrations and enhanced athletic ability.

Over the past few decades we have witnessed a handful of studies that have illustrated the diverse clinical applicability of the PSA assay and have shown its diagnostic potential in clinical conditions apart from prostate cancer. The regulation of PSA by androgenic hormones has major implications for its use in antidoping initiatives, particularly among females, for which PSA should generally be undetectable unless there is evidence of unusual androgenic activity. Consequently, we suspect that the new generation of ultrasensitive PSA assays will aid in the implementation of the PSA test on female athletes, which will hopefully in turn lead to more fair athletic competitions.

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