The XY female in sport: the controversy continues

L. J. Hipkin MD FRCPath
Department of Chemical Pathology, University of Liverpool, Liverpool, UK

The summer and winter Olympic Games have been accompanied by much press coverage of the controversy and confusion over sex tests for sportswomen. Much of this has centred on the eligibility of subjects with androgen insensitivity to compete in women’s events. The purpose of this paper is to review the process of sex differentiation and its abnormalities, highlighting those conditions in which biologically active testosterone is secreted which might confer an advantage in women’s sporting events.

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The historical basis for the need for sex testing has been reviewed by Professor Ferguson-Smith and Dr Ferris. They pointed out that initially inspection then manual examination of the external genitalia were carried out but these were replaced by sex chromatin testing by the buccal smear method in 1968. The authors heavily criticized this method since they thought it unfairly excluded those with androgen insensitivity or gonadal dysgenesis whom they believe account for the majority of XY females in sport. One problem is that competitors who fail the sex chromatin test withdraw without a diagnosis being made so statistics about those without virilization who are unfairly excluded are difficult to obtain. It is even possible that the proportion of XY females with virilization due to biologically active androgen secretion and a consequent advantage in competitive sport is greater than assumed. There are several conditions in which this occurs and in order for the nonspecialist to understand the pathogenesis, a review of normal sexual development and differentiation is helpful.

Normal sex development

Sexual differentiation is a complex process starting in the early embryo and being completed with the development of secondary sex characteristics at puberty. The early mammalian embryo has the potential to become either male or female with an inherent trend towards the latter. However, if the chromosomal sex is male then testes develop, and the hormonal secretions from these cause male differentiation of the internal and external genitalia. These processes will now be examined in more detail (for reviews see Bercovitz et al. or Batch et al.).

Chromosomes

In the human there are 46 chromosomes consisting of 22 pairs of autosomes and two sex chromosomes these being XX in females and XY in males (Figure 1). In the female, part of one X is inactivated and stains heterochromatically (Barr body) in nuclei from buccal scrapings (Figure 2). If more than 20% of these nuclei contain Barr bodies then it can be confidently predicted that at least two X chromosomes are present and the subject is chromatin positive. Chromatin negative subjects have a single X chromosome.

**Figure 1. Normal male chromosome constitution (46,XY)**

**Figure 2. Nucleus with a Barr body (chromatin positive) in a buccal smear from a normal female**
Gonadal development

Until the fetus is approximately 46 days old the gonads of males and females are indistinguishable. Thereafter it is thought that, in the male, a locus on the Y chromosome (SRY gene) is responsible for the expression of a male-specific cell membrane component called the H-Y antigen. This is disseminated by cells in the primitive gonad inducing its differentiation into a testis.

The ovary does not develop from the undifferentiated state until 3 months. Genes on both X chromosomes and possibly on an autosome are responsible, but whether there is a mechanism similar to the H-Y antigen in the male is uncertain.

Mechanism of testosterone synthesis and androgen action

Before discussing male differentiation of the internal and external genitalia it was important to discuss testosterone synthesis and the mechanism of androgen action.

Testosterone is synthesized from cholesterol in a series of enzymatic steps as depicted in Figure 3. The most potent androgen in the majority of tissues is not in fact testosterone but another steroid called dihydrotestosterone (DHT) which is formed from testosterone within androgen dependent cells by the action of the enzyme 5α-reductase (Figure 4).

Full biological expression of testosterone or DHT action requires the presence of an intra-cytoplasmic androgen receptor. Binding of the sex steroids to the receptor initiates the chain of cellular events which leads to the synthesis of androgen-induced proteins. Both sexes have the capacity to respond to androgen but this is limited in females because of the relatively low testosterone secretion.

![Figure 3. Enzymes involved in the synthesis of testosterone and oestadiol from cholesterol (1, cholesterol desmolase; 2, 3β-hydroxysteroid dehydrogenase; 3, 17α-hydroxylase; 4, 17,20-desmolase; 5, 17β-hydroxysteroid dehydrogenase; 6, aromatase)](image-url)

**Differentiation of the internal genitalia**

There are two sets of genital ducts in the 7-week-old fetus, the Wolffian ducts and the Müllerian ducts. During the first 3 months of pregnancy, the testicular Leydig cells of a male fetus are stimulated by chorionic gonadotrophin to produce testosterone. The high local levels of testosterone on each side causes the Wolffian duct (which lacks 5α-reductase) to differentiate into the epididymis, vas deferens, seminal vesicles and ejaculatory duct. Another testicular secretion called Müllerian inhibitory substance (MIS) or hormone causes the Müllerian system to degenerate.

In the female fetus, on the other hand, lack of testosterone leads to atrophy of the Wolffian system and the absence of Müllerian inhibitory substance allows maturation of these structures into the fallopian tubes, uterus and upper part of the vagina (Figure 5).

![Figure 5. Diagram showing internal genital development. In the male the testis produces testosterone and Müllerian inhibitory substance (MIS) which favours development of the Wolffian system. In the female these hormones are absent and the Müllerian ducts develop](image-url)
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Differentiation of the external genitalia is completed by 12–14 weeks. The tissues are rich in 5α-reductase and unlike differentiation of the male internal genitalia, that of the external genitalia is stimulated by DHT. The steroid causes closure of the inner folds to form a urethra which extends along the phallus, while fusion of the outer folds forms the scrotum and ventral skin of the penis. DHT stimulates the prostate to develop as an outgrowth of the urogenital sinus.

In females the folds which bound the sinus become the labia minora and majora while a septum develops which separates the urethral and vaginal openings. The vagina has a contribution from the Mullerian ducts already referred to.

Throughout gestation the penis lengthens while in females the phallus remains rudimentary and becomes the clitoris. Another difference between the sexes is that, after 28 weeks, the testes descend into the scrotum under androgen action stimulated by both pituitary and placental gonadotrophins.

Development of the secondary sex characteristics

Completion of sexual differentiation and development occurs at puberty. Although the mechanism for the onset of puberty is poorly understood, a feature is the pulsatile secretion of gonadotrophins from the pituitary, initially at night. This stimulates the gonads of both sexes to produce sex steroids.

In males testosterone causes muscular development, deepening of the voice and inhibition of breast development. The steroid causes some scrotal rugation and growth of the penis, the latter presumably because testosterone levels are much higher in relation to DHT at puberty than during fetal life. Testosterone is also a requirement for normal spermatogenesis and it seems to play a part in male psychosocial orientation at puberty but not before when environmental factors predominate. In the male, DHT is responsible for facial and body hair distribution as well as activating sebaceous glands which can lead to acne. Male pattern baldness is a DHT mediated event.

Females going through puberty experience breast development and redistribution of fat to give the female habitus. There is uterine and particularly endometrial growth, the latter undergoing cyclical shedding with the waxing and waning oestrogen levels to give rise to the menses. Female body hair develops following adrenal androgen action.

Non-virilized XY females

There are two main subgroups of females with an XY chromosome constitution and who have no evidence of androgen activity and consequently are not virilized. They are pure XY gonadal dysgenesis and complete androgen insensitivity. In the clinical domain patients usually present because they have never menstruated. Some are discovered when siblings of affected individuals are investigated because of the familial nature of the genetic defects, and some because of the finding of gonads in the labial folds.

Pure XY gonadal dysgenesis

In the complete form of pure XY gonadal dysgenesis, as well as amenorrhea there is sexual infantilism with eunuchoidism and lack of breast development. The external and internal genitalia are completely female. The gonads do not develop and are represented by streaks which are thought to be due to failure of H-Y antigen production or action. There is, therefore, no production of sex steroids or MIS which explains the clinical findings. The plasma testosterone and oestradiol are low even for females and the gonadotrophins are high through failure of the inhibitory feedback mechanism. Because the uterus, tubes and vagina are present, cyclical oestrogen–progestogen therapy results in withdrawal bleeding from the endometrium.

Complete androgen insensitivity

Subjects with complete androgen insensitivity, also sometimes referred to as testicular feminization, have a feminine appearance (Figure 6). The external genitalia are female (Figure 7) but with a short blind ending vagina, there is excellent breast development and delicate skin devoid of acne. However, the main characteristic is the absence or extreme sparsity of the axillary and pubic hair.

Figure 6. Patient with complete androgen insensitivity, aged 19 years. From Simmer, H. H., Pion, R. J. and Dignam, W. J., Testicular Feminization, 1965. Courtesy of Charles C. Thomas, Springfield, Illinois, USA
Partial androgen insensitivity

Insensitivity to androgen encompasses a wide spectrum of abnormalities from complete testicular feminization already described to a normal male habitus but with perineal hypospadias and gynaecomastia (Reifenstein’s syndrome). At the female end of this spectrum there are variants of testicular feminization which are caused by partial or incomplete androgen insensitivity. They are similar in every way to those with the complete form of the disorder except at birth there may be some enlargement of the clitoris and slight labioscrotal fusion. At puberty there is some breast development and normal growth of pubic and axillary hair. The derivatives of the Wolffian system are hypoplastic.

The androgen receptor abnormalities are similar to those found in the complete form but some have normal binding. The reason for androgen insensitivity in the latter is obscure.

Incomplete XY gonadal dysgenesis

In the incomplete form of pure gonadal dysgenesis there may be some evidence of androgen activity in fetal life in the form of sexual ambiguity but this becomes more apparent at puberty when ditoromegaly and facial hirsutes develop. The breasts do not develop.

The internal genitalia may be of either sex but they are rudimentary or hypoplastic. The gonads are dysgenetic testes which may be in streak form with islands of Leydig cells being found at the hilus. It is supposed that there is insufficient androgen from these cells to masculinize the fetus but the levels become significant at puberty under the influence of high levels of luteinizing hormone. The plasma testosterone is thus above the normal female range which differentiates the incomplete form from the complete in which testosterone levels are low.

Mixed or asymmetric gonadal dysgenesis

Although a 45,X0/46,XY mosaic chromosome constitution (chromatin negative) is sometimes found in pure gonadal dysgenesis, this genotype is more often found in mixed gonadal dysgenesis. The clinical features and hormone profile are similar to those in incomplete gonadal dysgenesis except individuals are short and stocky, resembling Turner’s syndrome because of the 45,X0 cell line.

Gonadal development is asymmetric, classically a rudimentary or normal testis with a vas is found on one side and a streak with a fallopian tube on the other. A small uterus and poorly developed vagina are present. The internal genitalia appearances are, therefore, those anticipated from the morphology of the ipsilateral gonad.

Defects in testosterone biosynthesis

All the enzymes involved in testosterone biosynthesis may be completely or partially deficient. In deficiency of cholesterol desmolase, 3β-hydroxysteroid dehydrogenase or 17α-hydroxylase, cortisol

Virilized XY females

There are several subgroups of females with an XY chromosome constitution or genotype who may have some ambiguity of the external genitalia and who may show some signs of virilization at puberty. Clinically it may be the effects of androgen activity which prompt referral rather than the amenorrhoea. These conditions have been reviewed by Savage.

Figure 7. External genitalia of a patient with complete androgen insensitivity, aged 48 years. From Simmer, H.H., Pion, R. J. and Dignam, W. J., Testicular Feminization, 1965. Courtesy of Charles C. Thomas, Publisher, Springfield, Illinois, USA
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synthesis is also impaired while lack of the first two enzymes decreases mineralocorticoid production. These are, therefore, forms of congenital adrenal hyperplasia which, without recognition and appropriate treatment at birth, cause death from lack of corticosteroids. With treatment some have survived to adulthood and together with deficiency of the other two enzymes involved in testosterone synthesis (17,20-desmolase and 17β-hydroxysteroid dehydrogenase) present a fairly similar clinical picture.

The external genitalia may vary from completely female to ambiguous with a small hypospadiac phallus and blind vaginal pouch. At puberty secondary sex characteristics may or may not develop but if they do the combination of virilization (Figure 8) and breast development is often recorded.

The Wolffian duct derivatives are absent, rudimentary or normal but female internal genitalia are never found. The gonads are testes, devoid of spermatogenesis and with Leydig cell hyperplasia (Figure 9). They are located in the abdomen, inguinal canal or labia.

The spectrum of abnormalities depends on the degree of enzyme deficiency, those in whom it is complete tending to have a female habitus with low steroid levels, while those with partial defects undergo some virilization. The picture is further complicated because precursors proximal to the enzyme block are produced excessively under stimulation from luteinizing hormone. These can be converted to androgen and oestrogen in the peripheral tissues, thus accounting for virilization and breast development at puberty.

Diagnosis is made after puberty by finding elevated plasma steroid levels on the proximal side of the block and low levels distally, e.g. a higher level of androstenedione and oestrone compared with testosterone and oestradiol is diagnostic of 17β-hydroxysteroid dehydrogenase deficiency (Figure 3). Before puberty the steroid pathways have to be stimulated with gonadotrophin or adrenocorticotropic hormone to unmask the block.

5α-Reductase deficiency

The effects of 5α-reductase deficiency are discussed by Imperato-McGinley et al.7. At birth, affected subjects have incompletely masculinized external genitalia. There is a small hypospadiac phallus resembling a clitoris, bifid scrotal folds, the sinus opens into the perineum and there is a blind vaginal pouch. Because of this phenotype rearing is in the female role.

At puberty varying degrees of masculinization occur. The phallus enlarges to form a small penis and the testes enlarge and appear in the inguinal region or in the scrotal folds which become pigmented and rugose. The voice deepens and muscular development is prominent. Normal female body hair develops but there is very little facial hair or acne. There is no breast development. The incidence of the disorder is relatively high in the Dominican Republic and many of those brought up there as females, at puberty adopt a male gender role.

There are no female internal genital structures. The male internal genitalia, except for the prostate which is tiny, are normal and the vas opens into the vaginal pouch. Normal spermatogenesis occurs and seminal fluid can reveal a normal sperm count.

All these somatic changes are predictable from the known effects of testosterone and DHT. Biochemically after puberty the diagnosis of 5α-reductase deficiency is suggested by the finding of a high testosterone:DHT ratio. The plasma testosterone level is normal or high for a male while plasma

Figure 8. Enlarged clitoris in a 19-year-old patient with 17β-hydroxysteroid dehydrogenase deficiency

Figure 9. Section of testis from a 19-year-old patient with 17β-hydroxysteroid dehydrogenase deficiency showing Leydig cell hyperplasia and no spermatogenesis
oestradiol is normal. Prepubertally the enzyme deficiency has to be unmasked with studies pre- and post-injection with chorionic gonadotrophin.

Confirmation of 5α-reductase deficiency can be made with enzyme studies on tissue or cultured fibroblasts from genital skin. The enzyme may be deficient or abnormal.

The reason for the masculinization of the external genitalia at puberty is obscure but may reflect a combination of the high plasma testosterone and low but detectable 5α-reductase activity.

**Discussion**

The purpose of sex verification testing is to ensure that sportswomen compete on an equal basis. The problem is that genetic testing, using either the traditional sex chromatin pattern or the recently introduced detection of the male determining gene on the Y chromosome, does not separate out only those who would have an unfair advantage in competition. The purpose of this review is to explain the differences between nonvirilized and virilized XY females. The former would theoretically compete on equal terms but the latter would have an unfair advantage because the excessive endogenous testosterone secretion is biologically active. Purely on the basis of genetic tests both groups would be disqualified.

A second problem is that such disorders are extremely rare in the population yet relatively much more frequent following testing of participants at various prestigious sports meetings. Since there is little follow-up information on diagnoses in these competitors, it is impossible to decide whether the discrepancy is due to excessive numbers of virilized XY females, which is of concern to the Medical Commission of the International Olympic Committee (IOC), or whether some other gene on the Y chromosome is conferring benefit. If the latter then the eligibility of non-virilized XY females may be questioned.

In spite of criticism of genetic testing for sex determination in sport, the IOC continued to use this method in their recent winter and summer Games. Although using the modern techniques they stated they were backing up questionable results with other evidence to determine sex.

The International Amateur Athletic Federation (IAAF) on the other hand, bowed to pressure from a number of quarters and announced that the buccal smear test would be abandoned. Instead all member federations were instructed to arrange for their male and female athletes to undergo a physical examination to ascertain their state of health and gender. The female athlete would then be issued with a gender certificate if not already on the IAAF femininity list.

It follows from the account of the differences between non-virilized and virilized XY females that physical examination, particularly searching for evidence of androgen activity is preferable to genetic testing. However, the latter had to be introduced and is still preferred by sportswomen because they found inspection of the genital region demeaning and they resented manual examination. It is for this reason there is the lack of clinical data referred to; chromatin negative sportswomen preferred to withdraw from competition rather than be investigated.

Not all sports medical officers agree with the IAAF approach, although it seems no different than the pre-employment medical examination required in many occupations. The British team doctor at the Tokyo World Championships refused to carry out such examinations, expressing confidence in his ability to assess the gender of team members with their clothes on. It is also rumoured that the IAAF is reconsidering whether to impose femininity testing and this has encouraged an Australian male-to-female transsexual to train for athletic competition.

Needless to say this controversy and confusion which has received much press and television coverage must cause sportswomen to lack confidence in gender determination methods and to lose faith in the medical profession. A statement that a sportswoman had to withdraw from competition because a routine test showed she was a 'man' only for her to be reinstated 3 years later does not help.

As pointed out many times the main problem is in defining sex, and in particular assigning sex on the basis of one characteristic. Based purely on genotype it is true to say that the female chromosome sex is XX and male XY. However, it is the physical appearance or phenotype which is the main determinant of the sex of rearing. Those with 5α-reductase deficiency regard themselves as female but some change to the male gender role at puberty when the external genitalia appearances change. Similarly there is a spectrum of androgen insensitivity states from testicular feminization through to Reifenstein's syndrome and sex of rearing is selected according to whether the genitalia are masculine or feminine. It is in this respect that throughout this review, the subjects have been referred to as XY females.

There is another aspect and that is psychosocial sex. Transsexuals have the genotype and phenotype of one sex but their sexual orientation is that of the opposite sex. Because of this difference between psyche and soma, transsexuals seek gender reassignment surgery, again showing the importance attached to phenotypic sex.

In clinical practice, XY females are never told they are really men but that they have abnormal gonads which need to be removed because of the risk of tumour development. Testosterone can also be produced excessively in XX females with such conditions as congenital adrenal hyperplasia and the far more common polycystic ovarian syndrome. These can also lead to virilization, so in some ways inspection of the genitalia is to assess androgen status rather than to determine sex. Whether sportswomen would accept this different interpretation for the need for genital inspection is open to speculation. It is of some relevance that there have been no adverse comments about the need for doping control independent sampling officers to view the source of urine during specimen collection.

If genetic testing unfairly disqualifies some female competitors and if women object to phenotypic sex...
being ascertained by genital inspection or examination, is there any other approach? The International Ski Federation (FIS) carries out serum testosterone estimations as part of their gender verification protocol (Dr Michael Turner, personal communication). This has two advantages; unlike tests of genotype it does not exclude those with complete gonadal dysgenesis, and it is a direct measure of a steroid that could be giving a female competitor an advantage. It has the disadvantage that suppressive medication could be employed to lower plasma testosterone levels. However, random drug control sampling would reveal this or, alternatively, long-term suppression of androgens would cause loss of their virilizing benefit. However, as with genetic tests, assay of plasma testosterone would only be a screen for those requiring physical examination.

Whether sportswomen will develop a different attitude to ‘femininity’ testing, with better education and with the knowledge there is a general desire to prove eligibility rather than to find grounds for disqualification, remains to be seen.

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References