

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

Kelly A. Mason,^{1,*} Melissa J. Schoelwer,^{1,*} and Alan D. Rogol¹

¹Department of Pediatrics, University of Virginia, Charlottesville, Virginia 22903

*Melissa J. Schoelwer and Kelly A. Mason contributed equally and are co-first authors of this article.

ORCID numbers: 0000-0001-6356-0353 (K. A. Mason); 0000-0002-7165-3434 (M. J. Schoelwer).

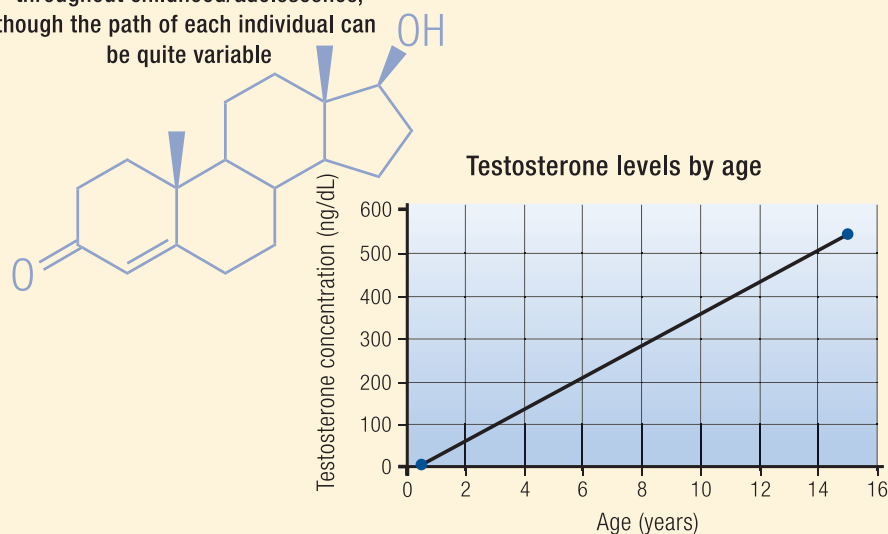
Abstract We provide an in-depth review of the role of androgens in male maturation and development, from the fetal stage through adolescence into emerging adulthood, and discuss the treatment of disorders of androgen production throughout these time periods. Testosterone, the primary androgen produced 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

Testosterone increases dramatically throughout childhood/adolescence, though the path of each individual can be quite variable



Key Words: androgens, testosterone, virilization, puberty, mini puberty, transgender

ISSN Print: 0163-769X

ISSN Online: 1945-7189

Printed in USA

© Endocrine Society 2020.

All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

Received: 23 June 2019

Accepted: 28 February 2020

First Published Online: 2 March 2020

Corrected and Typeset 30 March 2020.

ESSENTIAL POINTS

- Androgens play a central role throughout the male lifespan.
- Androgens induce virilization of the male fetus, influence body composition and fetal growth, and contribute to organizational changes within the developing nervous system.
- Androgen therapy is frequently used for micropenis and hypospadias during infancy.
- Apart from the treatment of micropenis and hypospadias, androgen therapy has limited applications during childhood, but has been studied in children with Klinefelter syndrome.
- 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.
- 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.
- 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 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 (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 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 (3β HSD) (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

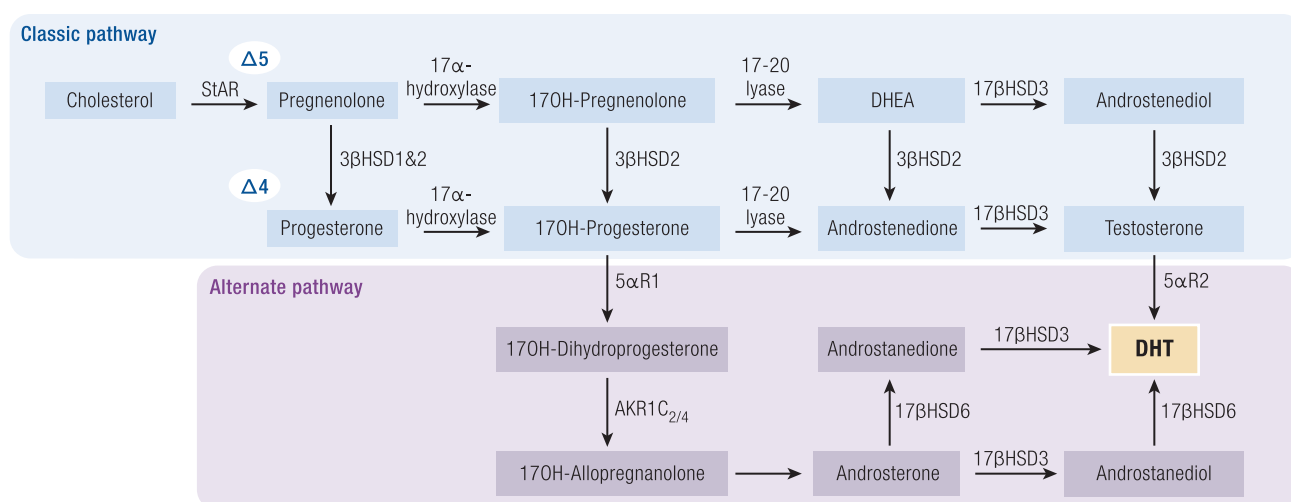


Figure 1. Diagram of the classic pathway (depicting both the $\Delta 4$ and $\Delta 5$ pathways) and alternate pathway for the production of dihydrotestosterone. Note that the alternate pathway bypasses testosterone as an intermediate. Not all intermediates, pathways, or enzymes may be shown. Abbreviations: 17 β HSD3, 17-hydroxysteroid dehydrogenase type 3; 17 β HSD6, 17-hydroxysteroid dehydrogenase type 6; 3 β HSD1&2, 3-hydroxysteroid dehydrogenase types 1 and 2; 5 α R1&2, 5 α -reductase types 1 and 2; DHT, dihydrotestosterone; StAR, steroidogenic acute regulatory protein.

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 5 α -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 5 α -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 5 α -reductase type 1 isoenzyme (41).

Mutations in 17 β -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 5 α -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 chondrocyte 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 5 α -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).

Table 1. Review of Published Data Using Androgen Therapy for Micropenis

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)	<ul style="list-style-type: none"> ■ ↑ 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/m ² /dose q2–4 wk × 3–4 injections	<ul style="list-style-type: none"> ■ ↑ 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	<ul style="list-style-type: none"> ■ ↑ 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	<ul style="list-style-type: none"> ■ ↑ Penile length from 2.5 to 3.5 cm (-2.74 to -0.72 SD) <ul style="list-style-type: none"> ○ Independent of CAG repeat and V89L polymorphisms (sequenced SRDSA2 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)	<ul style="list-style-type: none"> ■ ↑ 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	<ul style="list-style-type: none"> ■ ↑ 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

Reference	Study Design	Subjects	Regimen	Results
Arisaka et al. 2001 (118)	Case series	50 subjects 5 mo to 8 yr	5% T 0.2 g/day × 30 days	<ul style="list-style-type: none"> ■ ↑ 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	<ul style="list-style-type: none"> ■ ↑ 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

Abbreviations: adv, advancement; BA, bone age; d/c, discontinue/discontinuation; ea, each; HV, height velocity; IGF-1, insulin-like growth factor 1; mo, month(s); SDS, standard deviation score; T, testosterone; TID, 3 times a day; tx, treated/treatment; wk, week(s); yr, year(s).

1c. Topical Dihydrotestosterone

Reference	Study Design	Subjects	Regimen	Results
Choi et al. 1993 (120)	Case series	22 subjects 3-15 yr (9.7 yr)	<ul style="list-style-type: none"> ■ 12.5 mg × 8 wk if age < 10 yr ■ 25 mg × 8 wk if age > 10 yr 	<ul style="list-style-type: none"> ■ ↑ 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	<ul style="list-style-type: none"> ■ ↑ Penile length 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	<ul style="list-style-type: none"> ■ ↑ 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 <ul style="list-style-type: none"> ■ 5 mg in age >3 yr ■ 2.5 mg in age <3 yr 	<ul style="list-style-type: none"> ■ ↑ 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

Abbreviations: adv, advancement; alk phos, alkaline phosphatase; BA, bone age; d/c, discontinue/discontinuation; DHT, dihydrotestosterone; ea, each; HPG, hypothalamic-pituitary-gonadal (axis); HV, height velocity; mo, month(s); PAIS, partial androgen insensitivity syndrome; SDS, standard deviation score; T, testosterone; tx, treated/treatment; wk, week(s); yr, year(s).

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

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	<ul style="list-style-type: none"> ■ ↑ 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	<ul style="list-style-type: none"> ■ ↑ 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 (115)	17 Japanese subjects 0-5 yr (mean 1.4 yr)	25 mg q4 wk for 1-3 injections	<ul style="list-style-type: none"> ■ 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	<ul style="list-style-type: none"> ■ ↑ 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
Chalapati et al. 2003 (179)	Prospective control trial comparing topical vs intramuscular testosterone No comment on blinding or randomization	26 subjects age 1-10 yr (3.88 yr)	<i>Topical</i> Testoviron 2 mg/kg/wk applied BID × 3 wk <i>Parenteral</i> Testoviron 2 mg/kg weekly × 3 wk	<ul style="list-style-type: none"> ■ ↑ Penile growth (unstretched penile length) <ul style="list-style-type: none"> ○ Response not significantly different between groups ■ 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)	<ul style="list-style-type: none"> ■ "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 <ul style="list-style-type: none"> ■ n = 28 testosterone group (mean age 31.39 mo) ■ n = 24 E2 group ■ n = 17 placebo group 	1% T propionate BID × 30 days vs 0.01% E2 × 30 days vs placebo	<ul style="list-style-type: none"> ■ ↑ Penile length in testosterone group ($P = 0.005$); no significant increase in E2 or control group ■ ↑ T in testosterone group (not statistically significant) ■ More pubic hair and localized hyperpigmentation in testosterone than E2 group
Tsur et al. 1983 (182)	Prospective case series	7 subjects 2-6 yr	10% T propionate BID × 3 wk	<ul style="list-style-type: none"> ■ ↑ 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	<ul style="list-style-type: none"> ■ Improved cosmetic results <ul style="list-style-type: none"> ○ Less glandular dehiscence ($P < 0.05$) in DHT group vs control ○ Less mod/severe scarring ($P < 0.05$) 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 propionate 25 mg + T enanthate 110 mg (equivalent to 100 mg T)

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 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 biobehavioral, 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 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 $\mu\text{g}/\text{m}^2$ 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), 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 Edelsztejn 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 5 α -reductase or aromatized to 17 β -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 5 α -reductase, such as the prostate, where the effects of testosterone are amplified according to the enzymatic activity of 5 α -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 5 α -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

Table 3. Differential Diagnosis of Delayed Puberty in Males

<p>Hypogonadotropic hypogonadism</p> <p>Constitutional delay of growth and puberty (transient)</p> <p>Congenital Gonadotropin deficiency</p> <p>Kallmann syndrome (anosmia/hyposmia):</p> <ul style="list-style-type: none"> Anosmin 1 (<i>KAL1</i>) Fibroblast growth factor receptor 1 (<i>FGFR1</i>) Prokineticin 2 and receptor (<i>PROK2/PROKR2</i>) Fibroblast growth factor 8 (<i>FGF8</i>) Nasal embryonic LHRH factor (<i>NELF</i>) Heparan sulphate 6'O'sulphotransferase 2 (<i>HS6ST2</i>) <p>Mutations not associated with anosmia</p> <ul style="list-style-type: none"> Dosage-sensitive sex reversal, adrenal hypoplasia congenital, critical region on the X chromosome, gene 1 (<i>DAX1</i>) Gonadotropin releasing hormone receptor (<i>GNRHR</i>) Kisspeptin (<i>KISS</i>) G-protein coupled receptor 54 (<i>KISS1R</i> or <i>GPR54</i>) Tachykinin 3 and receptor (<i>TAC3/TACR3</i>) Gonadotropin releasing hormone 1 (<i>GNRH1</i>) Gonadotropin releasing hormone receptor (<i>GNRHR</i>) Leptin and Leptin receptor (<i>LEP/LEPR</i>) Luteinizing hormone β subunit (<i>LHβ</i>) Prohormone convertase 1 (<i>PCT1</i>) Homeobox protein prophet of Pit-1 (<i>PROPT1</i>) Homeobox expressed in ES cells 1 (<i>HESX1</i>) LIM/homeobox protein Lhx3 (<i>LHX3</i>) Transcription factor SOX-3 (<i>SOX3</i>) <p>Acquired gonadotropin deficiency</p> <ul style="list-style-type: none"> Brain tumor (craniopharyngioma, pituitary adenoma, germinoma) Brain irradiation Traumatic brain injury Infection (tuberculosis, syphilis) Infiltrative (hemochromatosis, Langerhans cell histiocytosis) <p>Isolated LH deficiency</p> <p>Genetic syndromes</p> <ul style="list-style-type: none"> Prader-Willi syndrome CHARGE syndrome Gordon Holmes syndrome Bardet-Biedl syndrome Moebius syndrome <p>Hyperprolactinemia</p> <p>Idiopathic</p>
<p>Hypergonadotropic hypogonadism</p> <p>Klinefelter syndrome</p> <p>XY gonadal dysgenesis</p> <p>Anorchia or vanishing testes syndrome</p> <p>LH resistance (mutation in LH receptor)</p> <p>Pseudohypoparathyroidism</p> <p>Autoimmune gonadal failure</p> <p>Acquired</p> <ul style="list-style-type: none"> Pelvic/testicular irradiation Chemotherapy (alkylating agents) Viral orchitis (mumps)
<p>Chronic disorders associated with pubertal delay</p> <ul style="list-style-type: none"> Diabetes mellitus Nephrotic syndrome Hypothyroidism Sickle cell disease Thalassemia Celiac disease Inflammatory bowel disease Juvenile arthritis Cystic fibrosis
<p>Stress/nutritional disorders</p> <ul style="list-style-type: none"> Excessive exercise Anorexia nervosa/bulimia Malnourishment

Table 3. Continued

<ul style="list-style-type: none"> iatrogenic/medication-induced Anabolic steroid abuse Glucocorticoids
--

Abbreviations: LH, luteinizing hormone

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 decanoate) (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 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 C_{max} 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

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				
<i>Short-acting combination</i>				
Testosterone propionate/testosterone phenylpropionate/testosterone isocaproate/testosterone decanoate (Sustanon)	25 mg/m ² every 2 weeks	250 mg every 3-4 weeks	<ul style="list-style-type: none"> • Low cost • Good adherence • Most data and clinical experience to support use in adolescents 	<ul style="list-style-type: none"> • Large swings in T concentration, not physiological • Painful injections • Fluctuations in mood and energy
<i>Intermediate-acting</i>				
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		
<i>Long-acting (3-months)</i> Testosterone undecanoate (Nebido)	Not reported	1000 mg every 12 weeks	<ul style="list-style-type: none"> • Stable serum T concentrations • Less frequent injections 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Mimics normal physiology 	<ul style="list-style-type: none"> • Potential transfer to other people • Daily administration may limit adherence
Testosterone patch <i>Scrotal</i> (Testoderm)	Not reported	4-6 mg/day	<ul style="list-style-type: none"> • Mimics normal physiology 	<ul style="list-style-type: none"> • Skin irritation • Patch is too large for prepubertal boys • High DHT levels • Daily administration may limit adherence • Lack of data in adolescents
<i>Nonscrotal</i> (Androderm or Andropatch)	2.5 mg × 12-24 hours/day or 5 mg × 8 hours/day	2.5-5 mg/day	<ul style="list-style-type: none"> • Mimics normal physiology 	<ul style="list-style-type: none"> • Skin irritation • Lack of data in adolescents • Daily administration may limit adherence
Oral				
Testosterone undecanoate (Restandol, Andriol, or Jatenzo)	40 mg daily, increasing to 40 mg twice daily	40-80 mg 2-3 times daily	<ul style="list-style-type: none"> • Oral, pain-free 	<ul style="list-style-type: none"> • Multiple doses needed per day • Fluctuating serum T concentrations
Buccal testosterone (Striant)	Not reported	30 mg twice daily	<ul style="list-style-type: none"> • Physiologic release of T 	<ul style="list-style-type: none"> • Altered taste • Gum irritation
Subcutaneous				
Testosterone cypionate	25 mg SQ every other week	50-70 mg SQ weekly	<ul style="list-style-type: none"> • Less pain than IM injections • Can administer at home 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Able to maintain normal T levels for months • Improved compliance compared with other formulations 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Oral, pain-free administration • Possible increase in height prediction 	<ul style="list-style-type: none"> • 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 <ul style="list-style-type: none"> • 26 treated with gel (mean age 14.3 yrs) • 25 treated with IM T • 22 not treated 	<ul style="list-style-type: none"> • Gel: 2% gel, 10 mg daily for 3 months • IM T: 50 mg T enanthate every 4 weeks for 3 months 	<ul style="list-style-type: none"> • 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)	<ul style="list-style-type: none"> • 2% gel; 10 mg/day • 1% gel 12.5 mg/day increasing up to 50 mg/d 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 <ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • All also treated with aromatase inhibitor. • Goal of treatment was fertility (all underwent testicular sperm extraction). • Successful sperm retrieval in 7 (70%).

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

5b. Oral Testosterone Undecanoate

Reference	Study Design	Subjects	Regimen	Results
Lawaetz et al. 2015	Retrospective observational study	159 boys with CDGP <ul style="list-style-type: none"> • 96 treated with oral TU (median age 15 yrs) • 63 untreated 	Average starting dose of 40 mg daily, increased to 40 mg twice daily, then up to max of 80 mg twice daily <ul style="list-style-type: none"> • Average treatment duration 0.8 yrs 	<ul style="list-style-type: none"> • Boys who were not treated had larger testes and higher concentrations of LH, FSH, and T at diagnosis. • 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)	<ul style="list-style-type: none"> • 40 mg 3x/week (4 subjects) • 40 mg daily × 1 year followed by 80 mg daily × 1 year (6 subjects) 	<ul style="list-style-type: none"> • 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) <ul style="list-style-type: none"> • 11 given oral TU • 12 placebo 	20 mg daily × 6 months	<ul style="list-style-type: none"> • 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) <ul style="list-style-type: none"> • 17 treated with oral TU • 16 treated with oxandrolone 	<ul style="list-style-type: none"> • TU: 40 mg daily × mean of 3.5 months • Oxandrolone: 2.5 mg/day 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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) <ul style="list-style-type: none"> • 3 treated with patch • 5 treated with monthly IM Sustanon 	<ul style="list-style-type: none"> • 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) 	<ul style="list-style-type: none"> • 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) <ul style="list-style-type: none"> • 1 had DM and 1 had CF 	5 mg patch applied for either 8 or 12 hrs overnight × 8 weeks (then crossover)	<ul style="list-style-type: none"> • 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)	<ul style="list-style-type: none"> • 14-16 yrs: 2.5 mg patch × 12 hrs overnight • 17-19 yrs: 2.5 mg × 24 hrs • 20+ yrs: 5 mg × 24 hrs 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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.

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 17 α -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

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
<i>Intramuscular^{a,b,c}</i> <ul style="list-style-type: none"> ■ 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 <i>Subcutaneous</i> <ul style="list-style-type: none"> ■ 25 mg q2 wk × 8 wk ■ 25 mg weekly × 4 wk ■ Up to 50 mg weekly if tolerated 	<i>Intramuscular^c</i> <ul style="list-style-type: none"> ■ 75 mg q2 wk × 6 mo ■ 125 mg q2 wk 	<i>Intramuscular</i> <ul style="list-style-type: none"> ■ T esters 100-200 mg q2 wk ■ T undecanoate 1000 mg q12wk <i>Subcutaneous</i> <ul style="list-style-type: none"> ■ 25-150 mg weekly (mean 46.4 mg/wk) <i>Topical</i> <ul style="list-style-type: none"> ■ Gel 1.6% 50-100 mg/day ■ Patch 2.5-7.5 mg/day 	<ul style="list-style-type: none"> ■ 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

^aIncreased every 6 months

^bAlternative regimen: Half the dose weekly or double the dose q4 weeks

^cSQ dosing roughly equivalent to 50% IM dosing

^dExpected 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).

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.

References

- Nieschlag E, Nieschlag S. Testosterone deficiency: a historical perspective. *Asian J Androl*. 2014;**16**(2):161–168.
- Loewe A, Voss HE. [update on the male sex hormone]. *Klin Wochenschr*. 1930;**9**:481–487.
- Butenandt A. The chemical investigation of the sex hormone. *Z Angew Chem*. 1931;**44**:905–908.
- David K, Dingemanse E, Freud J, Laquer E. Crystalline male hormone from the testes (testosterone) is more effective than androsterone derived from urine or cholesterol. *Hoppe-Seylers Z Physiol Chem*. 1935;**233**:281–282.
- Butenandt A, Hanisch G. Testosterone. The transformation of dehydroandrosterone into androstendiol and testosterone; a method for producing testosterone from cholesterol. *Hoppe-Seylers Z Physiol Chem*. 1935;**237**:89–98.
- Ruzicka L, Wettstein A. Synthesis of the testicular hormone (testosterone) (androsterone 3-on-17-ol). *Helv Chim Acta*. 1935;**18**:1264–1275.
- O’Shaughnessy PJ, Antignac JP, Le Bizec B, et al. Alternative (backdoor) androgen production and masculinization in the human fetus. *Plos Biol*. 2019;**17**(2):e3000002.
- Welsh M, Saunders PT, Fiskin M, et al. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. *J Clin Invest*. 2008;**118**(4):1479–1490.
- Hakim C, Padmanabhan V, Vyas AK. Gestational hyperandrogenism in developmental programming. *Endocrinology*. 2017;**158**(2):199–212.
- Conte FA, Grumbach MM, Ito Y, Fisher CR, Simpson ER. A syndrome of female pseudohermaphroditism, hypergonadotropic hypogonadism, and multicystic ovaries associated with missense mutations in the gene encoding aromatase (P450arom). *J Clin Endocrinol Metab*. 1994;**78**(6):1287–1292.
- Bulun SE. Aromatase and estrogen receptor α deficiency. *Fertil Steril*. 2014;**101**(2):323–329.
- Svechnikov K, Landreh L, Weisser J, et al. Origin, development and regulation of human Leydig cells. *Horm Res Paediatr*. 2010;**73**(2):93–101.
- Kuiri-Hänninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. *Horm Res Paediatr*. 2014;**82**(2):73–80.
- Bouvattier C, Maione L, Bouligand J, Dodé C, Guiochon-Mantel A, Young J. Neonatal gonadotropin therapy in male congenital hypogonadotropic hypogonadism. *Nat Rev Endocrinol*. 2011;**8**(3):172–182.
- Cimador M, Catalano P, Ortolano R, Giuffrè M. The inconspicuous penis in children. *Nat Rev Urol*. 2015;**12**(4):205–215.
- Riccetti L, Yvinec R, Klett D, et al. Human luteinizing hormone and chorionic gonadotropin display biased agonism at the LH and LH/CG receptors. *Sci Rep*. 2017;**7**(1):940.
- Müller T, Gromoll J, Simoni M. Absence of exon 10 of the human luteinizing hormone (LH) receptor impairs LH, but not human chorionic gonadotropin action. *J Clin Endocrinol Metab*. 2003;**88**(5):2242–2249.
- Szilágyi A, Benz R, Rossmanith WG. The human first-term placenta in vitro: regulation of hCG

- secretion by GnRH and its antagonist. *Gynecol Endocrinol*. 1992;**6**(4):293–300.
19. Pijnenborg R, Vercauteren L, Hanssens M. The uterine spiral arteries in human pregnancy: facts and controversies. *Placenta*. 2006;**27**(9–10):939–958.
 20. Reyes FI, Boroditsky RS, Winter JS, Faiman C. Studies on human sexual development. II. Fetal and maternal serum gonadotropin and sex steroid concentrations. *J Clin Endocrinol Metab*. 1974;**38**(4):612–617.
 21. Tapanainen J, Kellokumpu-Lehtinen P, Pelliniemi L, Huhtaniemi I. Age-related changes in endogenous steroids of human fetal testis during early and midpregnancy. *J Clin Endocrinol Metab*. 1981;**52**(1):98–102.
 22. Chemes HE, Rey RA, Nistal M, et al. Physiological androgen insensitivity of the fetal, neonatal, and early infantile testis is explained by the ontogeny of the androgen receptor expression in Sertoli cells. *J Clin Endocrinol Metab*. 2008;**93**(11):4408–4412.
 23. Aksglaede L, Sørensen K, Boas M, et al. Changes in anti-Müllerian hormone (AMH) throughout the life span: a population-based study of 1027 healthy males from birth (cord blood) to the age of 69 years. *J Clin Endocrinol Metab*. 2010;**95**(12):5357–5364.
 24. De Gendt K, Swinnen JW, Saunders PT, et al. A Sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. *Proc Natl Acad Sci U S A*. 2004;**101**(5):1327–1332.
 25. Siiteri PK, Wilson JD. Testosterone formation and metabolism during male sexual differentiation in the human embryo. *J Clin Endocrinol Metab*. 1974;**38**(1):113–125.
 26. Grumbach MM. A window of opportunity: the diagnosis of gonadotropin deficiency in the male infant. *J Clin Endocrinol Metab*. 2005;**90**(5):3122–3127.
 27. Lanciotti L, Cofini M, Leonardi A, Penta L, Esposito S. Up-to-date review about minipuberty and overview on hypothalamic-pituitary-gonadal axis activation in fetal and neonatal life. *Front Endocrinol (Lausanne)*. 2018;**9**:410.
 28. Corbier P, Dehennin L, Castanier M, Mebazaa A, Edwards DA, Roffi J. Sex differences in serum luteinizing hormone and testosterone in the human neonate during the first few hours after birth. *J Clin Endocrinol Metab*. 1990;**71**(5):1344–1348.
 29. Fukami M, Homma K, Hasegawa T, Ogata T. Backdoor pathway for dihydrotestosterone biosynthesis: implications for normal and abnormal human sex development. *Dev Dyn*. 2013;**242**(4):320–329.
 30. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev*. 2011;**32**(1):81–151.
 31. Savchuk I, Morvan ML, Antignac JP, et al. Ontogenesis of human fetal testicular steroidogenesis at early gestational age. *Steroids*. 2019;**141**:96–103.
 32. Flück CE, Meyer-Böni M, Pandey AV, et al. Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. *Am J Hum Genet*. 2011;**89**(2):201–218.
 33. Grino PB, Griffin JE, Wilson JD. Testosterone at high concentrations interacts with the human androgen receptor similarly to dihydrotestosterone. *Endocrinology*. 1990;**126**(2):1165–1172.
 34. Wilson JD. Testosterone uptake by the urogenital tract of the rabbit embryo. *Endocrinology*. 1973;**92**(4):1192–1199.
 35. Hannema SE, Hughes IA. Regulation of Wolffian duct development. *Horm Res*. 2007;**67**(3):142–151.
 36. Yeh S, Tsai MY, Xu Q, et al. Generation and characterization of androgen receptor knockout (ARKO) mice: an in vivo model for the study of androgen functions in selective tissues. *Proc Natl Acad Sci U S A*. 2002;**99**(21):13498–13503.
 37. Wilhelm D, Koopman P. The makings of maleness: towards an integrated view of male sexual development. *Nat Rev Genet*. 2006;**7**(8):620–631.
 38. Feldman KW, Smith DW. Fetal phallic growth and penile standards for newborn male infants. *J Pediatr*. 1975;**86**(3):395–398.
 39. Boas M, Boisen KA, Virtanen HE, et al. Postnatal penile length and growth rate correlate to serum testosterone levels: a longitudinal study of 1962 normal boys. *Eur J Endocrinol*. 2006;**154**(1):125–129.
 40. Bouvattier C, Carel JC, Lecointre C, et al. Postnatal changes of T, LH, and FSH in 46,XY infants with mutations in the AR gene. *J Clin Endocrinol Metab*. 2002;**87**(1):29–32.
 41. Nordenskjöld A, Ivansson SA. Molecular characterization of 5 alpha-reductase type 2 deficiency and fertility in a Swedish family. *J Clin Endocrinol Metab*. 1998;**83**(9):3236–3238.
 42. Lindqvist A, Hughes IA, Andersson S. Substitution mutation C268Y causes 17 beta-hydroxysteroid dehydrogenase 3 deficiency. *J Clin Endocrinol Metab*. 2001;**86**(2):921–923.
 43. Juul A, Almstrup K, Andersson AM, et al. Possible fetal determinants of male infertility. *Nat Rev Endocrinol*. 2014;**10**(9):553–562.
 44. Thankamony A, Ong KK, Dunger DB, Acerini CL, Hughes IA. Anogenital distance from birth to 2 years: a population study. *Environ Health Perspect*. 2009;**117**(11):1786–1790.
 45. Dean A, Sharpe RM. Clinical review: Anogenital distance or digit length ratio as measures of fetal androgen exposure: relationship to male reproductive development and its disorders. *J Clin Endocrinol Metab*. 2013;**98**(6):2230–2238.
 46. Fowler PA, Bhattacharya S, Flannigan S, Drake AJ, O'Shaughnessy PJ. Maternal cigarette smoking and effects on androgen action in male offspring: unexpected effects on second-trimester anogenital distance. *J Clin Endocrinol Metab*. 2011;**96**(9):E1502–E1506.
 47. Hsieh MH, Eisenberg ML, Hittelman AB, Wilson JM, Tasian GE, Baskin LS. Caucasian male infants and boys with hypospadias exhibit reduced anogenital distance. *Hum Reprod*. 2012;**27**(6):1577–1580.
 48. Abbott AD, Colman RJ, Tiefenthaler R, Dumesic DA, Abbott DH. Early-to-mid gestation fetal testosterone increases right hand 2D:4D finger length ratio in polycystic ovary syndrome-like monkeys. *PLoS One*. 2012;**7**(8):e42372.
 49. Eisenberg ML, Jensen TK, Walters RC, Skakkebaek NE, Lipshultz LI. The relationship between anogenital distance and reproductive hormone levels in adult men. *J Urol*. 2012;**187**(2):594–598.
 50. Eisenberg ML, Shy M, Walters RC, Lipshultz LI. The relationship between anogenital distance and azoospermia in adult men. *Int J Androl*. 2012;**35**(5):726–730.
 51. Scott HM, Mason JJ, Sharpe RM. Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds. *Endocr Rev*. 2009;**30**(7):883–925.
 52. Macleod DJ, Sharpe RM, Welsh M, et al. Androgen action in the masculinization programming window and development of male reproductive organs. *Int J Androl*. 2010;**33**(2):279–287.
 53. Zheng Z, Cohn MJ. Developmental basis of sexually dimorphic digit ratios. *Proc Natl Acad Sci U S A*. 2011;**108**(39):16289–16294.
 54. de Sanctis V, Soliman AT, Elseidfy H, Soliman N, Elalaily R, Di Maio S. Is the second to fourth digit ratio (2D:4D) a biomarker of sex-steroids activity? *Pediatr Endocrinol Rev*. 2017;**14**(4):378–386.
 55. Berenbaum SA, Bryk KK, Nowak N, Quigley CA, Moffat S. Fingers as a marker of prenatal androgen exposure. *Endocrinology*. 2009;**150**(11):5119–5124.
 56. Buck JJ, Williams RM, Hughes IA, Acerini CL. In-utero androgen exposure and 2nd to 4th digit length ratio-comparisons between healthy controls and females with classical congenital adrenal hyperplasia. *Hum Reprod*. 2003;**18**(5):976–979.
 57. Wong WI, Hines M. Interpreting digit ratio (2D:4D)-behavior correlations: 2D:4D sex difference, stability, and behavioral correlates and their replicability in young children. *Horm Behav*. 2016;**78**:86–94.
 58. Bloom MS, Houston AS, Mills JL, Molloy CA, Hediger ML. Finger bone immaturity and 2D:4D ratio measurement error in the assessment of the hyperandrogenic hypothesis for the etiology of autism spectrum disorders. *Physiol Behav*. 2010;**100**(3):221–224.
 59. McIntyre MH, Cohn BA, Ellison PT. Sex dimorphism in digital formulae of children. *Am J Phys Anthropol*. 2006;**129**(1):143–150.
 60. Malas MA, Dogan S, Evcil EH, Desdicoglu K. Fetal development of the hand, digits and digit ratio (2D:4D). *Early Hum Dev*. 2006;**82**(7):469–475.
 61. Nguyen TV, Monnier P, Muckle G, et al. Androgenic and estrogenic indices in human newborns and infants: the MIREC-ID study. *J Dev Orig Health Dis*. 2019;**10**(5):578–586.
 62. Gishiti O, Gaillard R, Manniesing R, et al. Fetal and infant growth patterns associated with total and abdominal fat distribution in school-age children. *J Clin Endocrinol Metab*. 2014;**99**(7):2557–2566.
 63. Sonksen P. Determination and regulation of body composition in elite athletes. *Br J Sports Med*. 2018;**52**(4):219–229.
 64. de Zegher F, Francois I, Boehmer AL, et al. Androgens and fetal growth. *Horm Res*. 1998;**50**(4):243–244.
 65. Christine Knickmeyer R, Baron-Cohen S. Fetal testosterone and sex differences. *Early Hum Dev*. 2006;**82**(12):755–760.
 66. McCarthy MM, Auger AP, Bale TL, et al. The epigenetics of sex differences in the brain. *J Neurosci*. 2009;**29**(41):12815–12823.
 67. Hines M, Golombok S, Rust J, Johnston KJ, Golding J, Avon Longitudinal Study of Parents and Children Study Team. Testosterone during pregnancy and gender role behavior of preschool children: a longitudinal, population study. *Child Dev*. 2002;**73**(6):1678–1687.
 68. Phoenix CH, Goy RW, Gerall AA, Young WC. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology*. 1959;**65**:369–382.
 69. Thornton J, Zehr JL, Loose MD. Effects of prenatal androgens on rhesus monkeys: a model system to explore the organizational hypothesis in primates. *Horm Behav*. 2009;**55**(5):633–645.
 70. Chura LR, Lombardo MV, Ashwin E, et al. Organizational effects of fetal testosterone on human corpus callosum size and asymmetry. *Psychoneuroendocrinology*. 2010;**35**(1):122–132.

71. Lombardo MV, Ashwin E, Auyeung B, et al. Fetal testosterone influences sexually dimorphic gray matter in the human brain. *J Neurosci*. 2012;**32**(2):674–680.
72. Ecker C, Andrews DS, Gudbrandsen CM, et al; Medical Research Council Autism Imaging Multicentre Study (MRC AIMS) Consortium. Association between the probability of autism spectrum disorder and normative sex-related phenotypic diversity in brain structure. *JAMA Psychiatry*. 2017;**74**(4):329–338.
73. Hönekopp J. Digit ratio 2D:4D in relation to autism spectrum disorders, empathizing, and systemizing: a quantitative review. *Autism Res*. 2012;**5**(4):221–230.
74. Knickmeyer R, Baron-Cohen S, Fane BA, et al. Androgens and autistic traits: A study of individuals with congenital adrenal hyperplasia. *Horm Behav*. 2006;**50**(1):148–153.
75. Baron-Cohen S, Lombardo MV, Auyeung B, Ashwin E, Chakrabarti B, Knickmeyer R. Why are autism spectrum conditions more prevalent in males? *Plos Biol*. 2011;**9**(6):e1001081.
76. Baron-Cohen S, Knickmeyer RC, Belmonte MK. Sex differences in the brain: implications for explaining autism. *Science*. 2005;**310**(5749):819–823.
77. Hollier LP, Mattes E, Maybery MT, Keelan JA, Hickey M, Whitehouse AJ. The association between perinatal testosterone concentration and early vocabulary development: a prospective cohort study. *Biol Psychol*. 2013;**92**(2):212–215.
78. Whitehouse AJ, Mattes E, Maybery MT, et al. Sex-specific associations between umbilical cord blood testosterone levels and language delay in early childhood. *J Child Psychol Psychiatry*. 2012;**53**(7):726–734.
79. Ohlsson Gottby V, Söder O, Frisén L, et al. Hypogonadotrophic hypogonadism, delayed puberty and risk for neurodevelopmental disorders. *J Neuroendocrinol*. 2019;**31**(11):e12803.
80. Sato T, Koizumi S. Effects of fetal androgen on childhood behavior. *Acta Paediatr Jpn*. 1991;**33**(5):639–644.
81. Auyeung B, Baron-Cohen S, Ashwin E, et al. Fetal testosterone predicts sexually differentiated childhood behavior in girls and in boys. *Psychol Sci*. 2009;**20**(2):144–148.
82. Mathews GA, Fane BA, Conway GS, Brook CG, Hines M. Personality and congenital adrenal hyperplasia: possible effects of prenatal androgen exposure. *Horm Behav*. 2009;**55**(2):285–291.
83. Beltz AM, Swanson JL, Berenbaum SA. Gendered occupational interests: prenatal androgen effects on psychological orientation to Things versus People. *Horm Behav*. 2011;**60**(4):313–317.
84. Berenbaum SA. Effects of early androgens on sex-typed activities and interests in adolescents with congenital adrenal hyperplasia. *Horm Behav*. 1999;**35**(1):102–110.
85. Auyeung B, Lombardo MV, Baron-Cohen S. Prenatal and postnatal hormone effects on the human brain and cognition. *Pflugers Arch*. 2013;**465**(5):557–571.
86. Dittmann RW, Kappes ME, Kappes MH. Sexual behavior in adolescent and adult females with congenital adrenal hyperplasia. *Psychoneuroendocrinology*. 1992;**17**(2-3):153–170.
87. Money J, Schwartz M, Lewis VG. Adult erotosexual status and fetal hormonal masculinization and demasculinization: 46,XX congenital virilizing adrenal hyperplasia and 46,XY androgen-insensitivity syndrome compared. *Psychoneuroendocrinology*. 1984;**9**(4):405–414.
88. Zucker KJ, Bradley SJ, Oliver G, Blake J, Fleming S, Hood J. Psychosexual development of women with congenital adrenal hyperplasia. *Horm Behav*. 1996;**30**(4):300–318.
89. Meyer-Bahlburg HF, Dolezal C, Baker SW, New MI. Sexual orientation in women with classical or non-classical congenital adrenal hyperplasia as a function of degree of prenatal androgen excess. *Arch Sex Behav*. 2008;**37**(1):85–99.
90. Reichman DE, White PC, New MI, Rosenwaks Z. Fertility in patients with congenital adrenal hyperplasia. *Fertil Steril*. 2014;**101**(2):301–309.
91. Shabir I, Khurana ML, Joseph AA, Eunice M, Mehta M, Ammini AC. Phenotype, genotype and gender identity in a large cohort of patients from India with 5 α -reductase 2 deficiency. *Andrology*. 2015;**3**(6):1132–1139.
92. Méndez JP, Ulloa-Aguirre A, Imperato-McGinley J, et al. Male pseudohermaphroditism due to primary 5 alpha-reductase deficiency: variation in gender identity reversal in seven Mexican patients from five different pedigrees. *J Endocrinol Invest*. 1995;**18**(3):205–213.
93. Meyer-Bahlburg HF. Gender assignment and re-assignment in 46,XY pseudohermaphroditism and related conditions. *J Clin Endocrinol Metab*. 1999;**84**(10):3455–3458.
94. Wilson JD. The role of androgens in male gender role behavior. *Endocr Rev*. 1999;**20**(5):726–737.
95. Kolesinska Z, Ahmed SF, Niedziela M, et al. Changes over time in sex assignment for disorders of sex development. *Pediatrics*. 2014;**134**(3):e710–e715.
96. Winter JS, Faiman C, Hobson WC, Prasad AV, Reyes FI. Pituitary-gonadal relations in infancy. I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *J Clin Endocrinol Metab*. 1975;**40**(4):545–551.
97. Schmidt H, Schwarz HP. Serum concentrations of LH and FSH in the healthy newborn. *Eur J Endocrinol*. 2000;**143**(2):213–215.
98. Bergadá I, Milani C, Bedecarrás P, et al. Time course of the serum gonadotropin surge, inhibins, and anti-Müllerian hormone in normal newborn males during the first month of life. *J Clin Endocrinol Metab*. 2006;**91**(10):4092–4098.
99. Johannsen TH, Main KM, Ljubicic ML, et al. Sex differences in reproductive hormones during mini-puberty in infants with normal and disordered sex development. *J Clin Endocrinol Metab*. 2018;**103**(8):3028–3037.
100. Forest MG, Sizonenko PC, Cathiard AM, Bertrand J. Hypophysio-gonadal function in humans during the first year of life. 1. Evidence for testicular activity in early infancy. *J Clin Invest*. 1974;**53**(3):819–828.
101. Pang S, Levine LS, Chow D, Sagiani F, Saenger P, New MI. Dihydrotestosterone and its relationship to testosterone in infancy and childhood. *J Clin Endocrinol Metab*. 1979;**48**(5):821–826.
102. Dhayat NA, Dick B, Frey BM, d'Uscio CH, Vogt B, Flück CE. Androgen biosynthesis during minipuberty favors the backdoor pathway over the classic pathway: Insights into enzyme activities and steroid fluxes in healthy infants during the first year of life from the urinary steroid metabolome. *J Steroid Biochem Mol Biol*. 2017;**165**(Pt B):312–322.
103. Kiviranta P, Kuirri-Hanninen T, Saari A, Lamidi ML, Dunkel L, Sankilampi U. Transient postnatal gonadal activation and growth velocity in infancy. *Pediatrics*. 2016;**138**(1):e20153561.
104. Varimo T, Hero M, Laitinen EM, et al. Childhood growth in boys with congenital hypogonadotropic hypogonadism. *Pediatr Res*. 2016;**79**(5):705–709.
105. Waxman DJ, Holloway MG. Sex differences in the expression of hepatic drug metabolizing enzymes. *Mol Pharmacol*. 2009;**76**(2):215–228.
106. Waxman DJ, Celenza JL. Sexual dimorphism of hepatic gene expression: novel biological role of KRAB zinc finger repressors revealed. *Genes Dev*. 2003;**17**(21):2607–2613.
107. Waxman DJ, O'Connor C. Growth hormone regulation of sex-dependent liver gene expression. *Mol Endocrinol*. 2006;**20**(11):2613–2629.
108. Waxman DJ. Growth hormone pulse-activated STAT5 signalling: a unique regulatory mechanism governing sexual dimorphism of liver gene expression. *Novartis Found Symp*. 2000;**227**:61–74; discussion 75.
109. Main KM, Schmidt IM, Skakkebaek NE. A possible role for reproductive hormones in newborn boys: progressive hypogonadism without the postnatal testosterone peak. *J Clin Endocrinol Metab*. 2000;**85**(12):4905–4907.
110. Chen C, Gong CX, Zhang WP. Effects of oral testosterone undecanoate treatment for severe hypogonadism. *Int Urol Nephrol*. 2015;**47**(6):875–880.
111. Iyer R, Mok SF, Savkovic S, et al. Pharmacokinetics of testosterone cream applied to scrotal skin. *Andrology*. 2017;**5**(4):725–731.
112. Burstein S, Grumbach MM, Kaplan SL. Early determination of androgen-responsiveness is important in the management of microphallus. *Lancet*. 1979;**2**(8150):983–986.
113. Zenaty D, Djoud F, Morel Y, et al. Bilateral anorchia in infancy: occurrence of micropenis and the effect of testosterone treatment. *J Pediatr*. 2006;**149**(5):687–691.
114. Guthrie RD, Smith DW, Graham CB. Testosterone treatment for micropenis during early childhood. *J Pediatr*. 1973;**83**(2):247–252.
115. Ishii T, Sasaki G, Hasegawa T, Sato S, Matsuo N, Ogata T. Testosterone enanthate therapy is effective and independent of SRD5A2 and AR gene polymorphisms in boys with micropenis. *J Urol*. 2004;**172**(1):319–324.
116. Bin-Abbas B, Conte FA, Grumbach MM, Kaplan SL. Congenital hypogonadotropic hypogonadism and micropenis: effect of testosterone treatment on adult penile size why sex reversal is not indicated. *J Pediatr*. 1999;**134**(5):579–583.
117. Nerli RB, Guntaka AK, Patne PB, Hiremath MB. Penile growth in response to hormone treatment in children with micropenis. *Indian J Urol*. 2013;**29**(4):288–291.
118. Arisaka O, Hoshi M, Kanazawa S, et al. Systemic effects of transdermal testosterone for the treatment of microphallus in children. *Pediatr Int*. 2001;**43**(2):134–136.
119. Ben-Galim E, Hillman RE, Weldon VV. Topically applied testosterone and phallic growth. Its effects in male children with hypopituitarism and microphallus. *Am J Dis Child*. 1980;**134**(3):296–298.
120. Choi SK, Han SW, Kim DH, de Lignieres B. Transdermal dihydrotestosterone therapy and its effects on patients with microphallus. *J Urol*. 1993;**150**(2 Pt 2):657–660.
121. Becker D, Wain LM, Chong YH, et al. Topical dihydrotestosterone to treat micropenis secondary to partial androgen insensitivity syndrome (PAIS) before, during, and after puberty - a case series. *J Pediatr Endocrinol Metab*. 2016;**29**(2):173–177.
122. Xu D, Lu L, Xi L, et al. Efficacy and safety of percutaneous administration of dihydrotestosterone

- in children of different genetic backgrounds with micropenis. *J Pediatr Endocrinol Metab*. 2017;**30**(12):1285–1291.
123. Charmandari E, Dattani MT, Perry LA, Hindmarsh PC, Brook CG. Kinetics and effect of percutaneous administration of dihydrotestosterone in children. *Horm Res*. 2001;**56**(5-6):177–181.
 124. Swee DS, Quinton R. Congenital hypogonadotrophic hypogonadism: minipuberty and the case for neonatal diagnosis. *Front Endocrinol (Lausanne)*. 2019;**10**:97.
 125. Boehm U, Bouloux PM, Dattani MT, et al. Expert consensus document: European Consensus Statement on congenital hypogonadotrophic hypogonadism—pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol*. 2015;**11**(9):547–564.
 126. Papadimitriou DT, Chrysis D, Nyktari G, et al. Replacement of male mini-puberty. *J Endocr Soc*. 2019;**3**(7):1275–1282.
 127. Bougnères P, François M, Pantalone L, et al. Effects of an early postnatal treatment of hypogonadotrophic hypogonadism with a continuous subcutaneous infusion of recombinant follicle-stimulating hormone and luteinizing hormone. *J Clin Endocrinol Metab*. 2008;**93**(6):2202–2205.
 128. Main KM, Schmidt IM, Toppari J, Skakkebaek NE. Early postnatal treatment of hypogonadotrophic hypogonadism with recombinant human FSH and LH. *Eur J Endocrinol*. 2002;**146**(1):75–79.
 129. Kuiri-Hänninen T, Seuri R, Tyrväinen E, et al. Increased activity of the hypothalamic-pituitary-testicular axis in infancy results in increased androgen action in premature boys. *J Clin Endocrinol Metab*. 2011;**96**(1):98–105.
 130. Mau Kai C, Main KM, Andersen AN, Loft A, Skakkebaek NE, Juul A. Reduced serum testosterone levels in infant boys conceived by intracytoplasmic sperm injection. *J Clin Endocrinol Metab*. 2007;**92**(7):2598–2603.
 131. Wosnitzer MS, Paduch DA. Endocrinological issues and hormonal manipulation in children and men with Klinefelter syndrome. *Am J Med Genet C Semin Med Genet*. 2013;**163C**(1):16–26.
 132. Aksglaede L, Skakkebaek NE, Almstrup K, Juul A. Clinical and biological parameters in 166 boys, adolescents and adults with nonmosaic Klinefelter syndrome: a Copenhagen experience. *Acta Paediatr*. 2011;**100**(6):793–806.
 133. Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *J Clin Endocrinol Metab*. 2003;**88**(2):622–626.
 134. Herlihy AS, Halliday JL, Cock ML, McLachlan RI. The prevalence and diagnosis rates of Klinefelter syndrome: an Australian comparison. *Med J Aust*. 2011;**194**(1):24–28.
 135. Bojesen A, Juul S, Birkebaek NH, Gravholt CH. Morbidity in Klinefelter syndrome: a Danish register study based on hospital discharge diagnoses. *J Clin Endocrinol Metab*. 2006;**91**(4):1254–1260.
 136. Bojesen A, Juul S, Birkebaek N, Gravholt CH. Increased mortality in Klinefelter syndrome. *J Clin Endocrinol Metab*. 2004;**89**(8):3830–3834.
 137. Committee on Practice Bulletins—Obstetrics, Committee on Genetics, and Society for Maternal–Fetal Medicine. Practice Bulletin No. 162 summary: prenatal diagnostic testing for genetic disorders. *Obstet Gynecol*. 2016;**127**(5):976–978.
 138. Simpson JL, de la Cruz F, Swerdloff RS, et al. Klinefelter syndrome: expanding the phenotype and identifying new research directions. *Genet Med*. 2003;**5**(6):460–468.
 139. Ross JL, Roeltgen DP, Stefanatos G, et al. Cognitive and motor development during childhood in boys with Klinefelter syndrome. *Am J Med Genet A*. 2008;**146A**(6):708–719.
 140. Tartaglia N, Cordeiro L, Howell S, Wilson R, Janusz J. The spectrum of the behavioral phenotype in boys and adolescents 47,XXY (Klinefelter syndrome). *Pediatr Endocrinol Rev*. 2010;**8 Suppl 1**:151–159.
 141. Rigamonti C, Vizzello P, Monti F, et al. Klinefelter Syndrome in preschool children: the importance of an early multidisciplinary approach for patients and families. *Minerva Pediatr*. 2019;**71**(5):395–403.
 142. Girardin CM, Van Vliet G. Counselling of a couple faced with a prenatal diagnosis of Klinefelter syndrome. *Acta Paediatr*. 2011;**100**(6):917–922.
 143. Cabrol S, Ross JL, Fennoy I, Bouvattier C, Roger M, Lahlou N. Assessment of Leydig and Sertoli cell functions in infants with nonmosaic Klinefelter syndrome: insulin-like peptide 3 levels are normal and positively correlated with LH levels. *J Clin Endocrinol Metab*. 2011;**96**(4):E746–E753.
 144. Aksglaede L, Petersen JH, Main KM, Skakkebaek NE, Juul A. High normal testosterone levels in infants with non-mosaic Klinefelter's syndrome. *Eur J Endocrinol*. 2007;**157**(3):345–350.
 145. Lahlou N, Fennoy I, Carel JC, Roger M. Inhibin B and anti-Müllerian hormone, but not testosterone levels, are normal in infants with nonmosaic Klinefelter syndrome. *J Clin Endocrinol Metab*. 2004;**89**(4):1864–1868.
 146. Lahlou N, Fennoy I, Ross JL, Bouvattier C, Roger M. Clinical and hormonal status of infants with nonmosaic XXY karyotype. *Acta Paediatr*. 2011;**100**(6):824–829.
 147. Samango-Sprouse C, Stapleton EJ, Lawson P, et al. Positive effects of early androgen therapy on the behavioral phenotype of boys with 47,XXY. *Am J Med Genet C Semin Med Genet*. 2015;**169**(2):150–157.
 148. Samango-Sprouse CA, Sadeghin T, Mitchell FL, et al. Positive effects of short course androgen therapy on the neurodevelopmental outcome in boys with 47,XXY syndrome at 36 and 72 months of age. *Am J Med Genet A*. 2013;**161A**(3):501–508.
 149. Saenz J, Alexander GM. Postnatal testosterone levels and disorder relevant behavior in the second year of life. *Biol Psychol*. 2013;**94**(1):152–159.
 150. Lamminmäki A, Hines M, Kuiri-Hänninen T, Kilpeläinen L, Dunkel L, Sankilampi U. Testosterone measured in infancy predicts subsequent sex-typed behavior in boys and in girls. *Horm Behav*. 2012;**61**(4):611–616.
 151. Pasterski V, Acerini CL, Dunger DB, et al. Postnatal penile growth concurrent with mini-puberty predicts later sex-typed play behavior: Evidence for neurobehavioral effects of the postnatal androgen surge in typically developing boys. *Horm Behav*. 2015;**69**:98–105.
 152. Schaadt G, Hesse V, Friederici AD. Sex hormones in early infancy seem to predict aspects of later language development. *Brain Lang*. 2015;**141**:70–76.
 153. Kung KT, Browne WV, Constantinescu M, Noorderhaven RM, Hines M. Early postnatal testosterone predicts sex-related differences in early expressive vocabulary. *Psychoneuroendocrinology*. 2016;**68**:111–116.
 154. Sisk CL, Foster DL. The neural basis of puberty and adolescence. *Nat Neurosci*. 2004;**7**(10):1040–1047.
 155. Mann DR, Johnson AO, Gimpel T, Castracane VD. Changes in circulating leptin, leptin receptor, and gonadal hormones from infancy until advanced age in humans. *J Clin Endocrinol Metab*. 2003;**88**(7):3339–3345.
 156. Farooqi IS, O'Rahilly S. Monogenic obesity in humans. *Annu Rev Med*. 2005;**56**:443–458.
 157. Fuqua JS, Rogol AD. Puberty: its role in adolescent maturation. In: O'Donohue WT, ed. *Handbook of Adolescent Health Psychology*. New York: Springer Science+Business Media; 2013.
 158. Mitamura R, Yano K, Suzuki N, Ito Y, Makita Y, Okuno A. Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, and testosterone secretion before the onset of male puberty. *J Clin Endocrinol Metab*. 1999;**84**(1):29–37.
 159. Crofton PM, Evans AE, Wallace AM, Groome NP, Kelnar CJ. Nocturnal secretory dynamics of inhibin B and testosterone in pre- and peripubertal boys. *J Clin Endocrinol Metab*. 2004;**89**(2):867–874.
 160. Manasco PK, Umbach DM, Muly SM, et al. Ontogeny of gonadotropin, testosterone, and inhibin secretion in normal boys through puberty based on overnight serial sampling. *J Clin Endocrinol Metab*. 1995;**80**(7):2046–2052.
 161. Wu FC, Brown DC, Butler GE, Stirling HF, Kelnar CJ. Early morning plasma testosterone is an accurate predictor of imminent pubertal development in prepubertal boys. *J Clin Endocrinol Metab*. 1993;**76**(1):26–31.
 162. Roth JC, Grumbach MM, Kaplan SL. Effect of synthetic luteinizing hormone-releasing factor on serum testosterone and gonadotropins in prepubertal, pubertal and adult males. *J Clin Endocrinol Metab*. 1973;**37**(5):680–686.
 163. Winter JS, Taraska S, Faiman C. The hormonal response to HCG stimulation in male children and adolescents. *J Clin Endocrinol Metab*. 1972;**34**(2):348–353.
 164. Kubini K, Zachmann M, Albers N, et al. Basal inhibin B and the testosterone response to human chorionic gonadotropin correlate in prepubertal boys. *J Clin Endocrinol Metab*. 2000;**85**(1):134–138.
 165. Ishii T, Matsuo N, Sato S, et al. Human chorionic gonadotropin stimulation test in prepubertal children with micropenis can accurately predict Leydig cell function in pubertal or postpubertal adolescents. *Horm Res Paediatr*. 2015;**84**(5):305–310.
 166. Andersson AM, Juul A, Petersen JH, Müller J, Groome NP, Skakkebaek NE. Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. *J Clin Endocrinol Metab*. 1997;**82**(12):3976–3981.
 167. Nguyen TV, McCracken JT, Ducharme S, et al. Interactive effects of dehydroepiandrosterone and testosterone on cortical thickness during early brain development. *J Neurosci*. 2013;**33**(26):10840–10848.
 168. Nguyen TV, McCracken JT, Albaugh MD, Botteron KN, Hudziak JJ, Ducharme S. A testosterone-related structural brain phenotype predicts aggressive behavior from childhood to adulthood. *Psychoneuroendocrinology*. 2016;**63**:109–118.
 169. Nguyen TV, Lew J, Albaugh MD, et al. Sex-specific associations of testosterone with prefrontal-hippocampal development and executive function. *Psychoneuroendocrinology*. 2017;**76**:206–217.
 170. Davis S, Lahlou N, Bardsley M, et al. Gonadal function is associated with cardiometabolic health in pre-pubertal boys with Klinefelter syndrome. *Andrology*. 2016;**4**(6):1169–1177.

171. Ross JL, Kushner H, Kowal K, Bardsley M, Davis S, Reiss AL, Tartaglia N, Roeltgen D. Androgen treatment effects on motor function, cognition, and behavior in boys with Klinefelter syndrome. *J Pediatr*. 2017;**185**:193–199.e194.
172. Davis SM, Lahlou N, Cox-Martin M, Kowal K, Zeitler PS, Ross JL. Oxandrolone treatment results in an increased risk of gonadarche in prepubertal boys with Klinefelter syndrome. *J Clin Endocrinol Metab*. 2018;**103**(9):3449–3455.
173. Fennoy I. Testosterone and the child (0-12 years) with Klinefelter syndrome (47XXY): a review. *Acta Paediatr*. 2011;**100**(6):846–850.
174. Roehrborn CG, Lange JL, George FW, Wilson JD. Changes in amount and intracellular distribution of androgen receptor in human foreskin as a function of age. *J Clin Invest*. 1987;**79**(1):44–47.
175. Luo CC, Lin JN, Chiu CH, Lo FS. Use of parenteral testosterone prior to hypospadias surgery. *Pediatr Surg Int*. 2003;**19**(1-2):82–84.
176. Davits RJ, van den Aker ES, Scholtmeijer RJ, de Muinck Keizer-Schrama SM, Nijman RJ. Effect of parenteral testosterone therapy on penile development in boys with hypospadias. *Br J Urol*. 1993;**71**(5):593–595.
177. Ishii T, Hayashi M, Suwanai A, Amano N, Hasegawa T. The effect of intramuscular testosterone enanthate treatment on stretched penile length in prepubertal boys with hypospadias. *Urology*. 2010;**76**(1):97–100.
178. Gearhart JP, Jeffs RD. The use of parenteral testosterone therapy in genital reconstructive surgery. *J Urol*. 1987;**138**(4 Pt 2):1077–1078.
179. Chalapathi G, Rao KL, Chowdhary SK, Narasimhan KL, Samujh R, Mahajan JK. Testosterone therapy in microphallic hypospadias: topical or parenteral? *J Pediatr Surg*. 2003;**38**(2):221–223.
180. Sakakibara N, Nonomura K, Koyanagi T, Imanaka K. Use of testosterone ointment before hypospadias repair. *Urol Int*. 1991;**47**(1):40–43.
181. Paiva KC, Bastos AN, Miana LP, et al. Biometry of the hypospadiac penis after hormone therapy (testosterone and estrogen): a randomized, double-blind controlled trial. *J Pediatr Urol*. 2016;**12**(4):200.e201–206.
182. Tsur H, Shafir R, Shachar J, Eshkol A. Microphallic hypospadias: testosterone therapy prior to surgical repair. *Br J Plast Surg*. 1983;**36**(3):398–400.
183. Kaya C, Bektic J, Radmayr C, Schwentner C, Bartsch G, Oswald J. The efficacy of dihydrotestosterone transdermal gel before primary hypospadias surgery: a prospective, controlled, randomized study. *J Urol*. 2008;**179**(2):684–688.
184. Lee PA, Danish RK, Mazur T, Migeon CJ. Micropenis. III. Primary hypogonadism, partial androgen insensitivity syndrome, and idiopathic disorders. *J Clin Endocrinol Metab*. 1980;**14**(5):175–181.
185. Rynja SP, de Jong T, Bosch J, de Kort LMO. Testosterone prior to hypospadias repair: Postoperative complication rates and long-term cosmetic results, penile length and body height. *J Pediatr Urol*. 2018;**14**:31.e31–31.e38.
186. Menon P, Rao KLN, Handu A, Balan L, Kakkar N. Outcome of urethroplasty after parenteral testosterone in children with distal hypospadias. *J Pediatr Urol*. 2017;**13**:292.e291–292.e297.
187. Martha PM Jr, Rogol AD, Veldhuis JD, Blizzard RM. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. III. The neuroendocrine growth hormone axis during late prepuberty. *J Clin Endocrinol Metab*. 1996;**81**(11):4068–4074.
188. Martínez AS, Domené HM, Ropelato MG, et al. Estrogen priming effect on growth hormone (GH) provocative test: a useful tool for the diagnosis of GH deficiency. *J Clin Endocrinol Metab*. 2000;**85**(11):4168–4172.
189. Marin G, Domené HM, Barnes KM, Blackwell BJ, Cassorla FG, Cutler GB Jr. The effects of estrogen priming and puberty on the growth hormone response to standardized treadmill exercise and arginine-insulin in normal girls and boys. *J Clin Endocrinol Metab*. 1994;**79**(2):537–541.
190. Metzger DL, Kerrigan JR. Androgen receptor blockade with flutamide enhances growth hormone secretion in late pubertal males: evidence for independent actions of estrogen and androgen. *J Clin Endocrinol Metab*. 1993;**76**(5):1147–1152.
191. Metzger DL, Kerrigan JR. Estrogen receptor blockade with tamoxifen diminishes growth hormone secretion in boys: evidence for a stimulatory role of endogenous estrogens during male adolescence. *J Clin Endocrinol Metab*. 1994;**79**(2):513–518.
192. Albrecht A, Penger T, Marx M, Hirsch K, Dörr HG. Short-term adverse effects of testosterone used for priming in prepubertal boys before growth hormone stimulation test. *J Pediatr Endocrinol Metab*. 2018;**31**(1):21–24.
193. Lazar L, Phillip M. Is sex hormone priming in peripubertal children prior to growth hormone stimulation tests still appropriate? *Horm Res Paediatr*. 2010;**73**(4):299–302.
194. Ramaswamy S, Weinbauer GF. Endocrine control of spermatogenesis: Role of FSH and LH/ testosterone. *Spermatogenesis*. 2014;**4**(2):e996025.
195. Jarow JP, Zirkin BR. The androgen microenvironment of the human testis and hormonal control of spermatogenesis. *Ann N Y Acad Sci*. 2005;**1061**:208–220.
196. Joustra SD, van der Plas EM, Goede J, et al. New reference charts for testicular volume in Dutch children and adolescents allow the calculation of standard deviation scores. *Acta Paediatr*. 2015;**104**(6):e271–e278.
197. Bertelloni S, Baroncelli GI, Garofalo P, Cianfarani S. Androgen therapy in hypogonadal adolescent males. *Horm Res Paediatr*. 2010;**74**(4):292–296.
198. Judd HL, Parker DC, Siler TM, Yen SS. The nocturnal rise of plasma testosterone in pubertal boys. *J Clin Endocrinol Metab*. 1974;**38**(4):710–713.
199. Marynick SP, Loriaux DL, Sherins RJ, Pita JC Jr, Lipsett MB. Evidence that testosterone can suppress pituitary gonadotropin secretion independently of peripheral aromatization. *J Clin Endocrinol Metab*. 1979;**49**(3):396–398.
200. Santen RJ. Is aromatization of testosterone to estradiol required for inhibition of luteinizing hormone secretion in men? *J Clin Invest*. 1975;**56**(6):1555–1563.
201. Albertsson-Wikland K, Rosberg S, Lannering B, Dunkel L, Selstam G, Norjavaara E. Twenty-four-hour profiles of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol levels: a semilongitudinal study throughout puberty in healthy boys. *J Clin Endocrinol Metab*. 1997;**82**(2):541–549.
202. Ankarberg-Lindgren C, Norjavaara E. Changes of diurnal rhythm and levels of total and free testosterone secretion from pre to late puberty in boys: testis size of 3 ml is a transition stage to puberty. *Eur J Endocrinol*. 2004;**151**(6):747–757.
203. Endocrine Sciences. *Endocrinology: expected values & S.I. Unit Conversion Tables*. Esoterix; 2015.
204. Camacho AM, Migeon CJ. Testosterone excretion and production rate in normal adults and in patients with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 1966;**26**(8):893–896.
205. Rivarola MA, Saez JM, Meyer WJ, Jenkins ME, Migeon CJ. Metabolic clearance rate and blood production rate of testosterone and androst-4-ene-3,17-dione under basal conditions, ACTH and HCG stimulation. Comparison with urinary production rate of testosterone. *J Clin Endocrinol Metab*. 1966;**26**(11):1208–1218.
206. Southren AL, Gordon GG, Tochimoto S, Pinzon G, Lane DR, Stypulkowski W. Mean plasma concentration, metabolic clearance and basal plasma production rates of testosterone in normal young men and women using a constant infusion procedure: effect of time of day and plasma concentration on the metabolic clearance rate of testosterone. *J Clin Endocrinol Metab*. 1967;**27**(5):686–694.
207. Wang C, Catlin DH, Starcevic B, et al. Testosterone metabolic clearance and production rates determined by stable isotope dilution/tandem mass spectrometry in normal men: influence of ethnicity and age. *J Clin Endocrinol Metab*. 2004;**89**(6):2936–2941.
208. Hudson B, Dulmanis A, Coghlan JP, Wintour M. The measurement of androgen production in normal subjects. *Australas Ann Med*. 1966;**15**(3):236–244.
209. Davis TE, Lipsett MB, Korenman SG. Suppression of testosterone production by physiologic doses of 2-alpha-methyl-dihydrotestosterone propionate. *J Clin Endocrinol Metab*. 1965;**25**:476–479.
210. Vandewalle S, De Schepper J, Kaufman JM. Androgens and obesity in male adolescents. *Curr Opin Endocrinol Diabetes Obes*. 2015;**22**(3):230–237.
211. Cooper LA, Page ST, Amory JK, Anawalt BD, Matsumoto AM. The association of obesity with sex hormone-binding globulin is stronger than the association with ageing—implications for the interpretation of total testosterone measurements. *Clin Endocrinol (Oxf)*. 2015;**83**(6):828–833.
212. Glass AR, Swerdloff RS, Bray GA, Dahms WT, Atkinson RL. Low serum testosterone and sex-hormone-binding-globulin in massively obese men. *J Clin Endocrinol Metab*. 1977;**45**(6):1211–1219.
213. Goldman AL, Bhasin S, Wu FCW, Krishna M, Matsumoto AM, Jasuja R. A reappraisal of testosterone's binding in circulation: physiological and clinical implications. *Endocr Rev*. 2017;**38**(4):302–324.
214. Handelsman DJ. Free testosterone: pumping up the tires or ending the free ride? *Endocr Rev*. 2017;**38**(4):297–301.
215. Lasnizki I, Franklin HR. The influence of serum on uptake, conversion and action of testosterone in rat prostate glands in organ culture. *J Endocrinol*. 1972;**54**(2):333–342.
216. Vos MJ, Mijnhout GS, Rondeel JM, Baron W, Groeneveld PH. Sex hormone binding globulin deficiency due to a homozygous missense mutation. *J Clin Endocrinol Metab*. 2014;**99**(9):E1798–E1802.
217. Edelsztein NY, Grinspon RP, Scheingart HF, Rey RA. Anti-Müllerian hormone as a marker of steroid and gonadotropin action in the testis of children and adolescents with disorders of the gonadal axis. *Int J Pediatr Endocrinol*. 2016;**2016**:20.
218. Rey R, Mebarki F, Forest MG, et al. Anti-Müllerian hormone in children with androgen insensitivity. *J Clin Endocrinol Metab*. 1994;**79**(4):960–964.
219. Grinspon RP, Andreone L, Bedecarrás P, et al. Male central precocious puberty: serum profile of anti-Müllerian hormone and inhibit B before, during,

- and after treatment with GnRH analogue. *Int J Endocrinol*. 2013;**2013**:823064.
220. Rey R, Lordereau-Richard I, Carel JC, et al. Anti-Müllerian hormone and testosterone serum levels are inversely during normal and precocious pubertal development. *J Clin Endocrinol Metab*. 1993;**77**(5):1220–1226.
221. Hero M, Tommiska J, Vaaralahti K, et al. Circulating antimüllerian hormone levels in boys decline during early puberty and correlate with inhibin B. *Fertil Steril*. 2012;**97**(5):1242–1247.
222. Cabrera SM, Rogol AD. Testosterone exposure in childhood: discerning pathology from physiology. *Expert Opin Drug Saf*. 2013;**12**(3):375–388.
223. Wei C, Crowne EC. Recent advances in the understanding and management of delayed puberty. *Arch Dis Child*. 2016;**101**(5):481–488.
224. Day FR, Thompson DJ, Helgason H, et al; LifeLines Cohort Study; InterAct Consortium; kConFab/AOCS Investigators; Endometrial Cancer Association Consortium; Ovarian Cancer Association Consortium; PRACTICAL consortium. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. *Nat Genet*. 2017;**49**(6):834–841.
225. Day FR, Bulik-Sullivan B, Hinds DA, et al. Shared genetic aetiology of puberty timing between sexes and with health-related outcomes. *Nat Commun*. 2015;**6**:8842.
226. Roseweir AK, Millar RP. The role of kisspeptin in the control of gonadotrophin secretion. *Hum Reprod Update*. 2009;**15**(2):203–212.
227. Leka-Emiri S, Chrousos GP, Kanaka-Gantenbein C. The mystery of puberty initiation: genetics and epigenetics of idiopathic central precocious puberty (ICPP). *J Endocrinol Invest*. 2017;**40**(8):789–802.
228. Rogol AD. Pubertal androgen therapy in boys. *Pediatr Endocrinol Rev*. 2005;**2**(3):383–390.
229. Stárka L, Pospíšilová H, Hill M. Free testosterone and free dihydrotestosterone throughout the life span of men. *J Steroid Biochem Mol Biol*. 2009;**116**(1–2):118–120.
230. Swerdloff RS, Dudley RE, Page ST, Wang C, Salameh WA. Dihydrotestosterone: biochemistry, physiology, and clinical implications of elevated blood levels. *Endocr Rev*. 2017;**38**(3):220–254.
231. Houchin LD, Rogol AD. Androgen replacement in children with constitutional delay of puberty: the case for aggressive therapy. *Baillieres Clin Endocrinol Metab*. 1998;**12**(3):427–440.
232. Kerrigan JR, Rogol AD. The impact of gonadal steroid hormone action on growth hormone secretion during childhood and adolescence. *Endocr Rev*. 1992;**13**(2):281–298.
233. Zacharin M. Pubertal induction in hypogonadism: Current approaches including use of gonadotrophins. *Best Pract Res Clin Endocrinol Metab*. 2015;**29**(3):367–383.
234. Sobel V, Schwartz B, Zhu YS, Cordero JJ, Imperato-McGinley J. Bone mineral density in the complete androgen insensitivity and 5 α -reductase-2 deficiency syndromes. *J Clin Endocrinol Metab*. 2006;**91**(8):3017–3023.
235. Hero M, Wickman S, Hanhijärvi R, Siimes MA, Dunkel L. Pubertal upregulation of erythropoiesis in boys is determined primarily by androgen. *J Pediatr*. 2005;**146**(2):245–252.
236. Mauras N, Rini A, Welch S, Sager B, Murphy SP. Synergistic effects of testosterone and growth hormone on protein metabolism and body composition in prepubertal boys. *Metabolism*. 2003;**52**(8):964–969.
237. Rogol AD. Androgens and puberty. *Mol Cell Endocrinol*. 2002;**198**(1–2):25–29.
238. Handelsman DJ. Sex differences in athletic performance emerge coinciding with the onset of male puberty. *Clin Endocrinol (Oxf)*. 2017;**87**(1):68–72.
239. Rogol AD, Cumming SP, Malina RM. Biobanding: a new paradigm for youth sports and training. *Pediatrics*. 2018;**142**(5):e20180423.
240. Malina RM, Cumming SP, Rogol AD, et al. Biobanding in youth sports: background, concept, and application. *Sports Med*. 2019;**49**(11):1671–1685.
241. Bradley B, Johnson D, Hill M, et al. Bio-banding in academy football: player's perceptions of a maturity matched tournament. *Ann Hum Biol*. 2019;**46**(5):400–408.
242. Berenbaum SA, Beltz AM, Corley R. The importance of puberty for adolescent development: conceptualization and measurement. *Adv Child Dev Behav*. 2015;**48**:53–92.
243. Pangelinan MM, Leonard G, Perron M, et al. Puberty and testosterone shape the corticospinal tract during male adolescence. *Brain Struct Funct*. 2016;**221**(2):1083–1094.
244. Bramen JE, Hranilovich JA, Dahl RE, et al. Sex matters during adolescence: testosterone-related cortical thickness maturation differs between boys and girls. *Plos One*. 2012;**7**(3):e33850.
245. Raznahan A, Lee Y, Stidd R, et al. Longitudinally mapping the influence of sex and androgen signaling on the dynamics of human cortical maturation in adolescence. *Proc Natl Acad Sci U S A*. 2010;**107**(39):16988–16993.
246. Duke SA, Balzer BW, Steinbeck KS. Testosterone and its effects on human male adolescent mood and behavior: a systematic review. *J Adolesc Health*. 2014;**55**(3):315–322.
247. Klinker K, Wrzus C, Rauers A, Boker SM, Riediger M. Within-person changes in salivary testosterone and physical characteristics of puberty predict boys' daily affect. *Horm Behav*. 2017;**95**:22–32.
248. Zhu J, Chan YM. Adult consequences of self-limited delayed puberty. *Pediatrics*. 2017;**139**(6):e20163177.
249. Chada M, Průša R, Bronský J, et al. Inhibin B, follicle stimulating hormone, luteinizing hormone and testosterone during childhood and puberty in males: changes in serum concentrations in relation to age and stage of puberty. *Physiol Res*. 2003;**52**(1):45–51.
250. Crofton PM, Evans AE, Groome NP, Taylor MR, Holland CV, Kelnar CJ. Inhibin B in boys from birth to adulthood: relationship with age, pubertal stage, FSH and testosterone. *Clin Endocrinol (Oxf)*. 2002;**56**(2):215–221.
251. Coutant R, Biette-Demeneix E, Bouvattier C, et al. Baseline inhibin B and anti-Müllerian hormone measurements for diagnosis of hypogonadotropic hypogonadism (HH) in boys with delayed puberty. *J Clin Endocrinol Metab*. 2010;**95**(12):5225–5232.
252. Harrington J, Palmert MR. Clinical review: distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: critical appraisal of available diagnostic tests. *J Clin Endocrinol Metab*. 2012;**97**(9):3056–3067.
253. Lewkowitz-Shpuntoff HM, Hughes VA, Plummer L, et al. Olfactory phenotypic spectrum in idiopathic hypogonadotropic hypogonadism: pathophysiological and genetic implications. *J Clin Endocrinol Metab*. 2012;**97**(1):E136–E144.
254. Young J, Xu C, Papadakis GE, et al. Clinical management of congenital hypogonadotropic hypogonadism. *Endocr Rev*. 2019;**40**(2):669–710.
255. Rogol AD, Tartaglia N. Considerations for androgen therapy in children and adolescents with Klinefelter syndrome (47, XXY). *Pediatr Endocrinol Rev*. 2010;**8 Suppl 1**:145–150.
256. Chemaitilly W, Li Z, Huang S, et al. Anterior hypopituitarism in adult survivors of childhood cancers treated with cranial radiotherapy: a report from the St Jude Lifetime Cohort study. *J Clin Oncol*. 2015;**33**(5):492–500.
257. Sklar CA, Antal Z, Chemaitilly W, et al. Hypothalamic-pituitary and growth disorders in survivors of childhood cancer: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2018;**103**(8):2761–2784.
258. Haas RJ, Janka G, Gaedicke G, Kohne E, Netzel B. Therapy of acute lymphocytic leukemia in childhood with intermediate dose methotrexate and CNS irradiation. A report of the ALL 77-02 study group. *Blut*. 1983;**47**(6):321–331.
259. Wallace WH. Oncofertility and preservation of reproductive capacity in children and young adults. *Cancer*. 2011;**117**(10 Suppl):2301–2310.
260. Howell SJ, Radford JA, Ryder WD, Shalet SM. Testicular function after cytotoxic chemotherapy: evidence of Leydig cell insufficiency. *J Clin Oncol*. 1999;**17**(5):1493–1498.
261. Green DM, Kawashima T, Stovall M, et al. Fertility of male survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. *J Clin Oncol*. 2010;**28**(2):332–339.
262. Rivkees SA, Crawford JD. The relationship of gonadal activity and chemotherapy-induced gonadal damage. *Jama*. 1988;**259**(14):2123–2125.
263. Watson AR, Rance CP, Bain J. Long term effects of cyclophosphamide on testicular function. *Br Med J (Clin Res Ed)*. 1985;**291**(6507):1457–1460.
264. Shalet SM, Horner A, Ahmed SR, Morris-Jones PH. Leydig cell damage after testicular irradiation for lymphoblastic leukaemia. *Med Pediatr Oncol*. 1985;**13**(2):65–68.
265. Taneja S, Rao A, Nussey S, Leiper A. Leydig cell failure with testicular radiation doses <20Gy: The clinical effects of total body irradiation conditioned haematopoietic stem cell transplantation for childhood leukaemia during long-term follow-up. *Clin Endocrinol*. 2019.
266. Howell SJ, Shalet SM. Spermatogenesis after cancer treatment: damage and recovery. *J Natl Cancer Inst Monogr*. 2005;2005(34):12–17.
267. Rowley MJ, Leach DR, Warner GA, Heller CG. Effect of graded doses of ionizing radiation on the human testis. *Radiat Res*. 1974;**59**(3):665–678.
268. Gross RT, Duke PM. The effect of early versus late physical maturation on adolescent behavior. *Pediatr Clin North Am*. 1980;**27**(1):71–77.
269. Duke PM CJ, Jennings D, Martin JA, Dornbusch SM, Gross RT, Siegel-Gorelick B. Educational correlates of early and late sexual maturation in adolescents. *J Pediatr*. 1982;**100**(4):633–637.
270. Conley CS, Rudolph KD. The emerging sex difference in adolescent depression: interacting contributions of puberty and peer stress. *Dev Psychopathol*. 2009;**21**(2):593–620.
271. Graber JA, Seeley JR, Brooks-Gunn J, Lewinsohn PM. Is pubertal timing associated with psychopathology in young adulthood. *J Am Acad Child Adolesc Psychiatry*. 2004;**43**(6):718–726.
272. Day FR, Elks CE, Murray A, Ong KK, Perry JR. Puberty timing associated with diabetes,

- cardiovascular disease and also diverse health outcomes in men and women: the UK Biobank study. *Sci Rep*. 2015;**5**:11208.
273. Crowne EC, Shalet SM, Wallace WH, Eminson DM, Price DA. Final height in boys with untreated constitutional delay in growth and puberty. *Arch Dis Child*. 1990;**65**(10):1109–1112.
 274. Jones M. Psychological correlates of somatic development. *Child Dev*. 1965;**36**(4):899–911.
 275. Stanhope R, Bommen M, Brook CG. Constitutional delay of growth and puberty in boys: the effect of a short course of treatment with fluoxymesterone. *Acta Paediatr Scand*. 1985;**74**(3):390–393.
 276. Rosenfeld RG, Northcraft GB, Hintz RL. A prospective, randomized study of testosterone treatment of constitutional delay of growth and development in male adolescents. *Pediatrics*. 1982;**69**(6):681–687.
 277. Soliman AT, Khadir MM, Asfour M. Testosterone treatment in adolescent boys with constitutional delay of growth and development. *Metabolism*. 1995;**44**(8):1013–1015.
 278. Finkelstein JS, Klibanski A, Neer RM. A longitudinal evaluation of bone mineral density in adult men with histories of delayed puberty. *J Clin Endocrinol Metab*. 1996;**81**(3):1152–1155.
 279. Bertelloni S, Baroncelli GI, Ferdeghini M, Perri G, Saggese G. Normal volumetric bone mineral density and bone turnover in young men with histories of constitutional delay of puberty. *J Clin Endocrinol Metab*. 1998;**83**(12):4280–4283.
 280. Yap F, Höglér W, Briody J, Moore B, Howman-Giles R, Cowell CT. The skeletal phenotype of men with previous constitutional delay of puberty. *J Clin Endocrinol Metab*. 2004;**89**(9):4306–4311.
 281. Kindblom JM, Lorentzon M, Norjawaara E, et al. Pubertal timing predicts previous fractures and BMD in young adult men: the GOOD study. *J Bone Miner Res*. 2006;**21**(5):790–795.
 282. Kuh D, Muthuri SG, Moore A, et al. Pubertal timing and bone phenotype in early old age: findings from a British birth cohort study. *Int J Epidemiol*. 2016;**45**(4):1113–1124.
 283. Kelly BP, Paterson WF, Donaldson MD. Final height outcome and value of height prediction in boys with constitutional delay in growth and adolescence treated with intramuscular testosterone 125 mg per month for 3 months. *Clin Endocrinol (Oxf)*. 2003;**58**(3):267–272.
 284. Arrigo T, Cistermino M, Luca De F, et al. Final height outcome in both untreated and testosterone-treated boys with constitutional delay of growth and puberty. *J Pediatr Endocrinol Metab*. 1996;**9**(5):511–517.
 285. Richman RA, Kirsch LR. Testosterone treatment in adolescent boys with constitutional delay in growth and development. *N Engl J Med*. 1988;**319**(24):1563–1567.
 286. Kulin HE, Finkelstein JW, D'Arcangelo MR, et al. Diversity of pubertal testosterone changes in boys with constitutional delay in growth and/or adolescence. *J Pediatr Endocrinol Metab*. 1997;**10**(4):395–400.
 287. Chioma L, Papucci G, Fintini D, Cappa M. Use of testosterone gel compared to intramuscular formulation for puberty induction in males with constitutional delay of growth and puberty: a preliminary study. *J Endocrinol Invest*. 2018;**41**(2):259–263.
 288. Lampit M, Hochberg Z. Androgen therapy in constitutional delay of growth. *Horm Res*. 2003;**59**(6):270–275.
 289. Kaplowitz P. Delayed puberty in obese boys: comparison with constitutional delayed puberty and response to testosterone therapy. *J Pediatr*. 1998;**133**(6):745–749.
 290. Bergadá I, Bergadá C. Long term treatment with low dose testosterone in constitutional delay of growth and puberty: effect on bone age maturation and pubertal progression. *J Pediatr Endocrinol Metab*. 1995;**8**(2):117–122.
 291. Uruena M, Pantisotou S, Preece MA, Stanhope R. Is testosterone therapy for boys with constitutional delay of growth and puberty associated with impaired final height and suppression of the hypothalamo-pituitary-gonadal axis? *Eur J Pediatr*. 1992;**151**(1):15–18.
 292. Kaplowitz PB. Diagnostic value of testosterone therapy in boys with delayed puberty. *Am J Dis Child*. 1989;**143**(1):116–120.
 293. Chalew SA, Udoff LC, Hanukoglu A, Bistrizter T, Armour KM, Kowarski AA. The effect of testosterone therapy on spontaneous growth hormone secretion in boys with constitutional delay. *Am J Dis Child*. 1988;**142**(12):1345–1348.
 294. Wilson DM, Kei J, Hintz RL, Rosenfeld RG. Effects of testosterone therapy for pubertal delay. *Am J Dis Child*. 1988;**142**(1):96–99.
 295. Drobac S, Rubin K, Rogol AD, Rosenfield RL. A workshop on pubertal hormone replacement options in the United States. *J Pediatr Endocrinol Metab*. 2006;**19**(1):55–64.
 296. Lucas-Herald AK, Mason E, Beaumont P, et al. Single-centre experience of testosterone therapy for boys with hypogonadism. *Horm Res Paediatr*. 2018;**90**(2):123–127.
 297. Büyükgökbay A. Treatment of constitutional delayed puberty with a combination of testosterone esters. *Horm Res*. 1995;**44** Suppl 3:32–34.
 298. Didi M, Morris-Jones PH, Gattamaneni HR, Shalet SM. Pubertal growth in response to testosterone replacement therapy for radiation-induced Leydig cell failure. *Med Pediatr Oncol*. 1994;**22**(4):250–254.
 299. Schnabel PG, Bagchus W, Lass H, Thomsen T, Geurts TB. The effect of food composition on serum testosterone levels after oral administration of Andriol Testocaps. *Clin Endocrinol (Oxf)*. 2007;**66**(4):579–585.
 300. Yin A, Alfidhli E, Htun M, et al. Dietary fat modulates the testosterone pharmacokinetics of a new self-emulsifying formulation of oral testosterone undecanoate in hypogonadal men. *J Androl*. 2012;**33**(6):1282–1290.
 301. Contreras MF, Raisingani M, Prasad K, Franklin B, Shah B. Transdermal testosterone gel for induction and continuation of puberty in adolescent boys with hepatic dysfunction. *J Pediatr Endocrinol Metab*. 2017;**30**(1):105–109.
 302. Rogol AD, Swerdloff RS, Reiter EO, et al. A multicenter, open-label, observational study of testosterone gel (1%) in the treatment of adolescent boys with Klinefelter syndrome or ororchia. *J Adolesc Health*. 2014;**54**(1):20–25.
 303. Mehta A, Clearman T, Paduch DA. Safety and efficacy of testosterone replacement therapy in adolescents with Klinefelter syndrome. *J Urol*. 2014;**191**(5 Suppl):1527–1531.
 304. Mehta A, Bolyakov A, Roosma J, Schlegel PN, Paduch DA. Successful testicular sperm retrieval in adolescents with Klinefelter syndrome treated with at least 1 year of topical testosterone and aromatase inhibitor. *Fertil Steril*. 2013;**100**(4):970–974.
 305. Lawaetz JG, Hagen CP, Mieritz MG, Blomberg Jensen M, Petersen JH, Juul A. Evaluation of 451 Danish boys with delayed puberty: diagnostic use of a new puberty nomogram and effects of oral testosterone therapy. *J Clin Endocrinol Metab*. 2015;**100**(4):1376–1385.
 306. Schmidt H, Knorr D, Schwarz HP. Oral testosterone undecanoate for the induction of puberty in anorchid boys. *Arch Dis Child*. 1998;**78**(4):397.
 307. Brown DC, Butler GE, Kelnar CJ, Wu FC. A double blind, placebo controlled study of the effects of low dose testosterone undecanoate on the growth of small for age, prepubertal boys. *Arch Dis Child*. 1995;**73**(2):131–135.
 308. Albanese A, Kewley GD, Long A, Pearl KN, Robins DG, Stanhope R. Oral treatment for constitutional delay of growth and puberty in boys: a randomised trial of an anabolic steroid or testosterone undecanoate. *Arch Dis Child*. 1994;**71**(4):315–317.
 309. Butler GE, Sellar RE, Walker RF, Hendry M, Kelnar CJ, Wu FC. Oral testosterone undecanoate in the management of delayed puberty in boys: pharmacokinetics and effects on sexual maturation and growth. *J Clin Endocrinol Metab*. 1992;**75**(1):37–44.
 310. Gregory JW, Greene SA, Thompson J, Scrimgeour CM, Rennie MJ. Effects of oral testosterone undecanoate on growth, body composition, strength and energy expenditure of adolescent boys. *Clin Endocrinol (Oxf)*. 1992;**37**(3):207–213.
 311. Mason A, Wong SC, McGrogan P, Ahmed SF. Effect of testosterone therapy for delayed growth and puberty in boys with inflammatory bowel disease. *Horm Res Paediatr*. 2011;**75**(1):8–13.
 312. Mayo A, Macintyre H, Wallace AM, Ahmed SF. Transdermal testosterone application: pharmacokinetics and effects on pubertal status, short-term growth, and bone turnover. *J Clin Endocrinol Metab*. 2004;**89**(2):681–687.
 313. De Sanctis V, Vullo C, Urso L, et al. Clinical experience using the Androderm testosterone transdermal system in hypogonadal adolescents and young men with beta-thalassaemia major. *J Pediatr Endocrinol Metab*. 1998;**11** Suppl 3:891–900.
 314. Moskovic DJ, Freundlich RE, Yazdani P, Lipshultz LI, Khera M. Subcutaneous implantable testosterone pellets overcome noncompliance in adolescents with Klinefelter syndrome. *J Androl*. 2012;**33**(4):570–573.
 315. Zacharin MR, Warne GL. Treatment of hypogonadal adolescent boys with long acting subcutaneous testosterone pellets. *Arch Dis Child*. 1997;**76**(6):495–499.
 316. McFarland J, Craig W, Clarke NJ, Spratt DI. Serum testosterone concentrations remain stable between injections in patients receiving subcutaneous testosterone. *J Endocr Soc*. 2017;**1**(8):1095–1103.
 317. Spratt DI, Stewart II, Savage C, et al. Subcutaneous injection of testosterone is an effective and preferred alternative to intramuscular injection: demonstration in female-to-male transgender patients. *J Clin Endocrinol Metab*. 2017;**102**(7):2349–2355.
 318. Nahata L, Yu RN, Bhasin S, Cohen LE. Management of testosterone therapy in adolescents and young men with hypogonadism: are we following adult clinical practice guidelines? *J Pediatr Endocrinol Metab*. 2015;**28**(5-6):635–640.
 319. Viswanathan V, Eugster EA. Etiology and treatment of hypogonadism in adolescents. *Pediatr Clin North Am*. 2011;**58**(5):1181–200, x.
 320. Han TS, Bouloux PM. What is the optimal therapy for young males with hypogonadotropic

- hypogonadism? *Clin Endocrinol (Oxf)*. 2010;**72**(6):731–737.
321. Ambler GR. Androgen therapy for delayed male puberty. *Curr Opin Endocrinol Diabetes Obes*. 2009;**16**(3):232–239.
 322. Delemarre EM, Feliuss B, Delemarre van de Waal HA. Inducing puberty. *Eur J Endocrinol*. 2008;**159**(Suppl 1):S9–15.
 323. Sokol RZ, Palacios A, Campfield LA, Saul C, Swerdloff RS. Comparison of the kinetics of injectable testosterone in eugonadal and hypogonadal men. *Fertil Steril*. 1982;**37**(3):425–430.
 324. Wu XY, Mao JF, Lu SY, Zhang Q, Shi YF. Testosterone replacement therapy improves insulin sensitivity and decreases high sensitivity C-reactive protein levels in hypogonadotropic hypogonadal young male patients. *Chin Med J (Engl)*. 2009;**122**(23):2846–2850.
 325. Scovell JM, Khera M. Testosterone replacement therapy versus clomiphene citrate in the young hypogonadal male. *Eur Urol Focus*. 2018;**4**(3):321–323.
 326. Shimon I, Lubina A, Gorfine M, Ilany J. Feedback inhibition of gonadotropins by testosterone in men with hypogonadotropic hypogonadism: comparison to the intact pituitary-testicular axis in primary hypogonadism. *J Androl*. 2006;**27**(3):358–364.
 327. Raivio T, Falardeau J, Dwyer A, et al. Reversal of idiopathic hypogonadotropic hypogonadism. *N Engl J Med*. 2007;**357**(9):863–873.
 328. Rosenfeld RG, Frane J, Attie KM, et al. Six-year results of a randomized, prospective trial of human growth hormone and oxandrolone in Turner syndrome. *J Pediatr*. 1992;**121**(1):49–55.
 329. Salehpour S, Alipour P, Razzaghy-Azar M, et al. A double-blind, placebo-controlled comparison of letrozole to oxandrolone effects upon growth and puberty of children with constitutional delay of puberty and idiopathic short stature. *Horm Res Paediatr*. 2010;**74**(6):428–435.
 330. Crowne EC, Wallace WH, Moore C, Mitchell R, Robertson WR, Shalet SM. Degree of activation of the pituitary-testicular axis in early pubertal boys with constitutional delay of growth and puberty determines the growth response to treatment with testosterone or oxandrolone. *J Clin Endocrinol Metab*. 1995;**80**(6):1869–1875.
 331. Nieschlag E, Vorona E. Doping with anabolic androgenic steroids (AAS): Adverse effects on non-reproductive organs and functions. *Rev Endocr Metab Disord*. 2015;**16**(3):199–211.
 332. Solimini R, Rotolo MC, Mastrobattista L, et al. Hepatotoxicity associated with illicit use of anabolic androgenic steroids in doping. *Eur Rev Med Pharmacol Sci*. 2017;**21**(1 Suppl):7–16.
 333. Stanhope R, Buchanan CR, Fenn GC, Preece MA. Double blind placebo controlled trial of low dose oxandrolone in the treatment of boys with constitutional delay of growth and puberty. *Arch Dis Child*. 1988;**63**(5):501–505.
 334. Stanhope R, Adams J, Brook CG. Disturbances of puberty. *Clin Obstet Gynaecol*. 1985;**12**(3):557–577.
 335. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child*. 1970;**45**(239):13–23.
 336. Li H, Guo Y, Yang Z, Roy M, Guo Q. The efficacy and safety of oxandrolone treatment for patients with severe burns: A systematic review and meta-analysis. *Burns*. 2016;**42**(4):717–727.
 337. Porro LJ, Herndon DN, Rodriguez NA, et al. Five-year outcomes after oxandrolone administration in severely burned children: a randomized clinical trial of safety and efficacy. *J Am Coll Surg*. 2012;**214**(4):489–502; discussion 502.
 338. Bi LX, Wiren KM, Zhang XW, et al. The effect of oxandrolone treatment on human osteoblastic cells. *J Burns Wounds*. 2007;**6**e4.
 339. Bistrizter T, Lunenfeld B, Passwell JH, Theodor R. Hormonal therapy and pubertal development in boys with selective hypogonadotropic hypogonadism. *Fertil Steril*. 1989;**52**(2):302–306.
 340. Barrio R, de Luis D, Alonso M, Lamas A, Moreno JC. Induction of puberty with human chorionic gonadotropin and follicle-stimulating hormone in adolescent males with hypogonadotropic hypogonadism. *Fertil Steril*. 1999;**71**(2):244–248.
 341. Raivio T, Wikström AM, Dunkel L. Treatment of gonadotropin-deficient boys with recombinant human FSH: long-term observation and outcome. *Eur J Endocrinol*. 2007;**156**(1):105–111.
 342. Dwyer AA, Sykiotis GP, Hayes FJ, et al. Trial of recombinant follicle-stimulating hormone pretreatment for GnRH-induced fertility in patients with congenital hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2013;**98**(11):E1790–E1795.
 343. Rohayem J, Hauffa BP, Zacharin M, Kliesch S, Zitzmann M; German Adolescent Hypogonadotropic Hypogonadism Study Group. Testicular growth and spermatogenesis: new goals for pubertal hormone replacement in boys with hypogonadotropic hypogonadism? - a multicentre prospective study of hCG/rFSH treatment outcomes during adolescence. *Clin Endocrinol (Oxf)*. 2017;**86**(1):75–87.
 344. Arrigo T, Crisafulli G, Salzano G, Zirilli G, De Luca F. High-flow priapism in testosterone-treated boys with constitutional delay of growth and puberty may occur even when very low doses are used. *J Endocrinol Invest*. 2005;**28**(4):390–391.
 345. Key LL, Myers MC, Kroovand RL, Kelly WS. Priapism following testosterone therapy for delayed puberty. *Am J Dis Child*. 1989;**143**(9):1001–1002.
 346. Ohlander SJ, Varghese B, Pastuszak AW. Erythrocytosis following testosterone therapy. *Sex Med Rev*. 2018;**6**(1):77–85.
 347. de Ronde W. Hyperandrogenism after transfer of topical testosterone gel: case report and review of published and unpublished studies. *Hum Reprod*. 2009;**24**(2):425–428.
 348. Rolf C, Knie U, Lemnitz G, Nieschlag E. Interpersonal testosterone transfer after topical application of a newly developed testosterone gel preparation. *Clin Endocrinol (Oxf)*. 2002;**56**(5):637–641.
 349. Reinehr T, Gueldensupp M, Wunsch R, Bramswig JH. Treatment of tall stature in boys: comparison of two different treatment regimens. *Horm Res Paediatr*. 2011;**76**(5):343–347.
 350. Bhasin S, Brito JP, Cunningham GR, et al. Testosterone therapy in men with hypogonadism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2018;**103**(5):1715–1744.
 351. Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an endocrine society clinical practice guideline. *Endocr Pract*. 2017;**23**(12):1437.
 352. Heylens G, De Cuyper G, Zucker KJ, et al. Gender identity disorder in twins: a review of the case report literature. *J Sex Med*. 2012;**9**(3):751–757.
 353. Skordis N, Butler G, de Vries MC, Main K, Hannema SE. ESPE and PES international survey of centers and clinicians delivering specialist care for children and adolescents with gender dysphoria. *Horm Res Paediatr*. 2018;**90**(5):326–331.
 354. Maraka S, Singh Ospina N, Rodriguez-Gutierrez R, et al. Sex steroids and cardiovascular outcomes in transgender individuals: a systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2017;**102**(11):3914–3923.
 355. Steensma TD, McGuire JK, Kreukels BP, Beekman AJ, Cohen-Kettenis PT. Factors associated with desistance and persistence of childhood gender dysphoria: a quantitative follow-up study. *J Am Acad Child Adolesc Psychiatry*. 2013;**52**(6):582–590.
 356. de Vries AL, McGuire JK, Steensma TD, Wagenaar EC, Doreleijers TA, Cohen-Kettenis PT. Young adult psychological outcome after puberty suppression and gender reassignment. *Pediatrics*. 2014;**134**(4):696–704.
 357. Olson J, Schrage SM, Clark LF, Dunlap SL, Belzer M. Subcutaneous testosterone: an effective delivery mechanism for masculinizing young transgender men. *LGBT Health*. 2014;**1**(3):165–167.
 358. Finlayson C, Johnson EK, Chen D, et al. Proceedings of the working group session on fertility preservation for individuals with gender and sex diversity. *Transgend Health*. 2016;**1**(1):99–107.
 359. Weinand JD, Safer JD. Hormone therapy in transgender adults is safe with provider supervision; a review of hormone therapy sequelae for transgender individuals. *J Clin Transl Endocrinol*. 2015;**2**(2):55–60.
 360. van Kesteren PJ, Asscheman H, Megens JA, Gooren LJ. Mortality and morbidity in transsexual subjects treated with cross-sex hormones. *Clin Endocrinol (Oxf)*. 1997;**47**(3):337–342.
 361. Singh-Ospina N, Maraka S, Rodriguez-Gutierrez R, et al. Effect of sex steroids on the bone health of transgender individuals: a systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2017;**102**(11):3904–3913.
 362. Braun H, Nash R, Tangpricha V, Brockman J, Ward K, Goodman M. Cancer in transgender people: evidence and methodological considerations. *Epidemiol Rev*. 2017;**39**(1):93–107.
 363. den Heijer M, Bakker A, Gooren L. Long term hormonal treatment for transgender people. *BMJ*. 2017;**359**:j5027.

Acknowledgments

We would like to thank Ms. Kate Joshua and the Claude Moore Medical Library as well as Drs. S. Faisal Ahmed, Christine Burt Solorzano, Stuart Howards, Ieuan Hughes, Alvin Matsumoto, Hershel Raff, Stephen Rosenthal, Richard Santen, Nicole Tartaglia, Jan-Maarten Wit, and Frederick Wu for their assistance and support.

There were no grants or fellowships that supported the writing of this paper.

Additional Information

Correspondence and Reprint Requests: Kelly Mason, MD, PO Box 800386, Charlottesville, VA 22908. E-mail: Kelly.Mason@Virginia.edu.

Disclosure Summary: K.A.M. receives support from the Cystic Fibrosis Foundation, but has no relevant disclosures to this manuscript. M.J.S. receives research support from Tandem Diabetes Care, but has no relevant disclosures to this manuscript. Competing interests of A.D.R. are as a consultant for the following companies which produce various forms of testosterone: Acerus Pharma, Anteres Pharma, Inc, Aytu BioScience, and Clarus Therapeutics. However, none of these companies were involved in the writing of this manuscript.

Abbreviations: 3 β HSD, 3 β -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.