Androgens During Infancy, Childhood, and Adolescence: Physiology and Use in Clinical Practice

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ABSTRACT

We provide an in-depth review of the role of androgens in male maturation and development, from the fetal stage through adolescence into emerging adulthood, and discuss the treatment of disorders of androgen production throughout these time periods. Testosterone, the primary androgen produced by males, has both anabolic and androgenic effects. Androgen exposure induces virilization and anabolic body composition changes during fetal development, influences growth and virilization during infancy, and stimulates development of secondary sexual characteristics, growth acceleration, bone mass accrual, and alterations of body composition during puberty.

Disorders of androgen production may be subdivided into hypo- or hypergonadotropic hypogonadism. Hypogonadotropic hypogonadism may be either congenital or acquired (resulting from cranial radiation, trauma, or less common causes). Hypergonadotropic hypogonadism occurs in males with Klinefelter syndrome and may occur in response to pelvic radiation, certain chemotherapeutic agents, and less common causes. These disorders all require testosterone replacement therapy during pubertal maturation and many require lifelong replacement.

Androgen (or gonadotropin) therapy is clearly beneficial in those with persistent hypogonadism and self-limited delayed puberty and is now widely used in transgender male adolescents. With more widespread use and newer formulations approved for adults, data from long-term randomized placebo-controlled trials are needed to enable pediatricians to identify the optimal age of initiation, route of administration, and dosing frequency to address the unique needs of their patients.

Graphical Abstract
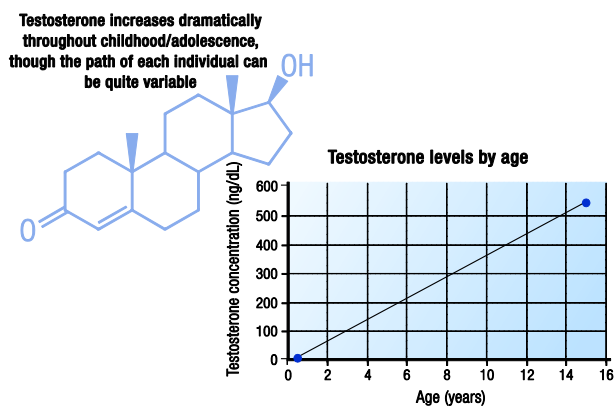
ESSENTIAL POINTS

1. Androgens play a central role throughout the male lifespan.

2. Androgens induce virilization of the male fetus, influence body composition and fetal growth, and contribute to organizational changes within the developing nervous system.

3. Androgen therapy is frequently used for micropenis and hypospadias during infancy.

4. Apart from the treatment of micropenis and hypospadias, androgen therapy has limited applications during childhood, but has been studied in children with Klinefelter syndrome.

5. At the time of puberty, the androgenic and anabolic effects of testosterone result in the development of secondary sexual characteristics along with increased growth, muscle mass, and bone mass as well as a reduction in fat mass.

6. Common disorders treated with testosterone replacement include constitutional delay of growth and puberty, Kallmann syndrome and other forms of congenital hypogonadotropic hypogonadism, and primary hypogonadism either from Klinefelter syndrome or secondary to cancer treatment.

7. While there are a number of testosterone formulations available, intramuscular testosterone esters remain the most widely used for the induction of puberty.

Androgens have been a source of study since Aristotle reported on the effects of castration. Based on these observations, ingestion of animal testes emerged as one of the

first therapies for the symptoms of testosterone deficiency, dating back to the Roman Empire (1). Despite evidence supporting the *systemic* effects of a substance within the testes, remedies such as testicular extracts and testicular transplantation became widely popular. Arnold Berthold was one of the first to recognize that the testes exerted effects "through their action on the blood, and then through the suitable ensuing action of the blood on the organism as a whole" in his 1849 report on an experiment in which he transplanted ectopic testes into roosters who had been castrated, comparing their behavior with that of roosters that had not been transplanted (1).

In 1931, Adolf Butenandt isolated the first androgen, androsterone, from the urine of young policemen using a bioassay for the size of the cock's comb (2, 3). Four years later, Ernest Lacqueur isolated a more potent androgen from bull testes, which he named "testosterone" and had the insight to realize that it must be produced in the testis and then metabolized (oxidized) by the time it appeared in the urine (4). In 1935, Butenandt and Hanish (5) and Ruzicka and Wettstein (6) published their methods for the chemical synthesis of testosterone and the pharmacological era for this product began. Following its discovery and synthesis, testosterone became available for clinical use, initially as pellets and later as esters (1).

Androgens play a central role throughout the male lifespan. We intend to review the role of androgens during fetal, neonatal (mini puberty), childhood, adolescent, and emerging adult stages, leading to full virilization and reproductive capacity. In each life segment we shall consider the appropriate physiology, including neurobehavioral effects, and specific disorders and appropriate therapies, with emphasis on the adolescent/emerging adult with delayed puberty. To perform a comprehensive review, we

searched PubMed using the terms (((*hormone replacement therapy*[MeSH] OR

therapy[TI]) AND (*testosterone*[MeSH] OR *testosterone*[TI] OR *"Androgens"*

[Pharmacological Action] OR *androgen*[ti])) NOT *("Estrogens"[Pharmacological*

Action] OR *"Estrogens"*[MeSH])) with the filters *English, Humans,* and *All Child* and

using the terms (*testosterone*[Majr] OR *testosterone*[TI] OR *"Androgens"*[Majr] OR

androgen[TI]) AND (*physiol**[TI] OR *physiology*[sh:noexp] OR "*growth and*

development"[sh] OR *physiology*[Majr] OR "*Sexual Maturation*"[MeSH] OR "*sexual*

maturation"[TI] OR "*pubertal induction*"[TI] OR *puberty*[MeSH]) with the filters

English, Humans, All Child, and *Male.* The initial search yielded 1480 references. Those

that were redundant or not relevant were eliminated and additional material was

identified upon searching the reference lists of relevant reviews and original articles.

Fetal

Androgens play a critical role in male development and are particularly important *in*

utero. Masculinization of the fetus is dependent on androgen action within a narrow

window of development, termed the masculinizing programming window (MPW) (7).

Interestingly, this critical window does not appear to coincide with the period of male

reproductive tract differentiation, but rather, the appropriate development of male

reproductive tissues is dependent on androgen exposure that occurs earlier in gestation as

summarized by Welsh and colleagues (8).

Although the fetal testes and adrenal glands are commonly thought of as the

primary sources for androgens *in utero*, there are some conditions in which maternal

androgens may also contribute. The changes in testosterone, androstenedione, and

dehydroepiandrosterone sulfate (DHEA-S) during pregnancy [AU: Please check the

accuracy of the added expansion for "DHEA-S" in the sentence beginning "The changes in testosterone, androstenedione, and dehydroepiandrosterone sulfate (DHEA-S) during pregnancy…".] are typically countered by compensatory mechanisms, preventing inappropriate fetal virilization. As reviewed by Hakim et al, these mechanisms include (1) a rise in sex hormone–binding globulin (SHBG) and [AU: Please check the accuracy of the added expansion for "SHBG" in the sentence beginning "As reviewed by Hakim et al, these mechanisms include (1) a rise in sex hormone–binding globulin (SHBG) and…".] (2) placental aromatase cytochrome P450 (9), which converts testosterone and androstenedione derived from fetal and maternal DHEA-S into estradiol that is then converted by the fetal liver into estriol to be excreted in maternal urine (10). The critical role of placental aromatase in protecting the fetus from hyperandrogenism is evident in cases of placental aromatase deficiency, in which the female fetus and mother become virilized in response to excess levels of androstenedione and testosterone (10). Virilization of the female fetus may also occur when the capacity of placental aromatase is exceeded by extremely high levels of androgens, as seen in ovarian tumors such as luteomas (11).

Throughout gestation, fetal Leydig cells undergo differentiation, maturation, and involution, as reviewed by Svechnikov et al (12). Prior to 14 weeks gestation, testosterone production is under the control of placental human chorionic gonadotropin (hCG), which, like the pituitary gonadotropin luteinizing hormone (LH), stimulates testosterone production via the LH/hCG receptor on Leydig cells beginning around 8 weeks gestation (13–15). Gonadotropin-releasing hormone (GnRH) neurons migrate from their origin in the olfactory placode to the hypothalamus early in gestation and after

a decline in placental hCG at 12 to 14 weeks, testosterone production becomes dependent on the hypothalamic-pituitary-gonadal (HPG) axis (14, 15). This may represent a biological turning point in that hCG and LH may not have the exact same biological activities at the LH/hCG receptor. *In vitro* differences have been found in potency, efficacy, and kinetics (16). It is likely that differences in binding conformation (physical changes) underlie the altered responses, since a mutated receptor (deletion of exon 10) impairs LH-induced cyclic adenosine monophosphate (cAMP) production [AU: Please check the accuracy of the added expansion for "cAMP" in the sentence beginning "It is likely that differences in binding conformation (physical changes) underlie the altered responses, since a mutated receptor (deletion of exon 10) impairs LH-induced cyclic adenosine monophosphate (cAMP) production…".] compared with that produced by hCG (17). This alteration may have biological consequences to the developing male fetus as the hCG stimulation to the testis wanes and that of LH increases.

GnRH is produced not only by the hypothalamus, but also by the placenta, which produces 1 of 2 forms of GnRH (GnRH-I and –II). Pulsatile GnRH, both physiologic and induced, regulates β-hCG biosynthesis (18). While it may influence fetal testosterone production, the primary function of pulsatile placental GnRH is likely to promote invasion of the trophoblast for the nutritive spiral arteries (19).

Shortly after testosterone production becomes dependent on LH secretion, serum and intratesticular testosterone (ITT) levels reach a peak, approximating 40 to 580 ng/dL or 1.39 to 20.13 nmol/L (11–17 weeks gestation) (20) and 1.9 to 2.7 ng/mg of tissue (11–14 weeks gestation) (21) respectively. Sertoli cells lack expression of the androgen receptor (AR) during fetal development (22). As complete spermatogenesis does not

occur in Sertoli-cell specific AR knockout rodent models, the lack of AR expression during fetal development may serve to prevent spermatogenesis in the setting of high ITT levels (23, 24). After reaching this peak, testosterone concentrations decrease from approximately 20 weeks to term (25), presumably in response to placental-derived estradiol and progesterone and inhibin B (INHB), which act on sex steroid receptors to suppress the GnRH pulse generator (14, 25–27). Within a few minutes of birth, there is a surge in LH. This is paralleled by an increase in serum testosterone over the first 3 hours. Testosterone concentrations remain elevated for 3 to 12 hours after birth (28).

Fetal testosterone may be produced through the Δ-4 pathway via synthesis of progesterone, 17-hydroxprogesterone, and androstenedione, or the Δ-5 pathway through conversion of pregnenolone to 17-hydroxy pregnenolone and DHEA, a route favored over the Δ-4 pathway due to the lower K_m for 17-hydroxylation than for reactions catalyzed by 3β-hydroxysteroid dehydrogenase (3βHSD) [AU: Please check the accuracy of the added expansion for "3βHSD" in the sentence ending "…a route favored over the Δ-4 pathway due to the lower K_m for 17-hydroxylation than for reactions catalyzed by 3β-hydroxysteroid dehydrogenase (3βHSD).".] (29, 30). Recent data, however, suggest differentiation of Leydig cell testosterone production with low levels produced via the Δ-4 pathway early in the first trimester and high levels of androgens produced via the Δ-5 pathway late in the first trimester (31).

Within target tissues, the enzyme 5α-reductase catalyzes the conversion of testosterone to the more potent dihydrotestosterone (DHT) through a process termed the *classic* or *frontdoor* pathway. DHT may also be produced through the *alternate* pathway, also known as the *backdoor* pathway, bypassing testosterone production altogether (Fig.

1). This was first fully described in the testes of the tammar wallaby pouch young and later in immature mice testes (29). Androsterone is the alternate pathway intermediate present in the highest concentration within human fetal serum. Androsterone and other alternate pathway intermediates are present in very low concentrations in the fetal testes and are found primarily in the fetal adrenal, liver, and placenta. Results from human fetal studies suggest that placental progesterone is an important source for the synthesis of these intermediates (7). In 46,XY infants, mutations in enzymes that participate in the alternate pathway may lead to disorders (differences) of sexual development (DSD), providing support for a role of the alternate pathway in virilization of the male fetus (32). This is clearly not the only means of male virilization, however, as evidenced by the undervirilization of XY infants with defects in 5α-reductase.

Both DHT and testosterone promote virilization through activity on the AR. The lower potency of testosterone compared with DHT is likely related to its weaker interaction with the AR, which may be overcome by very high concentrations (33). Studies of the uptake and metabolism of testosterone and intracellular assessments of testosterone and DHT in rabbit embryos highlight the differential effects of these androgens on the development of the male urogenital tract (34). Through its interaction with the AR, testosterone facilitates development of the Wolffian ducts between 9 and 13 weeks gestation as reviewed by Hannema et al (35), including the epididymis, vas deferens, seminal vesicles (36), and ejaculatory ducts, while DHT promotes development of the male external genitalia (25, 33). DHT in particular promotes formation of the prostate, stimulates labioscrotal fusion to form the scrotum, facilitates penile formation through elongation of the genital tubercle and fusion of the urethral folds, and promotes

penile growth after the first trimester as reviewed by Wilhelm et al and Cimador et al (15, 37). Androgens also facilitate the inguinoscrotal phase of testicular descent via regression of the cranial suspensory ligament between 27 and 35 weeks gestation (13, 14).

Any disruption within this intricate system may result in undervirilization. While disturbances early in gestation, presumably during the critical MPW, lead to abnormal formation of the external genitalia, those that occur later, once the penis is fully formed, lead to micropenis, a small, but normally-formed penis (8). By term, the stretched penile length measures 3.5 cm (38) (nonerect length 3.49 ± 0.4 cm) (39) and 1.1 ± 0.2 cm in diameter (38). A penile length of < 2.5 cm in a term infant is considered abnormal (26). Micropenis may result from a variety of disorders, including growth hormone deficiency, LH deficiency, or defects of the LH receptor, or may occur in various DSDs due to an inability to convert androstenedione to testosterone via the 17-β hydroxysteroid dehydrogenase type 3 enzyme, defects in the conversion of testosterone to DHT through 5-α reductase, or insufficient signaling through the AR (androgen insensitivity syndrome) (15).

Abnormal formation of the external genitalia (hypospadias with or without micropenis, clitoromegaly) may result from defects in testosterone production and/or action early in gestation, during the presumed MPW (8). Androgen insensitivity syndrome (AIS) is the most common cause of 46,XY DSD. In its complete form (CAIS), affected infants appear female with a short, blind-ending vagina and undescended testes. The appearance of the external genitalia can be quite variable in infants with partial AIS, ranging from isolated clitoromegaly to perineoscrotal hypospadias (40). The external genitalia of individuals with 5α-reductase deficiency is also broad, ranging from

hypospadias to female external genitalia with virilization at puberty, attributed to direct effects of testosterone and/or increased activity of the 5α-reductase type 1 isoenzyme (41).

Mutations in 17β-hydroxysteroid dehydrogenase type 3 prevent the conversion of androstenedione to testosterone within the testes. Affected infants have female external genitalia with normal to hypoplastic internal genitalia. As with 5α-reductase deficiency, significant virilization occurs during puberty due to the conversion of androstenedione to testosterone in peripheral tissues (42).

Evaluation of infants with such DSDs typically involves assessment of the anogenital distance (AGD), the distance between the junction of the scrotum and perineal skin to the midpoint of the anus (43). This sexually dimorphic measurement is 2 to 2.5 times greater in males than females (44). As summarized by Dean and Sharpe, the difference in AGD between males and females is first apparent from 11 to 13 weeks gestation, near the end of what many presume to be the MPW (45), with maximal differentiation apparent by 17 to 20 weeks (46). There are many lines of evidence to support the use of AGD as a reflection of intrauterine androgen exposure, such as the lack of significant difference between male rodents with CAIS and female rodents (36), increased, or more masculine AGD in girls with congenital adrenal hyperplasia (CAH) (45), reduced AGD in boys with hypospadias (47), and association with penile size at birth (44). Furthermore, when exposed to androgens during their presumed MPW, the AGD of female rhesus monkeys becomes masculinized (48). This suggests that androgen exposure during a critical period of gestation determines the AGD. In addition, fetal androgen exposure during the MPW likely contributes to later adult phenotypes, as

evidenced by the correlation between AGD and testosterone levels, penile size, and sperm count in adult men (49, 50). In fact, it is hypothesized that deficiencies in androgen production and/or action *in utero* may predispose individuals to testicular dysgenesis syndrome, characterized by cryptorchidism, hypospadias, testicular germ cell cancer and/or low sperm counts/testosterone concentrations, as reviewed by Scott et al and MacLeod et al (51, 52).

As with AGD, the ratio between the length of the second and fourth digit, the so-called 2D:4D, is also sexually dimorphic, potentially influenced by androgen-induced chrondrocyte proliferation during a narrow window in the first trimester (53). In males, the second digit is typically shorter than the fourth. In contrast, digit lengths are similar in females, who have a 2D:4D that is closer to 1. This sexual dimorphism has been attributed to intrauterine androgen exposure based on observations in individuals with CAH who have a more masculine 2D:4D and Klinefelter syndrome (54) and CAIS who have a more feminine 2D:4D (55). However, it should be noted that while the ratios between individuals with CAIS and male controls are different, there is significant between-group overlap (55); furthermore, while some studies report a lower 2D:4D in females with CAH, one of the largest studies demonstrated no significant difference between those with CAH and age-matched controls (56).

Many studies cite associations between 2D:4D and various conditions, implying effects from intrauterine androgen exposure. While some authors report high temporal stability of the 2D:4D (57), others suggest that ratios may vary over time, at least in some populations (58, 59), implying influences from postnatal factors. Moreover, the 2D:4D is relatively unchanged throughout fetal development despite rising testosterone levels that

peak between 11 and 17 weeks gestation (20) during the presumed MPW, raising doubt

that fetal androgen exposure is the sole predictor of 2D:4D (60). Finally, the 2D:4D is not

associated with other measures of prenatal androgen exposure, such as AGD (61); thus

caution should be employed when interpreting associations using 2D:4D as a marker of

intrauterine androgen exposure.

In addition to masculinization of the fetus, androgens may also influence other

aspects of fetal development. For example, males are larger at birth and have a greater

fat-free mass at least from the second trimester through the first 6 years of life (62). Thus,

males and females display different strategies for the relative allocation of energy input to

the somatic tissues and energy stores (63). Furthermore, birth weights of infants with AIS

are similar to those of females, supporting the role of androgens in intrauterine growth

(64).

Prenatal androgens may also affect subsequent behavior and development through

organizational changes, defined as permanent changes that occur during a critical window

of development. Such effects are in contrast to activational changes, or those that occur

later in life (65). There are several lines of evidence that support the role of androgens in

organizational changes during fetal development. As members of the nuclear

transcription factor superfamily, sex steroids possess the ability to recruit enzymes that

facilitate acetylation and methylation, thereby inducing epigenetic changes on the

developing nervous system (66). In addition, androgens may influence neural cell size

and survival, dendritic growth, and synapse formation (67). Animal models provide

further evidence for such functions. A landmark study by Phoenix et al in 1959

demonstrated that prenatal androgen exposure in guinea pigs influenced behavior into

adulthood (68). From these observations, the "organizational hypothesis" was born. As reviewed by Thornton et al (69), several subsequent studies in rhesus monkeys provide additional insight into the role of prenatal androgens in the development of permanent neurobehavioral changes. For example, female rhesus monkeys exposed to testosterone *in utero* develop masculinization of sexually dimorphic social behaviors.

Such organizational changes could involve effects on brain morphology. Amniotic testosterone concentrations have been associated with rightward asymmetry of the isthmus of the corpus callosum, a finding that may have implications for language processing, visuospatial cognition, and empathy (70). Prenatal testosterone concentrations have also been associated with sexually dimorphic gray matter regions, including those involved in social attention, empathy, and language. Taken together, these data suggest that intrauterine testosterone contributes to neuroanatomical sexual dimorphisms and may play a role in neurodevelopmental conditions that more commonly affect males, such as autism spectrum disorder (ASD) (71).

ASD is strongly biased toward males, with a prevalence 2- to 5-times higher in males than females (45, 72). Several biological theories exist, with a leading one showing evidence for an extreme expression of the psychological and physical attributes of the male brain from fetal testosterone levels and action. As mentioned above, the 2D:4D may be a marker of fetal testosterone exposure and is lower, or more masculine in children with ASD compared with controls (73). Fetal testosterone is also associated with ASD-like traits, and females virilized by CAH have more ASD-like traits than their unaffected sisters (74). Numerous animal studies note early exposure to fetal testosterone produces sexual differentiation in behavior, cognition, and brain structure (75). In humans, the first

testosterone surge occurs during gestational weeks 8 to 24, playing an organizational role in brain development (76). These changes are likely produced by the effects of fetal testosterone to avert cell death, influence neuronal connectivity, and alter neuroendocrine profiles (76) and may contribute, in some individuals, to the development of traits characteristic of ASD.

In addition to associations with ASD itself, fetal androgens have also been associated with altered language development, a characteristic feature of ASD. For example, males with higher umbilical cord testosterone levels have a smaller vocabulary during childhood (77) and are at increased risk for a clinically significant language delay in the first 3 years of life (78). Intrauterine androgen exposure has also been associated with degree of eye contact, restricted interest, and ability to empathize later in life (70).

Prenatal androgen exposure is unlikely to completely explain ASD and other neurodevelopmental disorders, such as attention deficit hyperactivity disorder (ADHD) and intellectual disability. A recent registry study based on the total Swedish population was performed to investigate whether conditions associated with low sex hormone levels, such as hypogonadotropic hypogonadism and delayed puberty, might also be associated with an increased risk for these conditions. In a matched cohort study linking several longitudinal, population-based registries in Sweden (79) in which 10 controls were matched to each "case", investigators sought to determine the prevalence of these neurodevelopmental conditions. The databases contained 264 individuals with hypogonadotropic hypogonadism (~1/3 girls) and 7447 with delayed puberty (~1/4 girls). Odds ratios for those with hypogonadotropic hypogonadism were 5.7 (confidence interval [CI], 2.6–12.6) for ASD, 3.0 (CI, 1.8–5.1) for ADHD, and 18.0 (CI, 8.9–36.3) for

intellectual disability compared with controls. For delayed puberty, the comparable odds ratios were 1.5 (CI, 1.3–1.7), 1.7 (CI, 1.6–1.9), and 3.0 (CI, 2.6–3.4). Thus, these neurodevelopmental conditions were most prevalent in those with hypogonadotropic hypogonadism, followed by delayed puberty, but both above the baseline for the population. These data call into question whether sex hormones play a definitive role in the development of ASD and contradict the notion that *only* elevated levels of prenatal sex hormones are associated with such neurodevelopmental conditions (79).

Intrauterine androgen exposure has also been implicated in gender role and play behavior. Play behavior is described as more feminine in children with disorders of androgen production, such as certain types of CAH or cholesterol side-chain cleavage deficiency, while those with hyperandrogenism due to CAH tend to display more male or bisexual play (80). Amniotic testosterone levels are positively correlated to male-typical scores on questionnaires of sex-typical play during childhood (81). Moreover, girls with CAH are described as less "tender-minded" with greater physical aggression compared with female controls (82) and have greater interest in occupations related to "things" over "people" compared with unaffected siblings (83). This lack of interest in gender-stereotyped activities and careers persists through adolescence and adulthood (84). Although prenatal androgen exposure likely contributes to toy, playmate, and activity preferences in females with CAH (67), influences intrinsic to the disease itself cannot be excluded (85). *In utero* androgen exposure may also influence sexual orientation. Several studies have reported reduced rates of exclusively heterosexual relationships in women with CAH (86–89). This may be one among many factors (reduced proportion of women attempting to conceive, virilization, impaired sexual functioning, impact of elevated

progesterone on endometrial tissue, and influence of hyperandrogenemia on the HPG axis) contributing to the reduced rate of fertility in this population, cited as 0% to 10% in women treated with corticosteroid therapy alone (90).

The influence of prenatal androgens on gender identity remains uncertain. Indeed, the frequency of gender reassignment in individuals with 5α-reductase deficiency who received a female gender assignment at birth is as high as 70% to 80% (91, 92). While prenatal androgen exposure may have contributed to the male gender identity of these individuals, the fact that a large portion of these transitions occurred during adolescence suggests effects from postnatal androgens as well (93). Of the 2 previously described cases of 46,XY infants who experienced penile ablation, underwent gonadectomy, and female gender reassignment, 1 underwent gender reassignment later in life while the other maintained her female gender identity (93). Thus, while prenatal androgen exposure may impact gender role and behavior, there are likely additional factors, such as postnatal androgen exposure and psychosocial influences that contribute to one's gender identity (93, 94).

Interestingly, the practice of assigning those with 46,XY DSD a female sex has been declining, at least in some regions. As in the past, sex assignment continues to be driven, in part, by the degree of masculinization of the external genitalia. However, additional factors may now have a greater contribution to sex assignment at birth. Such factors may include heavier reliance on karyotype, reports of adequate surgical outcomes in men with 46,XY DSD, a shift in social attitudes and/or perspectives, and greater attention to individuals with 46,XY DSD assigned the female sex at birth who undergo gender reassignment later in life (95).

Infancy

The GnRH pulse generator is reactivated by 6 to 10 days after birth (96, 97). This period, termed the mini puberty of infancy, was first described in the 1970s (26). During mini puberty, LH levels approximate pubertal concentrations, reaching a peak between 16 and 20 days of life (26, 96, 97). Serum testosterone levels rise in response to rising concentrations of LH, paralleling an increase in Leydig cell number (12) and testicular testosterone concentrations. Serum testosterone levels peak from 1 to 3 months (210 ± 130 ng/dL or 7.28 ± 4.51 nmol/L on day of life 30) (98) and decline by roughly 50% per month (99) reaching prepubertal levels by 7 to 12 months of age (100). DHT concentrations parallel the rise in testosterone, reaching pubertal values during the early postnatal period (101). This dramatic rise is due in large part to testicular DHT production via the alternate pathway, as determined by urine steroid profiling (102).

Other less potent androgens, such as dehydroepiandrosterone and androstenedione, are also secreted during the postnatal period as the fetal zone of the adrenal cortex involutes (13). Although these androgens are relatively weak, they may be converted to more potent androgens in peripheral tissues, such as skin, resulting in sebaceous gland hypertrophy and acne (13).

The mini puberty of infancy serves several important roles, including effects on linear growth. Postnatal height velocity is positively correlated with testosterone concentrations and is higher in boys than girls over the first 6 months of life with the greatest difference of 4.1 cm/year seen at 1 month of age during the peak of mini puberty (103). Furthermore, there is a deceleration of linear growth from 3 to 6 months of life in

infants with congenital hypogonadotropic hypogonadism (CHH) who fail to undergo mini puberty (104).

As with fetal testosterone exposure during a critical period of gestation, testosterone exposure during mini puberty may have impacts that do not become apparent until much later in life. As reviewed by Waxman and Holloway (105), neonatal testosterone is thought to imprint the hypothalamus the sexually dimorphic pattern of growth hormone (GH) pulsatility that becomes evident at puberty (106). In rodents, the relatively low frequency of GH pulses described in adult males induces a sexually dimorphic set of hepatic gene products, whereas the near continuous GH secretion found in the female adult induces a different set (107). This sexually dimorphic gene regulation is likely mediated by GH-dependent activation of STAT5 and includes induction or suppression of genes involved in steroid metabolism, such as the steroid hydroxylase P450 enzymes Cyp2c11 and Cyp2c12 (108). The full extent of this phenomenon and implications for humans remains uncertain.

Postnatal androgen exposure is also important for penile enlargement. Penile growth is positively correlated to testosterone levels with the greatest growth of 1 mm per month occurring between 0 and 3 months of age (39). Absence of the postnatal gonadotropin surge contributes to diminished penile growth (micropenis), wherein the stretched length from the pubis to the tip of the glans is > 2.5 standard deviations below the mean for age (26). Micropenis in infancy may be treated with gonadotropins, DHT, or testosterone. As reviewed in detail later, several formulations of testosterone are available and may be administered through parenteral, rectal (109), oral (110), or transdermal routes. Topical testosterone administration induces a significant rise in serum

concentrations of both testosterone and DHT, highlighting its systemic activity (111).

Androgen therapy for micropenis appears effective in increasing penile size with minimal permanent adverse effects. However, studies thus far are limited to case series with relatively small sample sizes as noted in Table 1 (112–123) and further study is warranted.

In addition to its role in penile growth, the mini puberty of infancy also serves to increase testicular volume through lengthening of the seminiferous tubules and an increase in Sertoli and germ cell numbers (26, 102).

Postnatal HPG reactivation may be blunted or absent in several conditions. Infants with CHH, for example, lack adequate GnRH pulse amplitude during mini puberty and may not have appropriate expansion of Leydig and Sertoli cell populations. Thus, conditions such as micropenis and cryptorchidism are not uncommon. In fact, cryptorchidism occurs in nearly a quarter of infants with CHH compared with less than 1% of the general population (124).

Hypogonadotropic hypogonadism may occur in the setting of multiple pituitary hormone deficiencies or in isolation. CHH is a widely heterogeneous disorder and may be associated with features such as cleft lip and/or palate, bimanual synkinesis, renal agenesis, ear anomalies, congenital hearing impairment, dental agenesis, and skeletal anomalies (125). At least 25 genes have been implicated in isolated CHH. When associated with anosmia or hyposmia, CHH is termed Kallmann syndrome (126), resulting from defective migration of GnRH neurons from the olfactory placode (14).

Although androgens have historically been used to induce virilization in infants with CHH, alternatives such as GnRH and gonadotropin therapy have been promising,

particularly given their stimulatory effect on gonadal development (14, 124–126). When administered subcutaneously via continuous pump or injections, recombinant LH and follicle stimulating hormone (FSH) not only increase testosterone concentrations and stimulate penile growth, but also normalize levels of INHB, anti-Müllerian hormone (AMH), and LH [AU: Please check the added expansion for the acronym "AMH" in the sentence that begins "When administered subcutaneously via continuous pump or injections, recombinant LH and follicle stimulating hormone (FSH) not only increase testosterone concentrations and stimulate penile growth, but also normalize levels of INHB, anti-Müllerian hormone (AMH), and LH…",] with supraphysiologic responses in concentrations of FSH (126–128). Gonadotropin therapy enhances testicular descent and increases testicular volume (126–128), presumably due to increased Sertoli cell mass. This suggests that early gonadotropin therapy may improve the response to spermatogenesis-inducing therapy in adulthood, although additional study is warranted (14).

As in those with hypogonadotropic hypogonadism, postnatal HPG reactivation may be blunted in infants with AIS. Although levels of LH and testosterone rise dramatically over the first 3 months of life in infants with partial androgen insensitivity syndrome (PAIS), the postnatal rise is largely absent and the LH response to GnRH poor in those with CAIS. This discrepancy suggests that postnatal HPG reactivation requires exposure of the gonadotropic axis to intrauterine androgens (40).

Although prematurity does not affect the onset of mini puberty, peak serum concentrations of LH and testosterone are higher (2.4-fold and 1.8-fold, respectively) and testicular and penile growth more rapid in preterm infants compared with those born at

term (129). Postnatal testicular activation lasts longer in boys born small for gestational age compared with those born at appropriate weight for gestational age (100). In contrast, serum testosterone concentrations are lower in infants conceived by intracytoplasmic sperm injection. Interestingly, levels are normal in those conceived via *in vitro* fertilization due to maternal subfertility, suggesting paternal heritability of Leydig cell dysfunction (130).

Data on postnatal testosterone concentrations in infants with Klinefelter syndrome (KS) are variable. KS is a disorder that occurs as a result of meiotic nondisjunction, resulting in excess X chromosome material, most often 47,XXY (131). It was first described in 1942 by Harry Klinefelter who reported a series of 9 boys with gynecomastia, small testes, azoospermia, and increased urinary FSH levels (132). Classic features include hypergonadotropic hypogonadism, tall stature, behavioral disorders, motor dysfunction, and mild to moderate deficits in language-based skills, attention and auditory processing (131). KS and other syndromes associated with supernumerary X chromosomes are associated with a demise of spermatogonia and hyalinization of seminiferous tubules at the onset of puberty (43). While some boys with KS are identified prenatally, the prevalence of postnatal diagnoses is much higher, up to 40/100 000 in some reports (133). Thus, the condition remains underdiagnosed, with approximately 25% to < 50% determined during the lifespan (133, 134). This has great implications for treatment with testosterone, care for comorbidities and even mortality (135, 136). The diagnosis may be delayed in adult men because the hypergonadotropic hypogonadism, testosterone deficiency, marked decrease in testicular size, and gynecomastia may go unnoticed (see "Delayed Puberty").

In contrast, the diagnostic journey now may be quite different given that prenatal screening may identify this condition in the asymptomatic child. Many boys have been diagnosed with KS by prenatal screening, given that it is strongly recommended, particularly in those of older maternal age (137). After birth, the phenotypic spectrum is broad and does not appear to be endocrine in nature. Rather, it is more neurodevelopmental, cognitive, and bio-behavioral, and it especially affects language development and speech (138–141). There are apparent differences within national cohorts as well as between pre- and postnatally-defined cohorts, with the former often having a less prominent phenotype (142).

Testosterone levels in infants with KS appear to be normally sensitive to LH (143), reaching a peak in the first few months of life. While small studies report higher postnatal testosterone concentrations compared with controls (144), several other studies have demonstrated lower levels (145, 146) in those with KS. This has generated interest in the effects of early testosterone administration, and nonrandomized studies have cited associations between testosterone treatment during infancy and subsequent neurodevelopmental outcomes and social skills (147, 148).

Early androgen exposure may also influence subsequent behavior and development in those without KS. For instance, testosterone concentrations in infancy have been associated with later sex-typed behaviors (149–151) and language development in the general population (152, 153). However, there is no current evidence for causality and more rigorous study is required.

Childhood

The mini puberty of infancy is followed by the juvenile pause, a quiescent period characterized by small, irregular, widely-spaced GnRH pulses and low gonadotropin levels (154). Before reactivation of the HPG axis occurs, a critical fat mass must be attained (155). Leptin, a cytokine produced by white adipose tissue, acts as a "metabolic barometer," signaling the central nervous system that pubertal maturation may ensue (155). Studies of individuals with disorders in leptin production or signaling highlight its critical role in pubertal initiation. Such disorders are characterized by delayed puberty and infertility, conditions that are reversed by leptin replacement therapy (155, 156). Throughout childhood, there is a gradual rise in leptin concentrations, coinciding with a reduction of the soluble leptin receptor (155). In late childhood, integrated inputs lead to a reactivation of the GnRH pulse generator as sensitivity to sex steroids decreases (157). This results in increased GnRH pulsatility, primarily at night.

Well before the clinical manifestations of puberty become apparent, concentrations of FSH, LH, and testosterone follow a circadian rhythm (158). Within 1 year of the onset of puberty, Leydig cells become responsive to LH (159) with an average of 2.2 LH and 2.1 testosterone pulses per night in prepubertal children (160). Thus, the rise in testosterone concentrations overnight (159) may be used to assess proximity to pubertal onset. In fact, in a study of prepubertal boys, 100% of those with morning testosterone levels ≥ 20.2 ng/dL (0.7 nmol/L) underwent puberty within 15 months, compared with only a quarter of those with levels < 20.2 ng/dL (0.7 nmol/L) (161).

Although earlier studies demonstrated minimal testosterone response to GnRH administration in prepubertal children (162), Leydig cell responsiveness to LH likely

depends on proximity to pubertal onset. Similarly, the response to hCG may provide insight into HPG function during childhood. Although infants may respond to hCG administration, the magnitude of rise in testosterone becomes greater with age (163) and may be used in the evaluation of children with gonadal dysgenesis or AIS (164) or to predict the ultimate requirement for testosterone replacement therapy in those with micropenis (165).

As puberty approaches, levels of LH and testosterone are positively correlated to INHB concentrations (166) and there is increased expression of Sertoli cell ARs (22). Taken together, these data suggest that Leydig cell factors influence the maturation of Sertoli cells that have been primed to respond to a rise in local androgen concentrations around the time of puberty.

Although the full extent of androgen action during childhood is unknown, testosterone levels, albeit relatively low throughout childhood, have been associated with neuroanatomical variations. Along with DHEA, testosterone concentrations have been associated with cortical thickness in children as young as 4 years, providing evidence for a role in cortical maturation (167). Testosterone also appears to regulate structural covariance, or the correlation between various regions of the brain. Specifically, testosterone levels are associated with the structural covariance of the amygdala and prefrontal cortex, a finding that may account for differences in aggressive behavior (168) and also with prefrontal-hippocampal covariance, with potential effects on executive functioning (169).

The effects of androgen therapy on prepubertal children with KS provide further evidence for potential neurodevelopmental influences. As with infants, testosterone levels

in children with KS are variable. Some studies report normal concentrations throughout childhood until the time of expected puberty, after which point most levels decline to a low-normal range (132). In contrast, others demonstrate low levels of testosterone in nearly half of children with KS (170). When administered to children with KS in a double-blind, placebo-controlled trial, oxandrolone, a nonaromatizable androgen, improved visual-motor performance, anxiety/depression, and social functioning but did not significantly affect cognition or attention (171). Despite such benefits, caution should be employed in prepubertal children, as the odds of reaching gonadarche within 2 years is 20.5 times higher in those treated with oxandrolone versus placebo (172) despite adjusting for baseline age. At this time, there is a paucity of risk/benefit data to support the clinical use of androgens in infants and children with KS except for those with micropenis or hypospadias (173).

Indeed androgen therapy has been utilized in the treatment of micropenis and hypospadias outside of infancy. The response in childhood may be limited by a nearly 40% reduction in the concentration of foreskin androgen receptors that occurs early in life (174). Nonetheless, several studies have demonstrated a significant response to therapy during early childhood as noted in Tables 1 (112–123) and [TS: Please link table 2.]2 (175–183). In general, the overall growth potential of the penis is thought to be greater in those with idiopathic micropenis compared with those with other disorders such as primary hypogonadism or PAIS (184).

The use of androgens prior to hypospadias repair remains an area of uncertainty. Although some studies reveal an increase in penile size after testosterone administration (181), others show no difference in cosmesis (185) with similar rates of urethrocutaneous

fistula and meatal stenosis to those who received no treatment (183, 186). In addition, testosterone has been associated with more wound dehiscence (186). At this time, there are no compelling data to support its generalized use for hypospadias repair. Instead, risks and benefits should be carefully examined and treatment individualized.

Apart from the treatment of micropenis and hypospadias, testosterone has limited applications during childhood. However, it may be used for priming prior to GH stimulation testing. Since sex steroid hormones cause an increase in GH secretion during puberty (187), it can be assumed that exposing prepubertal children to sex steroids (priming) facilitates GH release and may avoid false classification, although this process remains debated. Either testosterone or an estrogen can be used; for example, 100 mg depot testosterone 7 to 10 days before the stimulation test (188) or estradiol 40 µg/m^2 for 2 days (189), since the augmentation in GH release is an estrogen-dependent process (190, 191). Although side effects such as priapism and testicular pain are low (192), some authors feel its use should be limited to older boys, 13 to 13.5 years of age, who have little or no evidence of puberty prior to testing (193).

Adolescence

Puberty

Androgen production increases exponentially over the course of puberty, the term given to the transition from childhood to young adulthood that culminates in reproductive maturity. Testosterone is produced primarily by the testes, though a small amount is also made in the adrenal gland. Gonadarche refers to the onset of sex steroid production from the gonads and occurs in response to pulsatile production of GnRH from the

hypothalamus, which in turn stimulates production of LH and FSH from the pituitary gland. LH stimulates the Leydig cells to produce testosterone, whereas FSH stimulates the Sertoli cells to proliferate and initiate spermatogenesis. LH also indirectly impacts spermatogenesis through testosterone's action on the AR, although the role of testosterone in spermatogenesis is complex and not fully defined. As reviewed in Ramaswamy et al, ITT concentrations are far higher than serum concentrations across species; however, spermatogenesis can continue despite significant reductions in ITT in rodents (194). Although suppression of ITT in humans is associated with near-complete suppression of spermatogenesis, not all men become azoospermic, which highlights the potential for other undefined mechanisms in the regulation of spermatogenesis (195).

The first physical sign of central puberty in males is testicular enlargement, which is generally defined as a volume >3 mL as measured by a Prader orchidometer. Testicular ultrasonography may also be used to estimate testicular volume. Unlike the Prader orchidometer, testicular ultrasound eliminates contribution from the epididymis and overlying skin, providing a more accurate estimation, particularly at lower testicular volumes. However, orchidometer measurements correlate well with ultrasonography (196) and the Prader orchidometer remains readily available, cost-effective, and widely used amongst pediatric endocrinologists.

The progression of normal sexual maturation can be easily tracked with repeated testicular exams, with volume increasing up to 15–25 mL in adulthood. This increase in testicular volume is largely from development of Sertoli cells and seminiferous tubules, whereas the Leydig cells contribute significantly less to testicular size. Following testicular enlargement, there is thinning of the scrotum, pubic hair development, and

penile growth. The growth spurt tends to occur towards the end of puberty in males where height velocity increases to as much as 10 to 15 cm/year. Along with the appearance of secondary sexual characteristics, the hormonal changes during puberty result in the attainment of reproductive capabilities, development of an adult fat mass/muscle mass ratio, and increase in bone mass (197).

As previously noted, at the start of puberty, testosterone is produced primarily at night in response to an LH pulse during deep sleep (198), as sensitivity to the negative feedback to sex steroids wanes and the GnRH pulse generator is reactivated (156). Testosterone regulates its own secretion via negative feedback inhibition at both the hypothalamus and anterior pituitary. Testosterone or one of its metabolites suppresses gonadotropin secretion, in part independently of peripheral conversion to estradiol. Marynick and colleagues (199) noted that in young male volunteers, testosterone infusion at approximately twice the daily production rate would suppress LH by approximately 50%; however, when the aromatase enzyme was inhibited by testolactone, there was no change in peripheral estradiol concentration and LH was suppressed to a lesser degree. These data imply independent actions of testosterone or one of its metabolites and estradiol.

To further dissect the actions of testosterone on the feedback inhibition of LH, Santen carried out a series of experiments on various components of LH secretion: mean levels, pulsatile release, and response to exogenous GnRH (200). Various steroids were infused at twice their physiologic production rates to normal young male volunteers. Testosterone and estradiol both reduced the mean LH concentration by approximately 80% and were indistinguishable. When pulsatile LH release was investigated during and

after testosterone infusion, the investigator noted that the pulse amplitude increased by approximately 50% and the frequency slowed. In contrast, during and after the estradiol infusion the LH pulse amplitude decreased by approximately 50% and the frequency remained invariant. Infusion of the nonaromatizable androgen DHT led to a decrease in mean LH concentration and had intermediate effects between those of testosterone and estradiol on pulsatile LH release. To narrow the site of action to the hypothalamus or pituitary a bolus of GnRH was administered during the steroid infusions. Estradiol infusion markedly blunted the response to GnRH whereas testosterone infusion did not. Taken together, the data point to an effect of estradiol on feedback inhibition, at least at level of the pituitary.

As puberty progresses, testosterone is produced throughout the day, although with a distinct diurnal variation that mimics the diurnal variation of LH, with the peak occurring early in the morning and progressively declining over the course of the day (201). This diurnal variation is most pronounced during early and mid-puberty (202), although it does persist into adulthood.

Reference ranges exist for total testosterone levels for each Tanner stage, with levels measured by high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS), [AU: Please check edited sentence beginning "Reference ranges exist for total testosterone levels for each Tanner stage, with levels measured by high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS),…" for consistency with your meaning.] reaching approximately 350 to 950 ng/dL at the end of puberty (Tanner stage 5) (203). Although testosterone production increases throughout puberty as testicular volume increases, production remains fairly stable once testicular

volume reaches 15 mL (202). Daily plasma production rates of testosterone can be measured accurately by infusion of radioactive (204–206) or stable (207) isotopes of testosterone and calculated by the determined metabolic clearance rate or the urinary production. The daily output in normal men ranges between 4 and 12 mg/24 hours and averages 4 to 8 mg/24 hours. One group of investigators used both methods in the same men and found the blood production rate to be 6.1 ± 1.4 mg/day (4.3–8.7) versus 6.2 ± 1.6 mg/day (4.2–9.0) in the urine (208). Circulating levels of testosterone and its daily production rate are readily inhibited by administration of biologically active androgens (209). Both are greater in young compared with middle age (mean age 55 years) men (207).

Obesity plays an important role in interpreting total testosterone concentrations, with obese boys having normal or slightly higher levels in prepuberty and early puberty, but significantly lower levels in the later stages of puberty (210). This difference can be partially explained by higher levels of adrenal androgen precursors in early puberty along with lower SHBG across all stages in obese males (211, 212). This brings up the free hormone hypothesis, which has had divergent views in the scientific literature over the past 4 decades (213, 214). Support for the free hormone hypothesis comes from *in vitro* experiments in which radiolabeled testosterone entry into cells is impeded by serum containing SHBG (215). In addition, several families with disorders of SHBG production and normal levels of free testosterone have had normal male sexual development, normal gonadal function, and normal LH levels, despite low levels of total testosterone and SHBG (216). The European Male Aging Study (EMAS) noted that low free testosterone in aging men, even in the presence of normal levels of total testosterone, was associated

with sexual symptoms and evidence of androgen deficiency whereas low total testosterone in the presence of normal free testosterone was not.

Like testosterone, the gonadal glycoproteins AMH and INHB that are produced by the Sertoli cells can be tracked over the course of puberty. Prior to puberty, AMH can be used as a marker for the presence of immature Sertoli cells, and declining AMH at the start of puberty suggests normal maturation of Sertoli cells. Sertoli cells mature in response to rising ITT and at this time testosterone and AMH become negatively correlated. As reviewed by Edelsztein et al, AMH production likely decreases due to the synergistic action of increasing ITT as well as the presence of meiotic germ cells in the seminiferous tubules as spermatogenesis progresses, although this inverse correlation may not be causal (217). Testosterone is key in this downregulation as those with CAIS have persistently elevated AMH levels (218). Observed deviations from normal puberty have demonstrated that AMH rises with successful treatment in boys with central precocious puberty and remains elevated in boys with delayed puberty (219, 220). Conversely, INHB rises at the time AMH begins to fall in peripubertal boys in response to gonadotropin secretion (221). INHB levels can thus be used as a marker of testicular volume and spermatogenesis.

Pubertal timing

Pubertal onset between the ages of 9 to 14 years is considered normal in males, though the lower age boundary appears to be decreasing slightly over the past few decades, mirroring trends in females. The median age of pubertal onset in boys is between 11 and 12 years of age in the United States (222). There are a number of factors that influence the timing of puberty, including general health and environmental factors; nevertheless,

genetics are the largest determinant and account for 50% to 80% of the variation in pubertal timing (223). In population studies utilizing genomic analyses, Day et al identified hundreds of genes implicated in pubertal timing (224). Interestingly, some loci appear to show sex-divergent effects (225). While these studies are limited by participant self-reported recall of the timing of key pubertal events, they highlight the complexity of the regulation of pubertal maturation.

As previously discussed, adequate nutrition is considered necessary or permissive for the onset of puberty, and is signaled through the production of leptin by adipose tissue. Similarly, kisspeptin is a key regulator of pubertal onset through regulation of GnRH neurons (226). The exact mechanism of activation of the HPG axis remains largely unknown, although a multitude of neurotransmitters play a role (223). As reviewed by Leka-Emiri et al, gamma-aminobutyric acid (GABA) exerts inhibitory effects on GnRH neurons whereas glutamate, kisspeptin, and neurokinin B are excitatory (227). Interestingly, while obesity has been strongly linked to earlier puberty in girls, this trend is not as clear in boys, and it appears that very obese boys might develop later (210).

Androgen-mediated changes during puberty

The physical changes apparent in males during puberty are largely due to the anabolic and androgenic effects of testosterone. The androgenic effects include penile growth, development of the scrotum and prostate, and growth of pubic, axillary, and facial hair. Anabolic changes include linear growth acceleration, enlargement of the larynx with deepening of the voice, development of libido and sexual potency, increased muscle bulk and strength, and a reduction and redistribution of fat mass (228). Testosterone can be

converted to DHT via 5α-reductase or aromatized to 17β-estradiol. DHT has 3 to 6 times the biopotency of testosterone, and DHT levels increase in circulation at the time of puberty similarly to testosterone (229, 230). As reviewed by Swerdloff et al, DHT is primarily produced locally in tissues that express 5α-reductase, such as the prostate, where the effects of testosterone are amplified according to the enzymatic activity of 5α-reductase type 2 (230). Both DHT and testosterone bind to the intracellular AR on target cells to exert their effects. Testosterone can also act through its conversion to estradiol. As previously noted, estradiol produced from the conversion of testosterone plays an important role in augmenting GH and IGF-1 production, leading to the rapid linear growth characteristic of puberty (231, 232).

Testosterone exposure results in increased bone and muscle mass, along with decreased fat mass at the time of puberty. Bone mass nearly doubles during puberty and nearly 50% of lifetime bone mass is gained between the onset of puberty and late adolescence (233). Androgens have a direct effect on bone mineral density (BMD) independent of estrogen as evident by lower BMD in the setting of CAIS (234). This same study demonstrated that high levels of DHT do not appear to be necessary for attainment of optimal BMD, as men with 5α-reductase deficiency have normal BMD. In addition to effects on BMD, androgens are also directly responsible for the increase in hemoglobin concentration observed in males at the time of puberty (235).

Marked changes occur in body composition during puberty that lead to boys becoming stronger and more anabolic compared with female counterparts. Androgens alone increase both whole body and muscle protein synthesis, though this is increased further in the presence of GH (236). Similarly, GH works synergistically with

testosterone during puberty to reduce adiposity and increase lean muscle mass (236). Muscular strength accelerates rapidly after 13 or 14 years of age in response to rising testosterone and GH, which coincides with the gender divergence observed in athletic performance (237, 238). This has been tested in multiple venues based on the concept of bio-banding (239). In training sessions and competitions for adolescents, some use the state of pubertal maturation rather than chronological age, such that athletes train and compete based on their biological "age" as determined by their individual percentage of adult height attained at the date of the competition. As reviewed by Malina et al, the mid-pubertal height spurt is intense at about 90% of adult height (240). That indicates that the early maturing younger players who are bigger and stronger than their similar-age peers compete (play-up) with players their own size and degree of maturation, and the older chronologically and the later maturing compete with younger athletes at the same maturational state. Data for football (soccer) indicate good psychosocial outcomes on both accounts: the younger athletes need to use skill and strategy rather than "run over" their smaller similar-age peers, while the older athletes who played down could take on leadership roles as well as excel with technical skills (241). The physiological metrics were similar whether practices/competitions were bio-banded or age-grouped (240).

Along with changes in anatomy and physiology, the hormonal changes that occur during puberty contribute to psychological changes, both through direct effects on brain structure as well as indirectly through the adolescent's response to those physical changes (242). ARs are located in the central nervous system (CNS), enabling androgens to impact the neural circuitry of the brain causing organizational effects during a critical window of development with further influences (activational changes) at the time of

puberty. Some of these changes have been demonstrated by structural magnetic resonance imaging (MRI). Testosterone influences axonal growth and changes the appearance of white matter throughout the CNS and in the corticospinal tract (243). Testosterone also appears to affect cortical maturation with studies showing increased cortical thickness in areas with high levels of ARs in males compared with females during puberty (244). Thus, "masculinization" of the brain through cortical maturation during puberty appears to be linked to the AR (245).

Although structural changes in the brain occur at the time of puberty, little is known about how these anatomical alterations influence the psychological changes associated with puberty. Mood swings and changes in behavior, such as aggression and increased risk-taking, have long been attributed to hormonal fluctuations and rising testosterone levels that occur during puberty. Interestingly, cortical thickening in frontal subregions of the brain appears to be delayed in males compared with females, which is speculated to account for males participating in more impulsive and risk-taking behaviors (245). Although a systematic review of 27 articles concluded that there was a lack of sufficient data to determine the impact of testosterone on mood and behavior (246), a recent study demonstrated increased fluctuations in affect in adolescent boys who had greater increases in their testosterone concentrations over the study period (247).

Delayed puberty

As reviewed by Wei, pubertal maturation is considered delayed in boys if it has not started by age 14 years (223). The differential diagnosis of delayed puberty as listed in Table 3 is vast and essentially can be classified as to whether gonadotropin concentrations are low (hypogonadotropic or secondary hypogonadism) or elevated

(hypergonadotropic or primary hypogonadism). A distinct category to be considered is androgen resistance, as noted in AIS. By far the most common cause of delayed puberty in males is constitutional delay of growth and puberty (CDGP), or self-limited delayed puberty, which is a diagnosis of exclusion. CDGP is considered a variation of normal puberty, as children with CDGP will ultimately enter into puberty on their own without hormonal treatment. Boys with CDGP present with slowing of their growth rate (prepubertal nadir) along with short stature relative to their genetic potential, however they have significantly delayed bone maturation and thus a normal adult height prediction. There is often a family history of "late bloomers" and gonadotropin levels are low, making it indistinguishable from isolated hypogonadotropic hypogonadism. The majority of boys with CDGP will reach their genetic potential in terms of height, however not all do, particularly boys who also have short genetic potential (familial short stature) (248). The natural course of CDGP is normal HPG activation and pubertal progression that simply starts later than 14 years of age.

Persistent hypogonadotropic hypogonadism is likely if a patient thought to have CDGP lacks testicular enlargement by 17 or 18 years of age and should be suspected earlier in the presence of other features such as micropenis or cryptorchidism. Stimulation testing protocols using either hCG or GnRH have been used to differentiate CDGP from hypogonadotropic hypogonadism; however, their utility remains poor, as misclassification rates are fairly high (228). INHB, a direct marker for the presence and function of Sertoli cells, has recently been proposed as a useful marker in differentiating CDGP from persistent hypogonadotropic hypogonadism, as an increase in serum INHB precedes clinical puberty (249,250). Coutant et al reported that an INHB level of ≤ 35

pg/mL was 100% sensitive and specific for hypogonadotropic hypogonadism in Tanner genital stage 1 boys, whereas sensitivity ranged from 80% to 86% and specificity from 88% to 92% using an INHB level of ≤ 65 pg/mL in Tanner genital stage 2 boys (251). However, this testing remains imperfect, as overlap in serum concentrations between the 2 groups exists (252).

Complex neuronal signaling pathways control the HPG axis, and, as previously noted, a number of genes have been implicated in CHH. CHH can occur alone, as in Kallmann syndrome, or along with other pituitary hormone deficiencies. The ability to smell should be assessed in all males presenting with delayed puberty as anosmia or hyposmia is a hallmark of Kallmann syndrome, which results from defective migration of GnRH and olfactory neurons from the olfactory placode. Use of standardized, validated olfactory testing such as the University of Pennsylvania Smell Identification Test (UPSIT) is preferred, since a patient's self-assessment is often inaccurate unless they report anosmia (253). Abnormalities in the olfactory system as visualized by brain MRI are often detected in patients with anosmia or hyposmia (253). Other potential clinical features of Kallmann syndrome include bimanual synkinesis, where one hand mirrors the movements of the other hand, and midline facial defects such as cleft lip and/or palate. Although approximately half of patients with CHH have Kallmann syndrome, there is wide genetic and clinical heterogeneity even within families who carry the same mutation (254). As noted in Table 3, a number of genes have been implicated in Kallmann syndrome and can be inherited in both X-linked and autosomal forms depending on the gene involved. The majority of males with CHH have absent puberty, though partial puberty is observed in some (125).

The most common genetic cause of primary (hypergonadotropic) hypogonadism is Klinefelter syndrome (KS). Key features of KS include tall stature, eunuchoid body habitus, testes that are disproportionately small and firm for the degree of pubertal maturation, and psychosocial abnormalities. Most adolescents with KS will have spontaneous puberty, though testes rarely progress past 6 to 8 mL in volume. As described previously, due to normal growth and commencement of puberty, the majority of males with KS are not diagnosed until after adolescence, often upon presentation to a fertility clinic. While the hormonal profile of boys with KS is fairly normal early in puberty, LH, and FSH levels begin to rise, and AMH and INHB levels fall over time (255).

Finally, iatrogenic causes of hypogonadism are on the rise. As treatments improve for childhood cancers, there are increasing numbers of cancer patients surviving into adolescence and adulthood who are at risk for hypogonadism. Children who require resection of brain tumors, particularly craniopharyngiomas or germinomas, or those who require cranial irradiation are at high risk for pituitary dysfunction including hypogonadotropic hypogonadism. The estimated prevalence of hypogonadotropic hypogonadism among childhood cancer survivors is nearly 11% (256). Risk of hypogonadotropic hypogonadism increases with higher doses of radiation, with gonadotropin deficiency often developing after exposure ≥ 30 Gy (233). In addition, those receiving ≥ 50 Gy are at risk for developing hyperprolactinemia with resultant secondary hypogonadotropic hypogonadism. As such, recent guidelines published by the Endocrine Society recommend screening for hypogonadotropic hypogonadism in all childhood cancer survivors with a history of tumors or surgery near the hypothalamus or

pituitary as well as those exposed to radiation at doses of 30 Gy or higher (257). While screening is particularly important during the time of normal puberty, it is important to note that radiation-induced central hypogonadism may emerge even decades after treatment (258, 259).

Hypergonadotropic hypogonadism can likewise result from treatment with certain cytotoxic chemotherapeutic agents or following exposure to gonadal irradiation. The alkylating agents, such as cyclophosphamide, are often associated with germ cell dysfunction, although cytotoxic agents have also been shown to cause subclinical Leydig cell dysfunction as demonstrated by ongoing elevations in LH concentrations (260). Risk can be further stratified based on cumulative dose exposure, with prepubertal boys receiving > 200 mg/kg of cyclophosphamide being at highest risk for gonadotoxicity (261–263). The sensitivity of Leydig cells to damage from irradiation varies over the course of puberty and can occur at doses more than 20 Gy, although doses as low as 14 Gy can damage Leydig cells when treatment is combined with alkylating agents (260, 264). A recent study by Taneja et al reported that more than half of the boys who received testicular irradiation at doses as low as 9 to 14.4 Gy as part of total body irradiation prior to stem cell transplant ultimately required testosterone therapy despite having a history of spontaneous puberty (265). Germ cells are particularly sensitive to radiation, and spermatogenesis can be impaired at doses as low as 0.2 Gy, with variable rates of recovery depending on the dose and pubertal stage at the time of irradiation exposure (262, 266, 267). Ongoing screening for hypogonadism, including close monitoring of growth and pubertal progression, is essential for high-risk populations.

Psychological consequences of delayed puberty

Data on the psychological consequences of delayed puberty are fairly limited, although the majority of boys presenting for evaluation of pubertal delay report emotional distress. It can be challenging to differentiate whether the distress is related to pubertal delay or short stature or a combination of both. The Oakland Growth study reported that boys with delayed puberty experienced more feelings of inadequacy and rejection as teenagers and sought more social acceptance than their peers (268). Data from the National Health Examination Survey suggest that delayed puberty in boys negatively affects how parents and teachers rate intellect and educational expectations (269). More recent studies reported an association between late pubertal timing and higher levels of depression, disruptive behavior disorder, and substance abuse in adolescence (270, 271). It is unclear whether these negative psychosocial effects persist into adulthood. One study reported increased risk of anxiety and depression in adult men with a history of pubertal delay (272), whereas other studies have reported no long-standing psychological impact (273). Regardless of whether these changes persist, most boys express their desire to start treatment as soon as possible, and alleviation of psychological distress is one of the primary reasons treatment for delayed puberty is initiated, in addition to concerns about height.

Treatment of adolescent boys with delayed puberty

The most common pediatric indications for testosterone replacement therapy (TRT) are the induction of puberty in males with CDGP followed by maintenance of puberty for those with persistent hypogonadism. A rapidly emerging indication that will be discussed in more detail at the end of this review is cross-sex hormone therapy for transgender males. As mentioned above, the primary motivation for treating adolescents with CDGP

is the significant psychological distress experienced from being shorter and less physically developed than their peers, which in part stems from the Oakland Study, in which the 16 adolescents who were most accelerated in pubertal maturation were compared with the 16 most delayed. Those who matured early were perceived by adults as more poised, relaxed, good-natured, and unaffected than those maturing later. The latter were perceived by their *peers* as less good looking, less grown up, more attention seeking, bossy, and talkative (274). Short courses of androgens, oral fluoxymesterone, at approximately 5 mg daily for 3 to 6 months (275), or intramuscular (IM) testosterone enanthate, 200 mg every 3 weeks for a course of 4 doses (276), have been studied in the treatment of CDGP. In both studies, linear growth and weight gain accelerated, and there were psychosocial benefits without apparent compromise of adult height. Unfortunately, there is a lack of randomized, placebo-controlled trials and more studies are needed to fully elucidate the impact of TRT on the potential psychological distress in boys with delayed puberty. In one nonrandomized, prospective study, all boys with CDGP who received TRT with testosterone enanthate 100 mg IM monthly for 6 months (n = 148) reported that they were psychologically satisfied with their growth and increased muscle mass at 1 year, compared with only 40% of boys in an age-matched control group (n = 50) (277).

A secondary consideration for the treatment of CDGP is the possible negative impact on attainment of peak bone mass and subsequent risk for fractures (231). After approximately 20 years of age, optimal peak bone mass cannot be achieved and bone quality at the end of adolescence predicts fracture and osteoporosis risk in adulthood (233). However, studies published to date on the effects of untreated CDGP in

adolescence have been conflicting on whether BMD is actually decreased in adulthood (248). Three studies have reported significantly decreased BMD in men with a history of self-limited delayed puberty as compared with controls with normal puberty. The first study measured BMD by dual-energy x-ray absorptiometry (DXA) (278), whereas the other 2 measured volumetric BMD directly with peripheral quantitative computed topography (279, 280). However, more recent studies have found no significant decrease in BMD in men with a history of delayed puberty compared with controls (281, 282). Of note, delayed puberty was defined differently in each study and the mean age at which BMD was measured in adulthood varied from 19 to 64 years, making interpretation of the results difficult.

Finally, abnormal pubertal timing might result in increased risk for cardio-metabolic complications later in life. Interestingly, abnormal pubertal timing, both early and late, has been linked to an increased risk of a wide variety of diseases in adulthood in both men and women (272). Earlier pubertal timing appears to be associated with higher risks of adverse health outcomes when compared with delayed puberty. Large population studies have linked earlier pubertal timing to metabolic and cardiovascular outcomes (type 2 diabetes, angina, heart attack, obesity, and hypertension) in both men and women, whereas delayed puberty in men was associated with psychological distress (anxiety, panic attacks, depression) (225, 272).

TRT should ideally start around the time of normal average puberty (~12 years) in adolescents known to have a pubertal disorder, however most boys with CDGP present later and are started on treatment closer to 14 or 15 years of age. Certainly, well-adjusted boys with suspected CDGP could be monitored clinically (*watchful waiting*) without

choosing TRT, although treatment is safe and effective with high patient and parent satisfaction. Additionally, in studies reporting adult height in boys with CDGP, treatment with a short course of low-dose monthly IM testosterone does not appear to negatively impact growth when appropriate doses are used (283–285). As such, TRT can be considered in boys with suspected CDGP. Although there are no evidence-based recommendations on the ideal age TRT should be started in boys with CDGP, pediatric endocrinologists tend to wait until the bone age is at least 10.5 years, due to concerns about possible negative impact on height potential if started too early.

Most clinicians induce puberty in boys with CDGP with a short course of monthly intramuscular (IM) testosterone. Often TRT is only briefly necessary (3–6 months), however there can be variability in the pace and pattern of endogenous testosterone production following induction and some boys require extended treatment (286). There are wide differences in individual practice with no apparent superior dosing regimen as all appear to accomplish the same goals of successful induction of puberty, acceleration of height velocity without negatively impacting adult height, and easing of some of the emotional distress experienced by boys with CDGP (228, 277, 283–285, 287–294). Of note, standard induction doses of testosterone appear to be equally effective in the setting of obesity (289). TRT can be discontinued once testicular volume increases, typically up to 6 to 8 mL, which indicates significant activation of the HPG axis.

Treatment of permanent hypogonadism in adolescent males involves attempting to mimic normal pubertal physiology with gradual increases of testosterone over the course of approximately 2 to 3 years until adult doses are reached. This gradual progression is primarily indicated to allow for optimal growth. Adolescents with KS

often do not require pubertal induction, but do need maintenance of puberty once hypogonadism develops. Despite the need for incremental increases in testosterone exposure in pediatric patients, pediatric endocrinologists are limited by testosterone preparations that are metered for adults. Unfortunately, there is a lack of literature directly comparing different replacement protocols and as such, no evidence-based guidelines regarding optimal testosterone formulation and dosing for pubertal induction exist. Treatment should be individualized to the patient's response and needs, particularly with regard to height and growth potential, and newer testosterone formulations such as transdermal gels can be considered for ease of titration once mid-puberty is reached.

Pharmacologic options available for TRT are listed in Table 4, along with approximate starting doses, adult doses, and the advantages and disadvantages of each preparation. IM testosterone esters (testosterone cypionate and testosterone enanthate) remain the most widely used form in pediatrics, with 88% of pediatric endocrinologists in the United States reporting their use to treat hypogonadism in a survey on current practices in 2004, with only 10% and 7% reporting use of transdermal gels or patches, respectively (295). Since that survey more than 10 years ago, practices have not changed much, despite development of newer formulations. A recent single-center review from the United Kingdom reported that 89% of boys with hypogonadism were started on IM testosterone, followed by 9% taking oral and only 1% using gel (296). Other European countries presumably use more oral testosterone, as it is not available in the United States, however data on prescribing practices are lacking. Much of the initial research in pubertal induction was done using Sustanon, which consists of a mixture of 4 testosterone esters (30 mg testosterone propionate, 60 mg testosterone phenylpropionate, 60 mg

testosterone isocaproate, and 100 mg testosterone deconoate) (297, 298), whereas testosterone enanthate and testosterone cypionate are more widely used now.

As noted above, oral testosterone is not available in the United States, but it is used in other countries. Methyltestosterone and 17-α derivatives have a potential for hepatotoxicity and are no longer used. Testosterone undecanoate (TU) comes in an oral formulation that is not hepatotoxic; however, it is not widely available, has a short half-life necessitating multiple daily doses, and is unreliably absorbed with variable effects. Oral TU is taken up directly by the intestinal lymphatic system. Its absorption is sensitive to food intake, especially the lipid content of the meal. Schnabel and colleagues evaluated the effects of various meals on the absorption of TU in 24 healthy postmenopausal women who had very low endogenous testosterone (299). The women ingested 80 mg TU within 5 minutes of isocaloric meals of either < 0.7 g or 5 g lipid content. TU absorption was evaluated as the area under the curve (AUC) of testosterone over time [AU: Please check the added expansion for "AUC" in the sentence that begins "TU absorption was evaluated as the area under the curve (AUC) of testosterone over time…".] or as the maximal concentration of testosterone. The former was 2.5-fold greater with the higher fat content meal. With normal or high fat content meals, and thus much higher caloric density, there was much less difference in AUC or Cmax between the meals but almost a 10-fold greater amount than that noted in the 2 lower fat meal conditions. The investigators concluded that in a low-fat condition the lipid content of the meal was critical to the absorption of oral TU. They speculated that inconsistent absorption of TU, well described for decades, was due to variation in the meals preceding administration. A more recent formulation has been designed to foster more consistent

absorption and to be less sensitive to meal variance. The FDA recently approved a liquid, self-emulsifying formulation within a soft gel capsule for hypogonadal men, the absorption of which is less sensitive to low-fat meals (300).

Other testosterone formulations have been developed over the past 2 decades and include transdermal, subcutaneous, buccal, and nasal preparations. Reported use of non-IM formulations in the pediatric population is reviewed in Table 5 (287, 301–315). The limited data available in pediatric patients are reassuring in terms of short-term safety and efficacy, however randomized controlled trials are lacking. It is important to note that all of these formulations have been extensively studied in adults and are approved for use in adult men, and thus can certainly be considered once adult dosing is reached. As previously noted, injectable subcutaneous testosterone is emerging as a popular treatment strategy in the management of gender transition, although studies have yet to be done for the purpose of pubertal induction in other populations (316, 317).

Pediatric endocrinologists typically start pubertal induction with IM testosterone, given the increased data and clinical experience with this preparation, and will then consider transitioning to a newer, non-IM formulation once adult dosing is reached (197, 318–322). Although IM testosterone does not provide physiological replacement, since supraphysiological levels following injection fall substantially after 2 to 3 weeks, a theoretical benefit is the lack of persistent negative feedback on the HPG axis with low-dose monthly injections early in treatment, particularly when it remains unclear if the patient has CDGP or persistent hypogonadotropic hypogonadism. In fact, early pharmacokinetic studies of IM testosterone enanthate reported gonadotropin suppression for 11 days following injection of either 100 or 200 mg, which then normalized by day 14

(323). Although IM testosterone is frequently used, pretreatment counseling prior to TRT initiation should ideally include discussion of the risks and benefits of all available formulations.

TRT results in a number of clinical benefits, including improvement in insulin sensitivity (324), energy, and sense of wellbeing, as well as increasing BMD and sexual function. Importantly, while TRT results in the development of secondary sexual characteristics, such as penile growth and facial hair along with increased linear growth and attainment of muscle mass and bone mass, it will not result in testicular enlargement or spermatogenesis, which are dependent on FSH. In fact, TRT can suppress testicular enlargement in males with a normal HPG axis due to negative feedback inhibition of gonadotropin secretion, though testes will remain small in those with permanent hypogonadotropic hypogonadism regardless of treatment. Adolescents and their families should be counseled on the impact of testosterone on inhibiting spermatogenesis and thus fertility (325, 326). Ongoing monitoring of testicular volume as measured by a Prader orchidometer is essential while on treatment, even in those with suspected permanent hypogonadism. If testicular enlargement occurs while on TRT, treatment should be discontinued to determine if the patient has experienced hypogonadism reversal, which has been reported in approximately 10% of males diagnosed with idiopathic hypogonadotropic hypogonadism (327).

Oxandrolone, an orally active, nonaromatizable anabolic steroid has a long history of use in children and adolescents. Like testosterone, it has been used for growth acceleration in boys with CDGP and has also been used to augment growth in girls with Turner syndrome receiving growth hormone (328). It works directly at the AR to produce

anabolic effects without inducing significant androgenic changes. Studies suggest it is effective at increasing growth and is well tolerated (308, 329, 330) in appropriate doses and duration. This is in stark contrast to reports of oxandrolone used as a performance enhancing drug, where severe hepatic toxicity has been described in doses and durations that are largely unknown, often in the presence of polypharmacy (331, 332).

In a double-blind, placebo-controlled trial, 19 boys aged 12.9 to 16.3 years received 2.5 mg per day of oxandrolone or placebo for 3 months (the longest time permitted by the hospital ethics committee for those receiving placebo to start) (333). The height velocity accelerated markedly in all who received oxandrolone with few accelerating in the first 3 months of the placebo administration. For the second 3 months, virtually all those reverting to placebo had diminished height velocity. All in the oxandrolone group (following 3 months of placebo) had a marked upward trajectory in height velocity. Since the physiologic pubertal growth spurt usually occurs during genitalia stage 3 or 4 (in boys) or testicular volume of approximately 10 mL, oxandrolone therapy did not appear to accelerate them into the pubertal growth spurt prematurely (334, 335).

Oxandrolone has also been used as an anabolic agent for severe thermal injury where long-term safety data has been obtained. In summary, a meta-analysis of trials using oxandrolone noted no change in mortality, infection or hepatic function. Its use shortened the length of stay in the burn unit, the donor site healing time (and the ability to do the next graft sooner), the length of stay in the rehabilitation phase, and promoted additional gain in lean body mass in part by *decreasing* resting energy expenditure (336). In a very long-term (up to 5 years) placebo-controlled trial, 222 patients (mean age 8

years) with severe thermal injury ($\geq$ 30 % total body surface area) were randomized to receive oxandrolone 0.1 mg/kg or placebo twice daily for a full year (337). Those in the oxandrolone group had markedly reduced percentage of predicted resting energy rate, which was maintained for as long as 18 months. There were more children in the placebo group who were more than 2 SD below the mean normal height velocity, but this difference became smaller in the third to fifth years. Bone mineral content increased markedly in childhood and adolescence and was significantly greater than in those receiving placebo. The change in lean body mass was greater in the oxandrolone group than in the placebo group, but not as marked as the difference in bone mineral content between the groups.

The patients were monitored for adverse events for a period of up to a year following discontinuation of oxandrolone therapy. There were no signs of virilization in the female patients. Special emphasis was noted for monitoring liver function, given concerns of hepatotoxicity with oxandrolone, and while the alanine amino transferase and γ-glutamyl transferase levels were elevated in both groups during the acute period following the injury, they returned to normal within 3 to 6 months. In addition, *in vitro* experiments in proliferating bone cells demonstrate that oxandrolone can stimulate the production of osteoblastic differentiation, stimulating cellular type 1 collagen production, likely through an AR-dependent mechanism (338).

As reviewed above, several types of androgens and various formulations have been used for the treatment of delayed puberty and hypogonadism. Adolescents with CHH may similarly be treated with androgen replacement (TRT) or with alternate therapies such as pulsatile GnRH in those with isolated GnRH deficiency or hCG (as an

LH substitute) with or without FSH (322, 339). The major disadvantages of this therapy are the inconvenient administration and cost. FSH has been used in combination with hCG to optimize spermatogenesis and further increase testicular volume, however full adult testicular volume is rarely reached (340). In general, those with smaller testicular volumes at the start of treatment have lower volumes at the completion of treatment (322). Interestingly, males with acquired hypogonadotropic hypogonadism do achieve a normal adult testicular volume with this treatment, which suggests that gonadotropin exposure early in life might be necessary for this to occur, as discussed in the section "Infancy." Fertility is not guaranteed, particularly in the setting of cryptorchidism, although the majority of patients with CHH develop low-level sperm counts with long-term therapy (125, 341). Evidence suggests that spermatogenesis is best maintained in the presence of both FSH and LH-induced testosterone secretion. In a small but randomized study from Dwyer et al, all 7 men with hypogonadotropic hypogonadism treated with recombinant human FSH for 4 months prior to GnRH developed sperm in their ejaculate compared with only 67% in the group receiving pulsatile GnRH alone (342). Of note, boys previously treated with testosterone also respond to this treatment in terms of testicular enlargement and induction of spermatogenesis (343). However, gonadotropin therapy should not be used in the setting of CDGP, given its self-limited nature.

Adverse effects from TRT are rare but include aggression, mood swings with fluctuations in circulating testosterone, acne, oily skin, gynecomastia, priapism, and erythrocytosis (344, 345). Erythrocytosis occurs in up to 40% of men receiving testosterone therapy with highest risk for those using intermediate-acting IM testosterone

esters (346). A unique consideration for those using transdermal gels is the potential for transfer to anyone who comes in contact with the site of application or clothing that came into contact with the site of application (347, 348). There is also prevailing concern that TRT could result in rapid skeletal maturation with loss of adult height, particularly if supraphysiologic doses are given or if treatment is started too early. Early or excessive exposure to testosterone has clearly been shown to stunt growth in pathological conditions such as central precocious puberty or CAH. High-dose testosterone therapy (250–500 mg testosterone enanthate IM every 2 weeks) has also been used to decrease height potential in boys with constitutional tall stature (349). As noted previously, the data are reassuring that physiologic TRT in the treatment of adolescents with CDGP or permanent hypogonadism does not appear to negatively impact adult height.

As reviewed by Bertelloni et al, adolescents treated with testosterone should be followed routinely with close monitoring of growth, sexual maturation, and bone maturation and evaluation for side effects of treatment (197). Monitoring of serum testosterone concentration is not particularly useful while advancing toward adult dosing, although it should be assessed once adult dosing is reached. The ideal timing of measurement of testosterone depends upon the replacement formulation being used. For short-acting IM testosterone esters, testosterone levels can be obtained at the midway point between injections to monitor peak concentrations. Trough values obtained right before the next dose are also useful for guiding adjustments once adult dosing is reached. Lab monitoring for long-acting IM testosterone esters and buccal preparations should be performed just prior to the next dose. Testosterone concentrations should be measured 3 to 12 hours after application of a patch and any time after 1 to 2 weeks of consistent use

of a testosterone gel. Further analytes that require monitoring include red blood cell count, hematocrit, and lipid profile. It is recommended that therapy is temporarily discontinued or the interval between injections widened if the hematocrit exceeds 54%. While adolescents are still growing, a bone age radiograph can be useful to determine height prediction. Bone density as measured by DXA with appropriate adjustments for age, sex, and bone maturation might also be considered during the course of treatment. LH and FSH are not useful for monitoring in the setting of permanent hypogonadism; however, these can be informative after a short course of testosterone in adolescents with suspected CDGP to confirm activation of the HPG axis.

Transition to adult

Pediatric endocrinologists often treat adolescents with hypogonadism long after full adult dosing is reached. The Endocrine Society updated their Clinical Practice Guidelines on TRT in adults in 2018 (350). These guidelines recommend monitoring hematocrit at baseline, 3 to 6 months, and then annually along with DXA at baseline and every 1 to 2 years in hypogonadal men with osteoporosis or fracture considered to be from low-energy trauma. A recent study determined that pediatric endocrinologists who were treating hypogonadal adolescents and young adults did not consistently follow the adult guidelines, particularly with regard to monitoring of hematocrit and bone density, where 31% and 63% of patients reviewed were lacking these data (318). The timing of the transition of young adults to adult care depends on patient readiness and requires a structured approach with communication between providers to ensure there are no gaps in care.

Transgender Care

As mentioned above, androgens have also been used to induce puberty in adolescents with gender dysphoria, as defined by the American Psychiatric Association as a conflict between an individual's assigned natal gender and the gender with which they identify. Gender dysphoria was initially referred to as *transsexualism*, a term coined in 1923 to describe those who felt trapped in the wrong body (351).

The biologic basis for gender dysphoria is not well understood. Although some have hypothesized that circulating levels of sex steroids may influence gender identity, studies have failed to identify differences between transgender and nontransgender individuals (351). As previously noted, the full extent of early androgen exposure on the developing brain is unknown. Indeed the brain phenotype of those with gender dysphoria differs from controls and there is a higher prevalence of gender dysphoria/gender incongruence in 46,XX adults with high intrauterine androgen exposure due to CAH. However, early androgen exposure does not appear to contribute entirely to one's gender identity, based on observations in women with CAH who maintain female gender identity despite extensive prenatal virilization (351). Genetic factors may also play a role as suggested by data from twin studies (352).

Physicians such as Magnus Hirschfeld and Harry Benjamin pioneered the field of gender medicine, aimed to alleviate the suffering experienced by individuals who desired to live as their experienced, rather than assigned gender, eventually giving rise to hormone therapy in the latter half of the 20th century (351).

The number of referrals for adolescents seeking transgender care has increased dramatically over the past few years (353), with an estimated 2.6 out of 100,000 individuals pursuing transition to the male gender (354). Medical transition for these

individuals may involve masculinization through androgen therapy. Prior to initiating masculinizing therapy for transgender youth, the diagnosis of gender dysphoria/gender incongruence must be made by a qualified mental health provider. Data on the use of gender-affirming hormone therapy are limited in children under 14 years of age and practices vary greatly among centers across the world (353). Treatment may be considered on an individual basis in those who demonstrate sufficient mental capacity to provide informed consent (assent), typically achieved by age 16 (351). However, sex steroids are not recommended for prepubertal children given that only a minority experience persistent gender dysphoria into adolescence and adulthood (355). The intensity of gender dysphoria in childhood and female gender assignment at birth are associated with persistence into adolescence (355). Nonetheless, it is impossible to predict the course of a particular child, which may create a sense of reluctance on the part of parents and/or providers. However, in a study of adults treated with puberty blockers and cross-sex hormones during adolescence, none regretted treatment (356). Furthermore, the rate of desistance in studies to date may be overestimated due to the broad diagnostic criteria of the DSM-IV text revision, and children must be supported as their gender identity develops while the rates of persistence using the stricter DSM-5 criteria are being studied (351).

The child and parent(s)/guardian(s) should receive education on the expected masculinizing effects, anticipated time of onset, and possible side effects of testosterone therapy (Table 6) (317,351,357). This should include the potential for erythrocytosis, irreversible infertility, and options for fertility preservation, including oocyte or embryo banking or ovarian tissue cryopreservation (358). The threshold at which testosterone

impacts fertility is uncertain; thus, many feel that this should be discussed and pursued in those desiring fertility before testosterone is begun. However, fertility preservation has the potential to worsen body dysphoria due to a variety of factors, including delayed initiation of testosterone therapy, increased estrogen exposure in response to hormonal stimulation, and invasiveness of the procedures involved (358). Decisions surrounding fertility preservation may be very difficult and a multidisciplinary approach involving the support of mental health providers and reproductive specialists may be beneficial.

Families should also be counseled on the potential effects on lipid levels, as testosterone therapy has been shown to raise serum triglycerides and low-density lipoprotein (LDL) and to lower high-density lipoprotein (HDL) within 2 years of initiation (354). While baseline risk of cardiovascular disease should be assessed, several studies have failed to demonstrate an increase in cardiovascular events (351) and long-term data are needed to evaluate the clinical relevance of changes in lipid levels. Although the practice of lipid monitoring is not uniform among providers, periodic assessment has been recommended by the Endocrine Society (351).

Other potential effects include reduced insulin sensitivity and liver dysfunction, though these are uncommon (359) and the latter more commonly described in those taking methyltestosterone or other 17α-alkylated steroids (360). Although mild elevations in liver enzymes (within 2.5 times the upper limit of normal) have been reported in a small portion of transmale individuals treated with parenteral testosterone esters and/or oral TU, the incidence in untreated age-matched individuals was not reported and serious hepatic toxicity is not an anticipated side effect of such therapy (356). Testosterone does not appear to significantly impact BMD within the first 12 to 24 months of initiation

(361). However, given the difference in BMD between assigned natal men and women, testosterone therapy is likely to contribute to changes in BMD over time. Factors such as age at initiation and duration of treatment may influence the impact on BMD; thus, longitudinal studies assessing the effect of long-term testosterone therapy in various age groups are warranted.

The incidence of breast cancer in transmale individuals is similar to that of cisgender female individuals. Given case reports of breast cancer in subareolar tissue after mastectomy, however, routine breast exams are recommended even in those who have undergone mastectomy or "top surgery" (351). Ovarian cancer has also been reported in transgender men, but only 1 known case of endometrial cancer has been documented (362). This fairly low prevalence may be attributed to endometrial atrophy that occurs in response to testosterone treatment. In general, age-appropriate cancer screening is recommended for transgender males receiving testosterone.

As with other indications, testosterone may be administered to transgender males in many forms as outlined in Table 6. Pediatric endocrinologists tend to induce puberty with intermediate-acting testosterone esters starting at a dose of approximately 50 mg each month or 2-week interval, escalating to the full adult dose of approximately 200 mg every 2 weeks over a couple of years. Those who transition as adults often start near the full adult dose to quickly reduce endogenous sex hormone levels, abrogate menstruation, and attain a more masculine phenotype, inducing virilization (body shape, body composition, body and facial hair and more masculine voice) (363). However, given the limitations of various formulations, many are opting for subcutaneous administration. This approach is effective in suppressing menstruation in transgender male individuals

(317, 357), including those with an elevated body mass index (317). Subcutaneous injections allow for self-administration and are preferred by those previously treated with IM injections (317). Serum testosterone concentrations are stable for 7 days following injections, enabling providers to monitor levels at any time in relation to dosing (316).

Monitoring should include a physical exam with vital signs and potentially DXA and/or bone age as indicated during pubertal induction. Routine monitoring of testosterone levels is recommended with dose adjustments as needed to maintain levels in the physiologic range for adult males (351). Monitoring of additional parameters, including hemoglobin/hematocrit and lipids should also be considered.

Conclusion

Androgens play a crucial role in male development starting *in utero* and continuing on into infancy, childhood, and adolescence. Disorders of androgen production or action result in wide-ranging effects, not only in terms of virilization and development of secondary sexual characteristics, but also on growth, bone health, body composition, CNS structure, and psychosocial well-being. Androgen replacement with testosterone (or gonadotropins) is essential for males with hypogonadism; however, questions remain about the impact of timing of replacement, including need for replacement during infancy, as well as optimal testosterone formulation and dosing strategy. The development of newer testosterone formulations has provided more options for pediatric endocrinologists, although data on the use of these formulations, including about long-term outcomes, remain limited.

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Abbreviations: 3βHSD, 3β-hydroxysteroid dehydrogenase; ADHD, attention deficit hyperactivity disorder; AGD, anogenital distance; AIS, androgen insensitivity syndrome; AMH, anti-Müllerian hormone; AR, androgen receptor; ASD, autism spectrum disorder; BMD, bone mineral density; CAH, congenital adrenal hyperplasia; CAIS, complete androgen insensitivity syndrome; cAMP, cyclic adenosine monophosphate; CDGP, constitutional delay of growth and puberty; CHH, congenital hypogonadotropic hypogonadism; DHEA-S, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; DSD, differences/disorders of sex development; DXA, dual-energy x-ray absorptiometry; FSH, follicle-stimulating hormone; GH, growth hormone; GnRH, gonadotropin-releasing

hormone; hCG, human chorionic gonadotropin; HPG axis, hypothalamic-pituitary-gonadal axis; IM, intramuscular; INHB, inhibin B; ITT, intratesticular testosterone; KS, Klinefelter syndrome; LH, luteinizing hormone; MPW, masculinizing programming window; PAIS, partial androgen insensitivity syndrome; SHBG, sex hormone–binding globulin; TRT, testosterone replacement therapy; TU, testosterone undecanoate.

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Figure 1. Diagram of the classic pathway (depicting both the Δ4 and Δ5 pathways) and alternate pathway for the production of dihydrotestosterone. Note that the alternate pathway bypasses testosterone as an intermediate. Not all intermediates, pathways, or enzymes may be shown. Abbreviations: 17βHSD3, 17-hydroxysteroid dehydrogenase type 3; 17βHSD6, 17-hydroxysteroid dehydrogenase type 6; 3βHSD1&2, 3-hydroxysteroid dehydrogenase types 1 and 2; 5αR1&2, 5α-reductase types 1 and 2; DHT, dihydrotestosterone; StAR, steroidogenic acute regulatory protein.

Table 1. Review of Published Data Using Androgen Therapy for Micropenis [AU: Please check that all the Tables have been displayed correctly.]

1a. *Intramuscular Testosterone*

Reference	Study Design	Subjects	Regimen	Results
Burstein et al. 1979 (112)	Case series	14 subjects 1 wk to 11 yr 1 mo	25–50 mg/mo × 3 mo (1-2 courses)	▪ ↑Penile size by 1.99 cm w/ea course ▪ Transient ↑HV (resolved within 9 mo of d/c) ▪ No BA adv ▪ 1 transient scrotal rogation ▪ 1 transient pubic hair
Zenaty et al. 2006 (113)	Case series	19 subjects 0.08-1.4 yr (mean 0.7 yr)	50-150 mg/m2/dose q2-4 wk × 3-4 injections	▪ ↑Penile length >3 mo after tx by 1.9 SDS from baseline ($P = 0.0003$)
Guthrie et al. 1973 (114)	Case series	4 subjects 12-34 mo	25 mg q3wk × 4	▪ ↑Penile size to normal ▪ Transient ↑HV resolved 15 mo after d/c ▪ Transient BA adv ▪ 1 transient pubic hair ▪ 2 downy pubic hair
Ishii et al. 2004 (115)	Case series	53 Japanese subjects 0-13 yr	25 mg for 1-4 injections	▪ ↑Penile length from 2.5 to 3.5 cm (-2.74 to -0.72 SD)

Reference	Study Design	Subjects	Regimen	Results
				o Independent of CAG repeat and V89L polymorphisms (sequenced *SRD5A2* and *AR*)
Bin-Abbas et al. 1999 (116)	Case series	8 subjects 4 mo to 13 yr	25 or 50 mg/mo × 3 mo (1-2 courses)	▪ ↑ Penile length from 1.1 to 3.3 cm after 3 mo in <2 yo ▪ ↑ Penile length from 2.7 to 4.8 cm after 3 mo in 6-13 yo ▪ No virilization, ↑ HV, or BA adv
Nerli et al. 2013 (117)	Case series	11 subjects 3-14 yr (mean 5.09 yrs)	25 mg/mo × 3 mo	▪ ↑ Penile length from 1.55 to 3.72 cm after 8 weeks (*P* < 0.001) ▪ ↑ T levels

Abbreviations: adv, advancement; BA, bone age; d/c, discontinue/discontinuation; ea, each; HV, height velocity; mo, month(s); SDS, standard deviation score; T, testosterone; tx, treated/treatment; wk, week(s); yr, year(s).

1b. *Topical Testosterone* [AU: Please check and revise: In Table 1b, the first row, regimen column reads "5% T 0.2 gm/day × 30 days" but since "gm" is not a standard SI unit abbreviation, please specify as grams (g), mg, or something else.]

Reference	Study Design	Subjects	Regimen	Results
Arisaka et al. 2001 (118)	Case series	50 subjects 5 mo to 8 yr	5% T 0.2 gm/day × 30 days	▪ ↑ Penile length 1.89 to 2.73 cm ▪ ↑ T levels ▪ Slight ↑ in IGF-1; no change in BA ▪ Transient local hyperpigmentation (resolved 2-3 wk after d/c) in 5 subjects ▪ Mild pruritic eczema in 2
Ben-Galim et al. 1980 (119)	Case series	5 subjects 2 yr 6 mo to 11 yr 2 mo	5% T 2.5-4 mg applied to axilla (1) or penis (4) TID × 21 days	▪ ↑ Penile length to normal in all but 1 subject ▪ ↑ Serum T to adult levels (systemic action) ▪ No ↑ HV or BA adv ▪ 2 transient downy pubic hair ▪ 1 transient localized

				hyperpigmentation

1c. Topical Dihydrotestosterone

Reference	Study Design	Subjects	Regimen	Results
Choi et al. 1993 (120)	Case series	22 subjects 3-15 yr (9.7 yr)	▪ 12.5 mg × 8 wk if age <10 yr ▪ 25 mg × 8 wk if age >10 yr	▪ ↑Penile size ▪ Transient ↑DHT; HPG transiently suppressed ▪ Transient prostate enlargement during tx ▪ Transient localized hyperpigmentation ▪ Frequent erections in all ▪ Pubic hair in 6 (all pubertal age) ▪ Transient itching/redness in 6 ▪ ↑Bone specific alk phos ▪ No ↑HV or BA adv
Becker et al. 2016 (121)	Case series	3 related subjects with PAIS (2 children, 1 adult)	2.5% 0.3 mg/kg/day × 4 mo	▪ ↑Penile length from by 40 to 63% (in the 2 children) ▪ No adverse effects
Xu et al. 2017 (122)	Prospective case series	23 subjects mean 4.07 yr (2 AR mutations; 5 SRD5A2 mutations)	2.5% 0.1-0.3 mg/kg/day titrated to normal DHT serum reference ranges	▪ ↑Penile length from 1.68 to 2.9 cm at 6 mo ▪ 61% reached penile length >-2.5 SD ▪ Elevated DHT after tx ▪ No significant side effects ▪ 13% discontinued after 3 mo due to anxiety about potential effects
Charmandari et al. 2001 (123)	Prospective case series	6 subjects 1.9-8.3 yr	2.5% 0.15-0.33 mg/kg/day × 2.5-4 mo ▪ 5 mg in age >3 yr ▪ 2.5 mg in age <3 yr	▪ ↑Penile length by 0.5 to 2 cm after 3-4 months in all whose DHT was maintained in adult range ▪ DHT levels peak 2-8 hours after application ▪ No adverse effects

Table 2. Review of Published Data Using Testosterone for Hypospadias

2a. *Intramuscular Testosterone*

Reference	Study Design	Subjects	Regimen	Results
Luo et al. 2003 (175)	Prospective case series	25 subjects 9-12 mo	25 mg/mo × 1-3 mo	▪ ↑ Penile length (1.98 to 2.38 cm, *P* < 0.001) and glans circumference in all but 2 subjects ▪ No pubic hair or BA adv
Davits et al. 1993 (176)	Case series	40 subjects 13-74 mo (mean 27.5 mo)	Testoviron 2 mg/kg at 5 and 2 weeks preop	▪ ↑ Penile length by 2.4 cm ▪ No change in mean height ▪ Transient erections in 5, transient pubic hair in 2, transient acne in 1
Ishii et al. 2010 (177)	Retrospective case series Response compared with response in children with micropenis (Ishii 2004, 112)	17 Japanese subjects 0-5 yr (mean 1.4 yr)	25 mg q4 wk for 1-3 injections	▪ Change in penile length after 1st injection significantly lower in those with hypospadias vs micropenis (*P* = 0.02)
Gearhart et al. 1987 (178)	Case series	44 subjects (ages provided for only 4 subjects 2.3-4.2 yr)	2 mg/kg at 5 and 2 wk before surgery	▪ ↑ Penile length by 2.7 cm and circumference by 2.3 cm ▪ Negligible side effects

Abbreviations: adv, advancement; BA, bone age; mo, month(s); preop, preoperatively; T, testosterone; tx, treated/treatment; wk, week(s); yr, year(s).

2b. *Topical Testosterone/Dihydrotestosterone*

Reference	Study Design	Subjects	Regimen	Results
Chalapathi et al. 2003 (179)	Prospective control trial comparing topical vs	26 subjects age 1-10 yr (3.88 yr)	*Topical* Testoviron 2 mg/kg/wk applied BID × 3 wk *Parenteral*	▪ ↑ Penile growth (unstretched penile length) ▫ Response not significantly different between groups

	intramuscular testosterone No comment on blinding or randomization		Testoviron 2 mg/kg weekly × 3 wk	▪ Transient ↑ T from baseline in both groups ▪ Transient pubic hair in 2 of topical group
Sakakibara et al. 1991 (180)	Case series	15 subjects 2-9 yr	T ointment 0.2-0.4 mg/day × 3 wk then off × 1 wk (Repeated for ≥ 3 cycles unless adverse rxn)	▪ "Increase in overall penile size" ▪ Transient ↑ T levels ▪ Transient localized hyperpigmentation ▪ No significant pubic hair
Paiva et al. 2016 (181)	Randomized double blind placebo-controlled trial comparing topical T vs. topical E2 vs. placebo	69 total subjects ▪ n = 28 testosterone group (mean age 31.39 mo) ▪ n = 24 E$_2$ group ▪ n = 17 placebo group	1% T propionate BID × 30 days vs 0.01% E2 × 30 days vs placebo	▪ ↑ Penile length in testosterone group (P = 0.005); no significant increase in E$_2$ or control group ▪ ↑ T in testosterone group (not statistically significant) ▪ More pubic hair and localized hyperpigmentation in testosterone than E$_2$ group
Tsur et al. 1983 (182)	Prospective case series	7 subjects 2-6 yr	10% T propionate BID × 3 wk	▪ ↑ Penile length from 1.8-2.7 cm to 3.0-3.6 cm ▪ Transient ↑ T
Kaya et al. 2008 (183)	Prospective randomized control trial	75 subjects 10.6-159.1 mo (mean 33.4 mo)	2.5% DHT 0.2-0.3 mg/kg/day × 3 mo vs no tx	▪ Improved cosmetic results ○ Less glandular dehiscence (P < 0.05) in DHT group vs control ○ Less mod/severe scaring (P < 0.05) in DHT group vs. control ○ No significant difference in rates of postop urethrocutaneous fistulas or meatal stenosis ▪ Transient itching, redness in DHT group

				▪ Transient localized hyperpigmentation

Abbreviations: BID, twice a day; DHT, dihydrotestosterone; mo, month(s); postop, postoperatively; rxn, reaction; T, testosterone; tx, treated/treatment; wk, week(s); yr, year(s).

Testoviron: T proprionate 25 mg + T enanthate 110 mg (equivalent to 100 mg T)

Table 3. Differential Diagnosis of Delayed Puberty in Males

Hypogonadotropic hypogonadism

Constitutional delay of growth and puberty (transient)

Congenital Gonadotropin deficiency

Kallmann syndrome (anosmia/hyposmia):

Anosmin 1 *(KAL1)*

Fibroblast growth factor receptor 1 *(FGFR1)*

Prokineticin 2 and receptor (*PROK2/PROKR2*)

Fibroblast growth factor 8 (*FGF8*)

Nasal embryonic LHRH factor (*NELF*)

Heparan sulphate 6'O'sulphotransferase 2 (*HS6ST2*)

Mutations not associated with anosmia

Dosage-sensitive sex reversal, adrenal hypoplasia congenital, critical region on the X chromosome, gene 1 (*DAX1)*

Gonadotropin releasing hormone receptor (*GNRHR*)

Kisspeptin (*KISS)*

G-protein coupled receptor 54 (*KISS1R or GPR54)*

Tachykinin 3 and receptor (*TAC3/TACR3)*

Gonadotropin releasing hormone 1 (*GNRH1*)

Gonadotropin releasing hormone receptor (*GNRHR*)

Leptin and Leptin receptor (*LEP/LEPR)*

Luteinizing hormone β subunit (*LHβ)*

Prohormone convertase 1 (*PC1)*

Homeobox protein prophet of Pit-1 (*PROP1)*

Homeobox expressed in ES cells 1 (*HESX1)*

LIM/homeobox protein Lhx3 *(LHX3)*

Transcription factor SOX-3 (*SOX3)*

Acquired gonadotropin deficiency

Brain tumor (craniopharyngioma, pituitary adenoma, germinoma)

Brain irradiation

- Traumatic brain injury
- Infection (tuberculosis, syphilis)
- Infiltrative (hemochromatosis, Langerhans cell histiocytosis)
- Isolated LH deficiency
- Genetic syndromes
  - Prader-Willi syndrome
  - CHARGE syndrome
  - Gordon Holmes syndrome
  - Bardet-Biedl syndrome
  - Moebius syndrome
- Hyperprolactinemia
- Idiopathic

Hypergonadotropic hypogonadism
- Klinefelter syndrome
- XY gonadal dysgenesis
- Anorchia or vanishing testes syndrome
- LH resistance (mutation in LH receptor)
- Pseudohypoparathyroidism
- Autoimmune gonadal failure
- Acquired
  - Pelvic/testicular irradiation
  - Chemotherapy (alkylating agents)
  - Viral orchitis (mumps)

Chronic disorders associated with pubertal delay
- Diabetes mellitus
- Nephrotic syndrome
- Hypothyroidism
- Sickle cell disease
- Thalassemia
- Celiac disease
- Inflammatory bowel disease
- Juvenile arthritis
- Cystic fibrosis

Stress/nutritional disorders
- Excessive exercise
- Anorexia nervosa/bulimia
- Malnourishment

Iatrogenic/medication-induced

■ Anabolic steroid abuse

■ Glucocorticoids

Table 4. Advantages and Disadvantages of Different Testosterone Formulations

Along With Reported Dosing for Pubertal Induction

	Starting Dose for Pubertal Induction	Adult Dose	Advantages	Disadvantages
Intramuscular				
Short-acting combination				
■ Testosterone propionate/testosterone phenylpropionate/testosterone isocaproate/testosterone deconoate (Sustanon)	25 mg/m2 every 2 weeks	250 mg every 3-4 weeks	• Low cost • Good adherence • Most data and clinical experience to support use in adolescents	• Large swings in T concentration, not physiological • Painful injections • Fluctuations in mood and energy
Intermediate-acting				
■ Testosterone cypionate (Depo-Testosterone) Testosterone enanthate (Delatestryl)	25-100 mg every 2-4 weeks	75-100 mg weekly or 150-200 mg every 2 weeks		
Long-acting (3-months) ■ Testosterone undeconoate (Nebido)	Not reported	1000 mg every 12 weeks	• Stable serum T concentrations • Less frequent injections	• Painful injections • Expensive • Lack of data in adolescents
Transdermal				
■ Testosterone gel (AndroGel, Axiron, Fortesta, Testim, Tostrex 2%)	0.5 grams/day	1%: 5-10 mg/day 2%: 2-4 mg/day	• Mimics normal physiology	• Potential transfer to other people • Daily administration may limit adherence

	Starting dose	Maintenance dose	Advantages	Disadvantages
Testosterone patch *Scrotal* (Testoderm)	Not reported	4-6 mg/day	• Mimics normal physiology	• Skin irritation • Patch is too large for prepubertal boys • High DHT levels • Daily administration may limit adherence • Lack of data in adolescents
Nonscrotal (Androderm or Andropatch)	2.5 mg × 12-24 hours/day or 5 mg × 8 hours/day	2.5-5 mg/day	• Mimics normal physiology	• Skin irritation • Lack of data in adolescents • Daily administration may limit adherence
Oral				
Testosterone undeconoate (Restandol, Andriol, or Jatenzo)	40 mg daily, increasing to 40 mg twice daily	40-80 mg 2-3 times daily	• Oral, pain-free	• Multiple doses needed per day • Fluctuating serum T concentrations
Buccal testosterone (Striant)	Not reported	30 mg twice daily	• Physiologic release of T	• Altered taste • Gum irritation
Subcutaneous				
Testosterone cypionate	25 mg SQ every other week	50-70 mg SQ weekly	• Less pain than IM injections • Can administer at home	• Lack of data in adolescents (other than transgender protocols)
Subdermal testosterone pellets (Testopel)	8-10 mg/kg	3-4 pellets (75 mg each) every 4-6 months	• Able to maintain normal T levels for months • Improved compliance compared with other formulations	• Need for repeat implantations • Risk of extrusion, infection, fibrosis • Cost
Anabolic steroids				
Oxandrolone	0.1 mg/kg/d or 2.5 mg daily	Not applicable	• Oral, pain-free administration • Possible increase in height prediction	• Weak androgen • Cannot be converted to DHT or estradiol

Abbreviations: T, testosterone; DHT, dihydrotestosterone; IM, intramuscular; SQ, subcutaneous

Table 5. Review of Published Data Using Non-IM Testosterone Preparations in Pediatric Patients

5a. *Testosterone Gel*

Reference	Study Design	Subjects	Regimen	Results
Chioma et al. 2018	Retrospective review	73 boys with CDGP • 26 treated with gel (mean age 14.3 yrs) • 25 treated with IM T • 22 not treated	• Gel: 2% gel, 10 mg daily for 3 months • IM T: 50 mg T enanthate every 4 weeks for 3 months	• Same effectiveness between formulations. • No difference in bone age maturation between gel and IM after 6 months. • Growth velocity higher in both treatment groups at 6 months compared with control group.
Contreras et al. 2017	Retrospective case series	3 boys with CDGP and hepatic dysfunction (ages 16-17 yrs)	• 2% gel; 10 mg/day • 1% gel 12.5 mg/day increasing up to 50 mg/d	• Effective in raising serum T levels. • Liver enzymes decreased over time. • No AE.
Rogol et al. 2014	Retrospective analysis of observational studies	21 boys with KS (mean age 14.2 yrs) and 8 boys with anorchia (mean age 13.1 yrs)	0.5 g once daily of 1% gel, increasing up to 5 g/day as needed for up to 6 months	• Appropriate increase in measured serum T to pubertal range during treatment. • Compliance with daily application was 72%. • Most common AE reported were cough, headache, and acne.
Mehta et al. 2014	Retrospective cohort analysis	110 boys with KS • 104 treated with gel • 5 treated with IM T • 1 treated with pellets	No specifics given other than dose titrated to achieve a serum T in high-normal range	• 75 of the 110 were also treated with aromatase inhibitors. • Appropriate rise in mean serum T noted as age increased. • LH and FSH rose with age despite treatment (means 16.6 and 42 mIU/mL respectively). • No AE reported. • 25% reported dissatisfaction with gel application and 5% switched to injections due to poor compliance.
Mehta et al. 2013	Retrospective case series	10 boys with KS (mean age 15.5 yrs)	Treatment started when T <350 ng/dL and dose adjusted for goal T >400 ng/dL	• All also treated with aromatase inhibitor. • Goal of treatment was fertility (all underwent testicular sperm extraction). • Successful sperm retrieval in 7 (70%).

5b. *Oral Testosterone Undecanoate*

Reference	Study Design	Subjects	Regimen	Results
Lawaetz et al. 2015	Retrospective observational	159 boys with CDGP • 96 treated with oral	Average starting dose of 40 mg daily, increased to	• Boys who were not treated had larger testes and higher concentrations of LH, FSH, and T at diagnosis.

Reference	Study Design	Subjects	Regimen	Results
	study	TU (median age 15 yrs) • 63 untreated	40 mg twice daily, then up to max of 80 mg twice daily • Average treatment duration 0.8 yrs	• Adequate pubertal progression along with increased growth and IGF-1 in all boys who were treated. • PAH was not compromised with treatment. • Untreated boys also progressed in puberty, though reached Tanner 5 at later ages compared with those who were treated.
Schmidt et al. 1998	Case series presented in letter to editor	10 boys with anorchia (age 11-14 yrs)	• 40 mg 3×/week (4 subjects) • 40 mg daily × 1 year followed by 80 mg daily × 1 year (6 subjects)	• 40 mg thrice weekly deemed insufficient • Higher dose regimen resulted in pubic hair development, increased growth, and increased SPL. • No AE reported.
Brown et al. 1995	Randomized, double-blinded, placebo-controlled trial	23 prepubertal boys with short stature (age 11-14 years) • 11 given oral TU • 12 placebo	20 mg daily × 6 months	• Increased HV in boys taking TU compared with controls. • No change in T concentrations or BA advancement. • No AE reported.
Albanese et al. 1994	Randomized trial	33 boys with CDGP (age 12-16 years) • 17 treated with oral TU • 16 treated with oxandrolone	• TU: 40 mg daily × mean of 3.5 months • Oxandrolone: 2.5 mg/day	• Both treatments induced growth acceleration in all but 4 patients (3 with TU and 1 with oxandrolone), but all "non-responders" showed improved growth when treated with alternative sex steroid. • No significant difference in growth velocity or bone maturation between groups. • Increase in testicular volume seen in both groups at follow-up.
Butler et al. 1992	Prospective, noncontrolled, single-dose pharmacokinetics study	4 boys aged 12-17 years with CDGP and/or short stature	40 mg daily × 15-21 months	• All had pubertal progression and growth acceleration without change in PAH. • No AE. • Continued pubertal progression was evident after cessation of treatment.
Gregory et al. 1992	Double-blind, placebo-controlled study	18 boys with CDGP (mean age 13.2 yrs)	40 mg daily × 3 months	• Increase in HV and fat-free mass velocity compared with placebo after 6 months. • Decrease in PAH in treated group.

5c. *Testosterone Patch*

Reference	Study Design	Subjects	Regimen	Results

Mason et al. 2011	Retrospective review	8 boys with IBD (median age 14.8 yrs) • 3 treated with patch • 5 treated with monthly IM Sustanon	• 2.5 mg × 12 hours/day × 6 months (2 subjects) • 5 mg × 12 hours/day × 2 months, then 2.5 mg × 12 hours/day × 2 months (1 subject)	• 2 boys treated with patch had pubertal progression after 6 months and 1 did not. • All boys with IBD treated with IM T demonstrated an increase in HV >50%, whereas 2 boys treated with patch did not (possibly related to disease severity).
Mayo et al. 2004	Prospective, randomized, crossover study	8 boys with delayed puberty (median age 13.5 yrs) • 1 had DM and 1 had CF	5 mg patch applied for either 8 or 12 hrs overnight × 8 weeks (then crossover)	• Both doses simulated physiological T secretion and increased short-term growth and markers of bone turnover. • No AE other than minimal skin irritation.
De Sanctis et al. 1998	Prospective, not randomized (dose based on age)	9 males with beta-thalassemia major and hypogonadism (age 16-31 yrs)	• 14-16 yrs: 2.5 mg patch × 12 hrs overnight • 17-19 yrs: 2.5 mg × 24 hrs • 20+ yrs: 5 mg × 24 hrs	• Doses determined by age. • Well-tolerated with physiologically appropriate T levels. • Promoted growth, virilization, and increased BMD.

5d. *Subdermal Testosterone Pellets*

Reference	Study Design	Subjects	Regimen	Results
Moskovic et al. 2012	Retrospective case series	4 males with KS and history of noncompliance with other T formulations (age 14-20 yrs)	Initial dose of 4-10 pellets, dose increased with subsequent implantations every 3-4 months based on laboratory results	• All subjects presumed to be Tanner 5 at baseline. • Appropriate increase in serum T observed though wide variability noted. • No AE other than minimal discomfort with procedure.
Zacharin et al. 1997	Prospective study, nonrandomized	16 boys with hypogonadism and 2 boys treated for tall stature (age 13-17 yrs)	8-10 mg/kg every 6 months	• All had received some other form of T prior to study. • Normal pubertal and growth progression. T levels maintained for 4-6 months after implantation. • No AE reported and all reportedly preferred to IM.

Abbreviations: AE, adverse events; BA, bone age; BMD, bone mineral density; CDGP, constitutional delay of growth and puberty; CF, cystic fibrosis; DM, diabetes mellitus; hrs, hours; IBD, inflammatory bowel disease; IM, intramuscular; KS, Klinefelter syndrome; PAH, predicted adult height; SPL, stretched penile length; T, testosterone; TU, testosterone undecanoate; yrs, years.

Table 6. Testosterone Regimens and Masculinizing Effects in Transgender Males

Pubertal Induction Adolescents (testosterone esters)	Therapy in Postpubertal Adolescents (testosterone esters)	Adult Therapy	Effects[d]
Intramuscular[a,b,c] ▪ 25 mg/m2/q2 wk ▪ 50 mg/m2/q2 wk ▪ 75 mg/m2/q2 wk ▪ 100 mg/m2/q2 wk ▪ 100-200 mg q2 wk *Subcutaneous* ▪ 25 mg q2 wk × 8 wk ▪ 25 mg weekly × 4 wk ▪ Up to 50 mg weekly if tolerated	*Intramuscular*[c] ▪ 75 mg q2 wk × 6 mo ▪ 125 mg q2 wk	*Intramuscular* ▪ T esters 100-200 mg q2 wk ▪ T undecanoate 1000 mg q12wk *Subcutaneous* 25-150 mg weekly (mean 46.4 mg/wk) *Topical* ▪ Gel 1.6% 50-100 mg/day ▪ Patch 2.5-7.5 mg/day	▪ Acne/skin oiliness 1-6 mo (1-2 yr) ▪ Clitoromegaly 1-6 mo (1-2 yr) ▪ ↓Fat mass 1-6 mo (2-5 yr) ▪ Cessation of menses (usually) 1-6 mo ▪ ↑Muscle mass 6-12 mo (2-5 yr) ▪ ↑Facial and body hair (particularly face, chest, abdomen) 6-12 mo (4-5 yr) ▪ Male pattern baldness (esp. in those genetically predisposed) 6-12 mo ▪ Voice deepening 6-12 mo (1-2 yr) ▪ ↑Sexual desire ▪ Temporary or permanent ↓fertility

[a]Increased every 6 months

[b]Alternative regimen: Half the dose weekly or double the dose q4 weeks

[c]SQ dosing roughly equivalent to 50% IM dosing

[d]Expected onset (maximum onset)

SQ T drawn into 1 mL syringe with 20-25G needle; 25G 5/8" needle used for injection (177)

Abbreviations: esp (especially); IM, intramuscular(ly); mo, month(s); SQ, subcutaneous(ly); T, testosterone; wk, week(s); yr, year(s).