

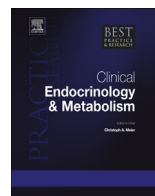


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### Puberty: Normal physiology (brief overview)



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Puberty is a defining phase of human development where growth ends and the ability to reproduce begins. An understanding of the events leading up to puberty highlights the fact that this is the culmination of a process of skeletal and gonadal activity that has been ongoing since conception. Although there is natural variation in the timing of events in and around puberty the basic underlying processes are common to all healthy human beings. This chapter is intended to outline the mechanisms underlying normal growth and development before and during puberty. By understanding normality the pathological processes that give rise to abnormalities of pubertal development can be understood more easily.

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## Overview of puberty

### *Introduction*

Puberty is the physiological process whereby adolescents reach sexual maturity and become capable of reproduction. The key underlying mechanism behind this transition is gonadal stimulation by rising levels of gonadotrophin (Gn) released in a pulsatile manner from the pituitary gland. Changes in central nervous system function (CNS) and the hypothalamo-pituitary (HP) axis in particular underpin the process of Gn (luteinising hormone, LH and follicle stimulating hormone, FSH) release. The rise in gonadal sex steroid production following Gn stimulation is responsible for changes in many tissues including the genitalia, skin, breast, brain, muscle and bone.

Whilst puberty is characterised by profound changes in Gn and sex steroid production, this is not the first time that activation of the HP axis has stimulated gonadal function. Gn and/or gonadal sex steroid production have an important influence on genital and CNS development in utero, particularly in males, and hypothalamo-pituitary-gonadal (HPG) activity is an important component of gonadal development between birth and puberty. An understanding of HPG activity and the growth pattern in infancy and childhood is useful because it places the physical changes associated with puberty into context.

### *Growth and the growth plate*

Linear growth occurs because bones elongate. Endochondral ossification is the process by which bone tissue is created in the long bones of the skeleton. It is driven by chondrocytes (cartilage cells) within the growth plate (GP) and is the process underlying bone formation and longitudinal growth of the majority of the skeleton.

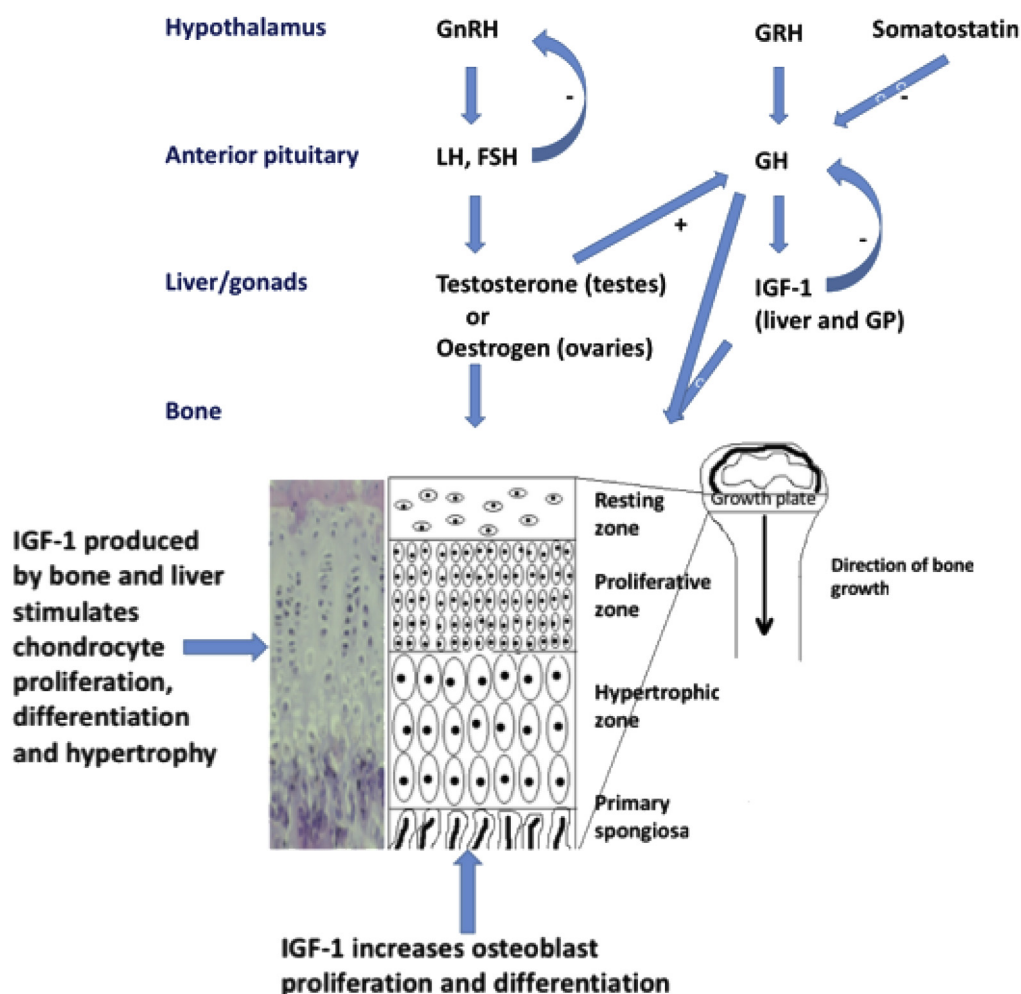
During the initial phase of skeletal development in utero, mesenchymal cells condense into tissue elements at specific sites that form the structure of future bones [1]. By 5 weeks gestation, these pre-cartilaginous anlagen or 'buds' reflect the shape, size, position and number of skeletal elements that will be present in the mature skeleton [2]. Following this, differentiation into either chondrocytes or osteoblasts occurs. Chondrocytes within each tissue organise into GPs and move through their associated orderly pattern of resting, proliferative and hypertrophic phases [3] (Fig. 1).

The resting zone contains small, scattered chondrocytes that are not actively involved in bone growth and replenish the pool of proliferative chondrocytes when required. The proliferative zone chondrocytes are neatly stacked and are able to rapidly replicate. During the hypertrophic phase, chondrocytes increase their height between 5 and 10-fold but remain orientated in columns. Once they reach the hypertrophic phase, chondrocytes promote invasion of blood vessels and the production of an extracellular matrix (ECM) that is rich in type 2 collagen, aggrecan and cytokines. The presence of growth factors facilitates vascular invasion and gradual mineralisation of the matrix. The size of the hypertrophic chondrocyte and rate of bone growth are highly correlated.

The cartilaginous ECM is gradually replaced by a bony ECM (rich in type 1 collagen), when apoptosis of the hypertrophic chondrocytes occurs and osteoblasts invade the cartilaginous scaffold. As osteoblasts lay down new bone to form the periosteum, the primary ossification centre expands towards the ends of the cartilage model. In long bones, a secondary ossification centre subsequently forms at the end of the bone, leaving a cartilaginous GP in between the two ossification centres where growth occurs.

### *Pre-pubertal childhood growth*

The most rapid phase of growth occurs in utero and growth rate declines rapidly after birth (Fig. 2). If newborns were to continue at their in utero growth rate they would reach adult height before two years of age. Linear growth slows during childhood and then rapidly increases during the 'pubertal growth spurt'. The physiological growth pattern prior to the onset of puberty follows a predictable pattern that can be divided into an individual's chronological stage of life; prenatal, infancy and childhood. After puberty, once the primary ossification centre meets the secondary ossification centre,



**Fig. 1.** Diagram summarising the complex endocrine regulation of endochondral bone formation during the pubertal growth spurt.

the GP becomes replaced by bone (known as epiphyseal fusion) and growth stops. The factors that influence growth rate are diverse and vary in terms of their relative contribution throughout childhood (Table 1). An understanding of the pattern of normal childhood linear growth provides a foundation for deciding when puberty is normal or abnormal.

#### *Prenatal*

Intrauterine growth rate is dependent on a variety of fetal, maternal and paternal factors, which act as either positive or negative regulators of the growth process. Important maternal factors include nutritional status, body size, blood pressure, placental size and uterine growth potential. Hyperglycaemia with the associated increased carbohydrate delivery and fetal hyperinsulinaemia will promote growth, resulting in increased length and weight in utero [4]. Exposure to toxins such as alcohol and tobacco smoke will impair fetal growth [5]. Changes in a single gene can impact on birthweight as shown by studies of the glucokinase gene [6]. Nutritional status is a key factor in the prenatal phase alongside hormonal regulators [7], notably insulin-like growth factors 1 and 2 (IGF-1 -2) [8,9]. Thyroid hormones also have an important role in the regulation of fetal growth, as demonstrated by the

## Schematic representation of the postnatal growth velocity curve

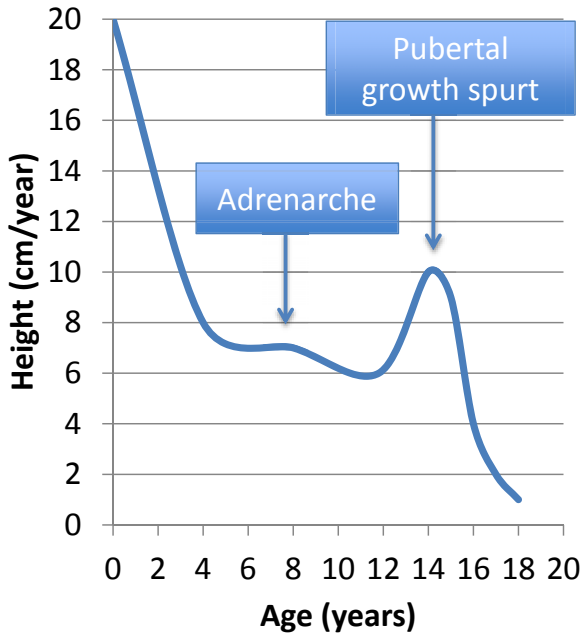


Fig. 2. Graph illustrating the changing growth velocity postnatally.

positive relationship between umbilical thyroxine concentrations and body weight and length at birth [10]. In utero androgen status does not have a significant impact on birth size [11]. The peak prenatal growth velocity usually occurs between gestational weeks 20–24, reaching around 2.5 cm per week [12]. Fetal growth pattern is related to longer term health parameters as shown by Barker and colleagues who noted that small size at birth is associated with an increased risk of cardiovascular disease many years later [13].

### Postnatal

Postnatal growth follows a specific pattern with a high growth rate after birth, followed by a deceleration that continues until around the age of 3 years, with a slower phase of deceleration until puberty [14]. The Karlberg Infancy, Childhood and Puberty (ICP) model (Fig. 3) [15] describes three main stages of postnatal growth, with each phase linked to the changing hormonal regulators involved. This model can be used to confirm growth normality as well acting as a foundation for the identification of abnormalities of growth and puberty.

### Infancy

Growth rate during infancy is more rapid than at any other stage of postnatal life, averaging 25 cm per year [16]. Growth rate at this stage of life is largely dependent on nutritional status as it was in the latter stages of pregnancy. During the first 2 years of life growth hormone (GH) has an increasingly important role in longitudinal growth; children with isolated GH deficiency have a near-normal birth length but are typically short by 2 years of age. It is not unusual for healthy infants to cross centile charts in this phase of growth with some showing evidence of 'catch up' (crossing the height centiles upwards as they grow) or 'catch down' growth (crossing the height centiles downwards as they grow).

**Table 1**

The predominant factors influencing pre-natal, childhood and pubertal growth rate.

| Growth phase     | Predominant factors |          | Hormonal regulators |       |       |         |     |             |
|------------------|---------------------|----------|---------------------|-------|-------|---------|-----|-------------|
|                  | Nutrition           | Genetics | GH                  | IGF-1 | IGF-2 | Insulin | TH  | Sex steroid |
| <i>Prenatal</i>  | +++                 | +        | +                   | +     | ++    | ++      | +   |             |
| <i>Infancy</i>   | +++                 | ++       | ++                  | ++    | +     | ++      | ++  |             |
| <i>Childhood</i> | ++                  | +++      | ++                  | ++    | +     | +       | +++ |             |
| <i>Pubertal</i>  | ++                  | +++      | +++                 | ++    | +     | +       | ++  | +++         |

GH: growth hormone, IGF-1: insulin-like growth factor 1, IGF-2: insulin-like growth factor 2, TH: thyroid hormone, +/++/+++ increasing level of influence.

Hence crossing centiles at this stage does not usually suggest underlying pathology and may simply reflect the genetic contribution of factors such as parental stature. Infants who develop an increased fat mass at this stage of life tend to be long (relatively tall) [17]. The growth pattern in infancy establishes the growth trajectory or 'centile' leading into puberty [18].

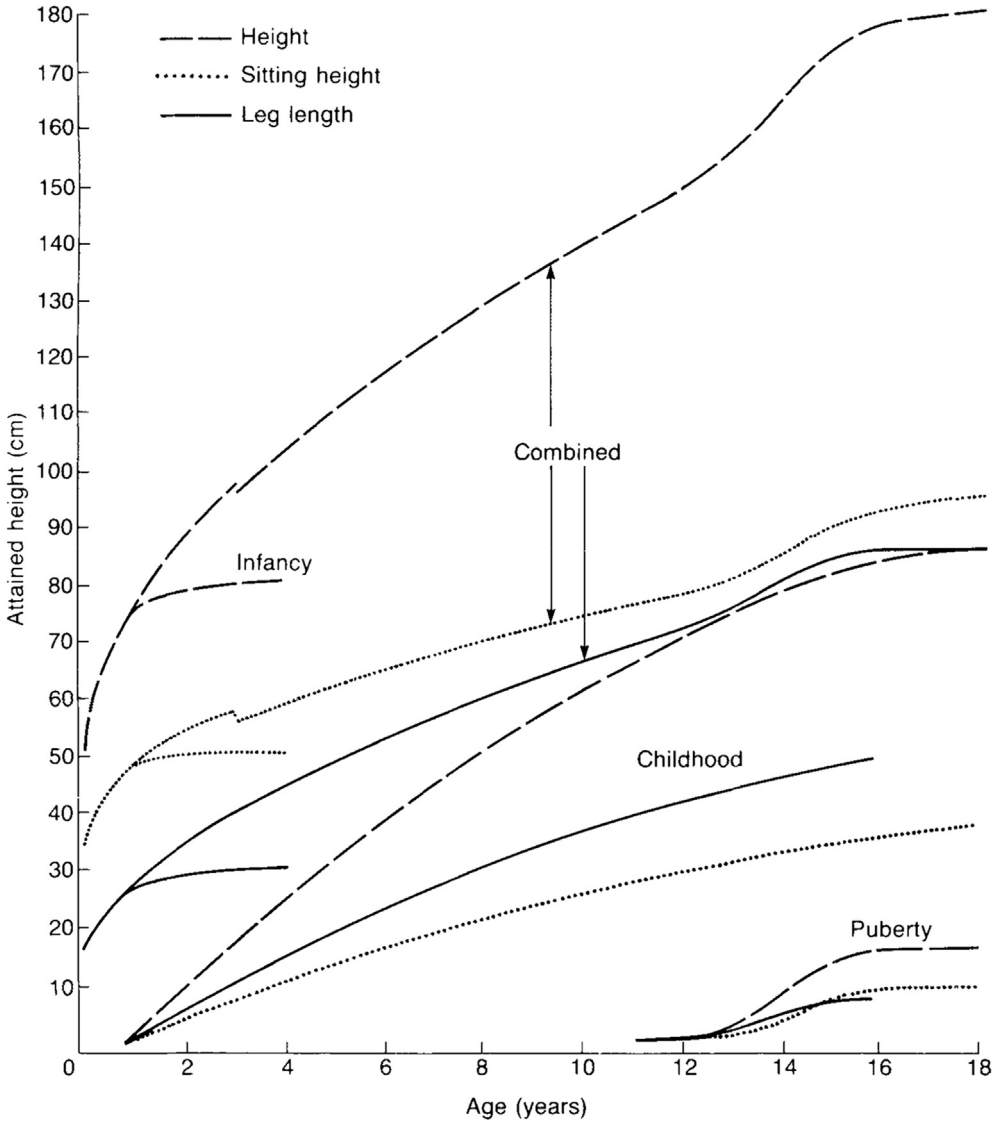
### Childhood

By 2–3 years of age both boys and girls tend to grow at a rate that results in them following a particular centile [14]. It is during this phase of growth that height velocity is affected less by nutritional status and more by hormonal regulators [19]. Adequate nutrition in childhood remains an important component of general well-being and continues to influence growth. A high dietary intake of protein has been shown to increase serum IGF-1 concentration by 25% [20] with the anabolic action of IGF-1 increasing protein synthesis, bone mineral density and muscle mass [21]. On the other hand, children with malnutrition have been shown to have lower IGF-1 levels [22]. From the ages of 2–6 years height velocity is typically around 6–8 cm per year but there is then a subtle increase in growth rate at adrenarche (described later in this chapter).

Chronic illness, a reduction in the normal production or action of hormonal regulators such as growth hormone (GH) and IGF-1 or an abnormal skeleton can be associated with slow growth. Conversely, excessive secretion of GH and thyroxine can result in a child growing abnormally quickly. Pathological factors like chronic illness that impact on pre-pubertal growth can delay puberty and reduce final adult height.

### Growth patterns and pubertal timing

Knowledge of a child's growth pattern in the years prior to the onset of puberty can help to predict pubertal timing. The centiles on population growth charts diverge, demonstrating that short children grow more slowly than their taller counterparts. However, a child's growth centile prior to puberty does not correlate closely with their centile at final adult height and some shorter children can overtake their peers and ultimately be taller adults. These individuals are more likely to have a 'younger' or relatively immature skeleton which is reflected by bone age delay; a radiograph of the wrist will demonstrate a discrepancy between chronological age and skeletal age when compared to radiological standards [23]. A child with a delayed bone age will have more growth 'potential' than a child with a skeletal age that is equivalent to chronological age and they are more likely to progress into puberty late, reflecting the fact that pubertal timing is more closely linked to skeletal maturation than chronological age [24]. Shorter children with bone age delay will therefore tend to have a more prolonged period of pre-pubertal growth than children without bone age delay which helps to explain why they can overtake taller peers – even if the magnitude of the pubertal growth spurt is similar. The term 'constitutional delay of growth and puberty' (CDGP) is frequently used to describe the growth pattern of an individual who grows relatively slowly for physiological reasons, has bone delay and as a result progresses into puberty relatively late. This term helpfully links the relatively slow growth of many short children to their relatively late progress into puberty. In such children there is frequently a family history of a similar growth pattern with late progress into puberty. Conversely, those children that have relatively rapid growth after birth, reaching a higher centile by 2–4 years of age, and have an advanced bone age are more likely to progress into puberty at an earlier stage. This pattern can be described as constitutional advancement of growth.



**Fig. 3.** The ICP model for attained size (height, sitting height and leg length): the mean values for each component (infancy, childhood, puberty) and their sum (combined growth) are plotted. Reproduced from Karlberg [15], with the permission of John Wiley and Sons.

### Sex steroid production prior to puberty

#### Gonadotrophin and sex steroid production in utero

Whilst puberty is frequently seen as the key stage at which gonadal sex steroid production begins in earnest, the gonad and HPG axis play a fundamental role in fetal and childhood development. Placental human chorionic gonadotrophin (hCG) binds to the LH receptor and is responsible for the androgen (primarily testosterone and dihydrotestosterone) production that virilises the male fetus in utero from around week 7. In the third trimester Gn release and androgen production facilitate testicular descent

and increase genital development. Gn production also facilitates the proliferation of the Germ and Sertoli cell populations.

#### *Gn and sex steroid production in infancy and the 'mini-puberty'*

After birth, Gn and sex steroid levels reach a nadir, only to rise again by 1–3 months of age. There is a pronounced sexual dimorphism with LH concentrations higher in males and FSH concentrations higher in females [25]. There is considerable overlap in terms of absolute concentrations although the LH/FSH ratio is almost always higher in infant males [26]. Whilst oestradiol concentrations are similar in male and female infants, testosterone concentrations are much higher in males. Oestradiol concentrations are relatively low in female infants when compared to adults [25] but testosterone concentrations reach mid-pubertal levels of around 2–10 nmol/l in males at around 2–3 months of age. Gn production at this stage facilitates ongoing testicular descent and the further maturation of the gonadal cell populations. This phase is often termed the 'mini-puberty'. Following this period of HPG axis activity there is a phase of relative (but not complete) quiescence until puberty. The fact that sex steroid production by the testes does not have a more profound impact on genital appearance including hair development in early infancy highlights the fact that phenotype reflects tissue sensitivity as well as the amount of hormone produced.

#### *Adrenarche*

At around 5–8 years of age the zona reticularis (the innermost layer of the adrenal cortex) develops and produces weak androgen. This process, known as adrenarche, involves the reactivation of the adrenal cortex with the production of adrenal androgens, specifically dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) and androstenedione. In females, DHEAS concentrations increase around 24 months before breast development whilst androstenedione and estrone usually rise between 12 and 18 months before breast development [27]. The presence of pubic hair is related to testosterone concentrations, particularly when measured by a sensitive liquid chromatography-mass spectrometry (LC-MS/MS) method [28].

One of the more frequent reasons for referral to a paediatric endocrine service is the development of relatively subtle signs of androgen production in a child – usually a girl – in pre-puberty. Parents and some physicians perceive this to be indicative of 'true' puberty occurring early, prompting a referral. Signs of adrenarche typically include the development of body odour, greasy hair, mild acne, axillary and pubic hair (pubarche). In the great majority of these children the clinical picture reflects the physiological rise in adrenal androgen production. This is a process that occurs in all healthy children although the manifestations in terms of timing and magnitude vary from person to person. In some individuals the process will occur without generating any concern whilst in other people – perhaps at the more pronounced end of the spectrum in terms of age of onset and magnitude – the impression is that something is wrong. Adrenal androgen production can be associated with a subtle increase in prepubertal growth rate as highlighted earlier in this chapter.

Adrenarche tends to occur at a younger age in patients with a history of being born small for gestational age and in young people who become obese. This may reflect increased androgen generation by the enzyme 17,20 lyase that converts 17-OH pregnenolone to DHEA and hence facilitates the generation of androstenedione and testosterone [29]. The phenotype can also be more profound where there is a family history of hyperandrogenism, such as polycystic ovarian syndrome. This background can help to place the phenotype into context although the key differential from a pathological perspective are disorders associated with androgen excess, notably non-classical congenital adrenal hyperplasia and virilising adrenal tumours. The hyperandrogenism associated with an adrenal tumour tends to occur in younger children who are often pre-school and is often florid and rapidly progressive, with pronounced signs of virilisation when compared to physiological adrenarche.

#### *What controls the onset of puberty?*

Scientists have searched for a key factor that initiates puberty but to date it has not been possible to identify a single initiating genetic mechanism. Indeed, it is highly unlikely that there is a single trigger and instead, as outlined in chapter 1 it appears that a range of genetic signals act in sequence and harmony to initiate hypothalamic Gn-releasing hormone (GnRH) production and pituitary FSH/LH

secretion. Kisspeptin is known to be a key player in pubertal induction and it binds to the G-protein coupled receptor, (KISS1R) to initiate a sequence of downstream events. The stimulus for kisspeptin release remains unknown at present and other pathways also contribute; for example neurokinin B signalling is also important for GnRH activation [30].

The timing of puberty is influenced by genetic and environmental factors. It is estimated that between 50 and 80% of the variation in pubertal timing is genetically determined and individuals progressing into puberty late will frequently have a parent who followed a similar pattern. However, other factors are also important. For example, recent studies have shown that Indian, Bangladeshi and Pakistani children tend to progress into puberty relatively early [31]. To what extent this reflects genetic factors as opposed to confounders such as socioeconomic and nutritional status is unclear. More recently the association between poverty and an increased BMI and obesity may have impacted on the timing of puberty at the population level by increasing the tempo of growth and puberty. Other studies have identified a role of behavioural and emotional circumstances on pubertal onset with acute stress delaying puberty and more chronic adversity associated with early puberty [32,33]. Endocrine disrupting compounds are predominantly man-made chemicals that can interfere with hormonal activity and have been shown to alter pubertal timing. Their effects are sexually dimorphic and depend on the compound in question as well as the window of exposure. For example, polychlorinated biphenyls (PCB's) have been shown to cause delayed puberty in males but earlier menarche in females [34]. The average age for the onset of puberty has gradually decreased during the twentieth century, probably as a result of improved socioeconomic conditions and hygiene, although this secular trend now appears to be levelling off [35].

Paediatricians will be aware from their clinical practice of the many variables that impact on pubertal onset. Chronic inflammation is a potent suppressor of Gn release with diseases such as inflammatory bowel disease a notable cause of delayed puberty. The role of nutritional status is evident from the delayed pubertal onset seen in anorexia nervosa. There is an inherent logic in not progressing into puberty and hence demonstrating reproductive 'readiness' in the absence of appropriate levels of nutrition and well-being. Circulating leptin concentrations are associated with pubertal onset with low leptin concentrations in the context of anorexia nervosa linked to pubertal delay. The paediatrician will also be aware of more specific, individual factors that can facilitate or delay puberty onset. The existence of 'higher centre' mechanisms that influence pubertal timing is illustrated by the susceptibility to early puberty in patients with hydrocephalus or central nervous system (as opposed to pituitary gland) irradiation.

According to the Buckler/Tanner revised data of 1995 the average age of pubertal onset in girls (defined by Tanner stage B2) in the UK is between 11 and 11.5 years and ranges between 9 years (+2SD) and just prior to 14.0 years of age (-2SD). The average age of pubertal onset in boys in the UK (defined by the attainment of a testicular volume of 4 mls) is around 12.0 years and ranges between 10.0 years (+2SD) and 14.0 years of age (-2SD) [36].

### *The Tanner staging system of pubertal development*

The ability to assess pubertal stage is essential to understand the normal progression of adolescent physical development. The Tanner staging system is an established means of assessing pubertal development and as such is a key component of the medical curriculum. The Tanner staging process (Fig. 4) describes the pattern of genital and pubic hair development in boys as the testes enlarge and the pattern of breast and pubic hair development in girls as the ovaries enlarge.

#### *Males*

The marker of pubertal onset in boys is the attainment of a testicular volume of 4 mls. This is a marker of Gn production although there are notable pathological causes of Gn independent gonadal enlargement such as familial male limited precocious puberty due to LH receptor activating mutations, TSH stimulation in the context of primary hypothyroidism and stimulation of the LH receptor (LHR) by hCG in certain germ cell tumours (see chapter 1). The pubertal growth spurt commences at a testicular volume of around 8–10 ml. It is around this time that circulating oestradiol concentrations start to rise. Most of the testicular volume reflects the process of spermatogenesis and hence reflects tissues such as seminiferous tubules rather than Leydig cells.

Females

The marker of pubertal onset in girls is the development of breast tissue. This is, as with males, a marker of Gn production acting on the gonad. Girls start to grow more quickly soon afterwards as a result of rapidly increasing oestrogen levels. Peak height velocity in girls occurs around B4 with menarche typically occurring as growth rate falls and the long bone epiphyses fuse.

Some of the key events in terms of sex steroid production and puberty are summarised in [Table 2](#).

The physiology of pubertal growth

One of the hallmarks of puberty is the rapid growth spurt that takes place at this stage of development. Compared with childhood growth, the hormonal regulation of pubertal growth is more complex. Alongside the requirement for normal levels of factors such as thyroid hormone, cortisol and insulin, there are important interactions between sex steroids (oestrogens and androgens) and the GH-IGF-1 axis which are crucial for normal growth and sexual development and occur uniquely during puberty. GH and IGF-1 concentrations rise in response primarily to the increase in oestrogen levels and when puberty is complete, GH secretion and hence circulating IGF-1 concentrations start to fall. The importance of this synergy is demonstrated by patients with either a deficiency of sex steroid hormone production (such as in hypogonadotropic hypogonadism) or GH. Both groups of patient will have a reduced pubertal growth spurt.

There are sex-specific patterns in terms of the timing of the growth spurt as well as age at skeletal maturity. The rise in oestrogen concentrations that drives an increase in growth rate is an early feature of female puberty. In females, the growth spurt typically occurs at Tanner breast stage 2–3 and the individual will gain approximately 20–25 cm in height during this phase of growth. The increase in growth velocity in males also reflects rising oestrogen concentrations although this does not occur to a significant degree until mid to late puberty. Males attain peak height velocity on average 2 years later than females, usually at Tanner genital stage 4 [37] and gain approximately 28 cm (25–30 cm) in height.

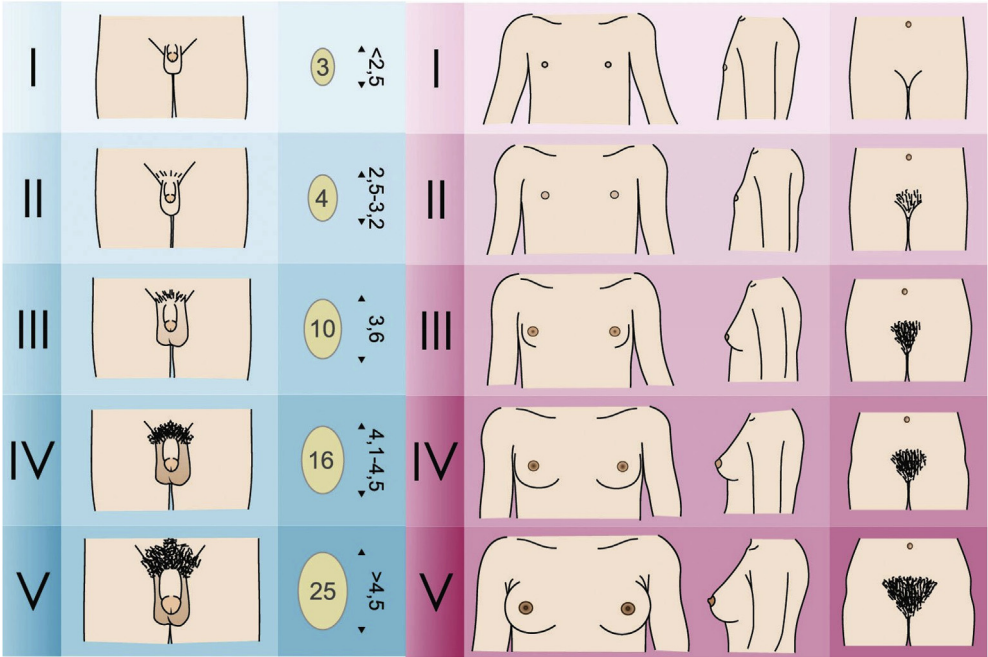


Fig. 4. The Tanner staging system used to assess stage of pubertal development.

*The role of GH and IGF-1 in pubertal growth*

GH and IGF-1 are fundamental regulators of longitudinal bone growth and have interdependent roles in the regulation of growth and skeletal function. The rapid linear growth seen during the pubertal growth spurt is a result of a 1.5–3-fold increase in pulsatile GH secretion and a greater than 3-fold increase in IGF-1 secretion. GH is secreted by the anterior pituitary gland under the control of hypothalamic peptides; growth hormone-releasing hormone (GHRH) and ghrelin stimulate GH release and somatostatin inhibits GH release. Whilst it was previously thought that GH only acted through IGF-1, it has now been shown that both hormones act synergistically in addition to exerting independent effects on growth. The precise mechanism of GH and IGF-1 action on the epiphyseal GP remains unknown and there are conflicting data regarding the relative contribution of each hormone on overall bone growth [38–40]. One theory is ‘the dual effector theory’, whereby GH induces both the expression and release of IGF-1 from the liver and local IGF-1 at the level of the GP [41]. The presence of local GH recruits resting chondrocytes into a proliferative state and stimulates local IGF-1 production. Once activated, the chondrocytes also become responsive to IGF-1. IGF-1 is expressed in all maturational GP zones and acts mainly on the hypertrophic zone, enabling clonal expansion of

**Table 2**  
Summary of the key events and associations during growth and puberty.

|                | What happens                                                                                                                 | When                                                                                                                                         | Factors involved                                                                                                                                                                   |
|----------------|------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Mini-puberty   | Gn and sex steroid levels rise after nadir at birth. Facilitates testicular descent in M and gonadal cell populations mature | 2–3 months of age                                                                                                                            | T levels in M reach mid-pubertal levels. Oestradiol levels similar in M and F.                                                                                                     |
| Adrenarche     | ‘Awakening of adrenal gland’ causes development of body odour, oily skin and hair, pubic hair (pubarche)                     | Usually begins at 6–8 years of age and is independent of true central puberty- usually precedes gonadarche by approx. 2 years                | Production of androgens by adrenal cortex including DHEA, DHEAS and androstenedione                                                                                                |
| Thelarche (F)  | Onset of female breast development, or budding (Tanner stage 2), usually first sign of puberty in girls                      | Usually occurs after 8 years of age (mean 10–11, range 8–13 years)                                                                           | Rising oestrogen levels                                                                                                                                                            |
| Gonadarche     | Growth of ovaries and testes and increased sex steroid production (true central puberty).                                    | Testicular enlargement in M usually signals pubertal development. Ovarian growth can't be directly seen but usually coincides with thelarche | Activation of gonads by LH and FSH to increase T and oestrogen levels                                                                                                              |
| Pubarche       | Development of first pubic hair                                                                                              | First pubic hair occurs at adrenarche (can be transient) and then again at Tanner stage 3. In F usually 6 m after thelarche.                 | Rising levels of androgens (during adrenarche) and sex steroids during central puberty                                                                                             |
| Growth spurt   | Peak growth velocity seen in childhood after infancy. Occurs shortly before final height is reached.                         | Usually occurs at Tanner stage 2 in F and 3–4 in M. Always occurs before menarche in F.                                                      | Multiple hormones involved. Rising Oestrogen levels cause GH secretion and act directly at GP. Occurs later in M as T needed in higher conc to convert to oestradiol via aromatase |
| Spermarche (M) | Development of sperm in the testicle and first ejaculation                                                                   | Usually coincides with development of secondary sexual characteristics in mid-puberty                                                        | Increased T levels from Leydig cells and nocturnal LH surge                                                                                                                        |
| Menarche (F)   | Onset of menstruation (Often thought of as culmination of pubertal development in F)                                         | Usually occurs 2 years after thelarche and soon after growth spurt                                                                           | Oestrogen stimulated growth of uterus and vascularity of endometrium, leading to sloughing of part of the lining. Most menstrual cycles are initially anovulatory.                 |

DHEA: dehydroepiandrosterone, DHEAS: dehydroepiandrosterone sulfate, F: female, FSH: follicle stimulating hormone, GH: growth hormone, GP: growth plate, LH: luteinising hormone, M: male, T: testosterone.

chondrocyte columns to occur [42]. It appears that GH also has a direct effect on chondrocytes, which is mediated via the growth hormone (GH) receptor. IGF-1 is also anabolic to bone; through the phosphoinositide-3-kinase (PI3K) pathway, it enhances Wntless/Integrated (Wnt) dependent activity and probably increases both osteoblast proliferation and osteoblast differentiation. Increases in IGF-1 also occur independently from GH as a result of increased testosterone levels during puberty. The prepubertal growth advantage shown by obese children is offset during puberty when obese children show a decreased peak height velocity compare to their normal weight peers. This is probably because obese children have reduced levels of GH secretion and because they are more likely to have an advanced BA.

#### *The role of sex steroid production in pubertal growth*

Gonadotrophins (LH and FSH) are released from the pituitary gland in a pulsatile manner in response to GnRH stimulation. Sensitive assays have shown that Gn and sex steroid are released in a diurnal pattern both before and during puberty and it is the amplitude of Gn pulses that increases at puberty. The increase in amplitude of LH and FSH secretion in turn increases the production of sex steroid hormones, primarily oestrogens in females and androgens in males. The LH surge occurs mainly at night initially and causes increased sex steroid hormone production early in the day. As puberty advances the LH pulses also increase in the daytime thereby promoting more sustained steroid hormone release.

Many of the growth enhancing effects of sex steroids are mediated by oestrogens produced by the gonad (ovary and testis) or generated from androgens in peripheral tissues such as fat by the enzyme aromatase. Oestrogen is primarily responsible for the increase in GH secretion by the pituitary gland at puberty with the increase in GH release primarily a pulse amplitude related phenomenon. The increase in GH release is an early feature of female puberty but occurs later in males. In both instances growth acceleration correlates with a rise in circulating oestrogen concentrations. However, rising testosterone levels have also been shown to directly augment GH secretion [43] and it is thought that testosterone itself may also contribute to bone formation via a direct effect on chondrocytes [44].

Oestrogen receptors are expressed throughout all GP zones [45] and oestrogen has a biphasic effect on bone growth in both sexes. Activation of the GH/IGF-1 axis with low doses of oestrogen is essential for endochondral bone formation during the pubertal growth spurt, but when higher concentrations of oestrogen bind to their receptors in the GP towards the end of puberty, GP senescence accelerates [46]. The exact cellular mechanism causing oestrogen-driven epiphyseal fusion remains unknown. As androgens mainly stimulate skeletal growth indirectly via their aromatisation into oestrogens and subsequent pituitary GH secretion, the relatively lower oestrogen concentration in males for a prolonged period during puberty may partly explain the increased final height and bone mass. The increase in lean muscle mass may also enhance mechanical loading which acts as a stimulus for bone growth [47].

The fundamental role of oestrogen in men and women has been illustrated by the absence of a pubertal growth spurt in a male with an oestrogen receptor mutation [48] and by the presence of a growth spurt in individuals with complete androgen insensitivity [49]. Whilst oestrogen is responsible for the pubertal growth spurt directly via its actions on the epiphysis and indirectly via its' actions on GH release, it is also responsible for epiphyseal fusion. Hence individuals who cannot generate oestrogen from androgen because of aromatase deficiency or an inability to respond to oestrogen due to an oestrogen receptor mutation tend to be tall. Excess oestrogen generation in the context of aromatase excess results in gynaecomastia but not necessarily compromised final adult height [50].

#### *Sex steroid production – the importance of oestrogen and androgen in both males and females*

Oestrogens and androgens have important roles in both males and females. A summary of tissue sex steroid production in males and females is shown in Figs. 5 and 6. In addition to the role of oestrogen on growth rate, breast development, CNS function, the skeleton and uterus in females, oestrogen also has a role in ensuring normal skeletal mineralisation and fertility in males. Conversely androgens have a profound impact on virilisation and brain development in males but androgens also have an impact on well-being as well as the development of pubic and axillary hair in females. The fact that males

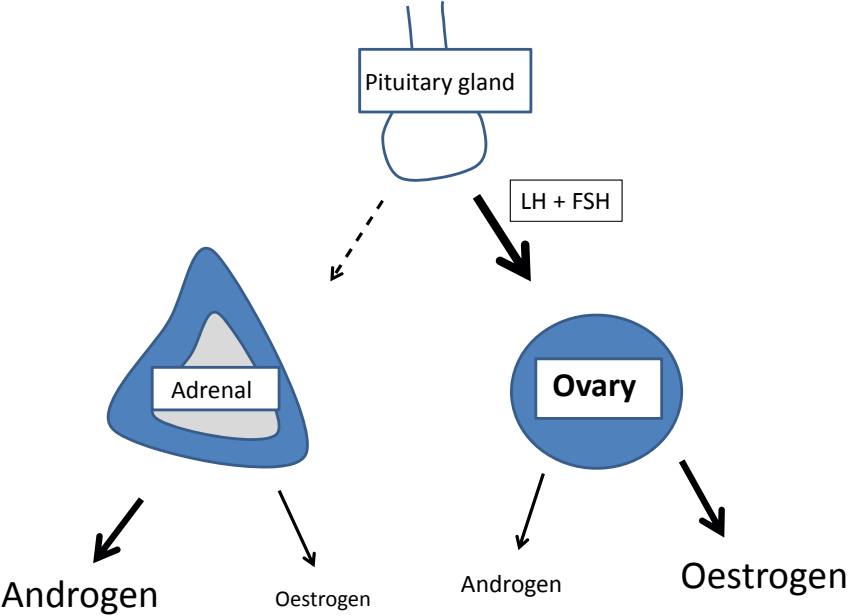


Fig. 5. Gonadal and adrenal sex steroid production in females.

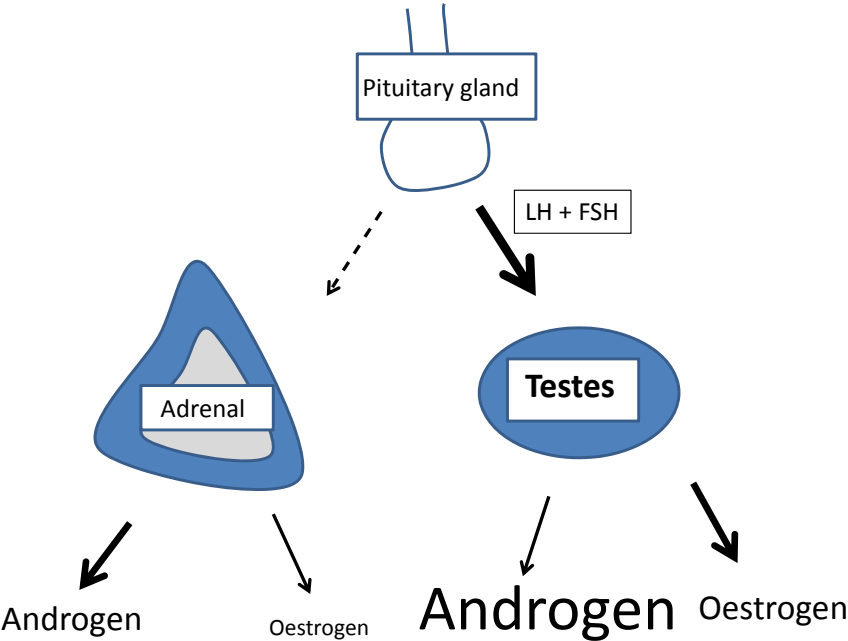


Fig. 6. Gonadal and adrenal sex steroid production in males.

normally make oestrogen in significant quantities is clear from the presence of gynaecomastia in around 50% of boys [51]. Boys with physiological gynaecomastia reach peak height velocity at a younger age and tend to have higher testosterone and oestradiol levels during puberty.

Changes in body composition accompany the rapid growth phase and are sexually dimorphic. In prepuberty the differences in body composition are relatively small. Under the influence of sex steroids and GH, increases in bone mineral content and muscle mass occur. Testosterone secretion causes a significant increase in lean muscle mass alongside a loss of adipose tissue. As height velocity declines towards the end of puberty, fat accumulation continues in both sexes, but at twice the rate in females. The net effect of changes in sex steroids and associated alterations in the GH/IGF-I axis is that the young woman has approximately 25% body fat in the 'gynoid' distribution while the male has 13% fat with much more muscle in the shoulders and upper body [52].

#### *Additional markers of gonadal development and function*

##### *Anti-Müllerian Hormone (AMH)*

AMH, a glycoprotein dimer hormone, has a fundamental role in fetal sex differentiation by inducing regression of the Müllerian ducts at approximately 8 weeks' gestation [53]. In males, AMH is secreted by the prepubertal Sertoli cells under the influence of FSH, with circulating levels increasing during the 'mini-puberty', before stabilising and then declining in parallel with the onset of puberty and the associated increase in testosterone secretion from the Leydig cells [54]. The negative correlation between testosterone and AMH can be explained by the progressive increase in expression of androgen receptors that appear in the Sertoli cell during pubertal development. These receptors are responsive to the increasing intratesticular testosterone concentration which predominate over the stimulating effect of FSH and inhibit AMH production [55]. In contrast to the immature pre-pubertal Sertoli cell whose primary function is to secrete AMH, puberty induces Sertoli cell maturation which supports spermatogenesis. AMH is a useful Sertoli cell-specific biomarker of testicular function in newborn infants with ambiguous genitalia or impalpable testes, where a measurable level can distinguish between anorchia and cryptorchidism. A very rare exception is if there was a mutation in the AMH gene, where there is unmeasurable AMH with abdominal testis in boys [56]. AMH has also been used to diagnose hypogonadotropic hypogonadism because reduced FSH secretion will result in low levels of AMH (and inhibin B) [57]. The physiological role of AMH beyond infancy remains to be determined.

In females, AMH is secreted by the ovarian granulosa cells of preantral follicles and is first detected at 36 weeks' gestation [53]. Similar to boys, AMH levels increase during the 'mini-puberty', remain stable through childhood, with a slight decline at pubertal onset and a more rapid decline from the mid-twenties. AMH appears to regulate folliculogenesis by inhibiting recruitment of follicles from the resting pool so that the dominant follicle can develop. AMH concentrations are relatively stable across the menstrual cycle and because of their relationship to ovarian follicle development are used as a marker of ovarian reserve. Alongside inhibin B, AMH has been used to predict the rate of pubertal progression in girls with central precocious puberty [58].

##### *Inhibin B*

Inhibin B is a heterodimeric glycoprotein and similar to AMH, is a product of Sertoli and ovarian Granulosa cells under the stimulus of FSH. Both *in vitro* and *in vivo* studies have demonstrated the important role of inhibin in the negative feedback regulation of FSH [59,60].

In male infants, inhibin B concentrations are measurable in the postnatal period and rise during the first week of life. Unlike AMH which decreases with pubertal onset, inhibin B increases progressively as boys approach the onset of puberty, increasing during pubertal stages G1 to G3 and declining slightly thereafter [61]. Less is known about the role of inhibin B in girls during childhood and puberty, however like their male counterparts they follow a similar pattern of secretion, albeit at significantly reduced concentrations [25]. In contrast to AMH in females, inhibin B concentrations vary across the menstrual cycle, with higher levels found in the follicular phase, peaking after the FSH surge, followed by a decline during the luteal phase. Clinical studies have suggested inhibin B as a useful marker to help distinguish between CDGP and hypogonadotropic hypogonadism (HH) in both males and females, where the absence of stimulating FSH in HH results in low inhibin B levels [62,63].

### *Insulin-like factor 3 (INSL3)*

In males, INSL3 is secreted by the Leydig cells during fetal and immediate postnatal life, before declining during childhood and increasing at pubertal onset. INSL3, regulated by hCG and LH, has an important role in the first phase of fetal testicular descent during the second trimester [64]. Less is known about its function in postnatal life, but it is thought to act via a G-protein-coupled receptor called RXFP2 to modulate steroidogenesis and support spermatogenesis [64]. INSL3 is constitutively expressed by the Leydig cells and, unlike testosterone, is not regulated by the hormones of the HPG axis. INSL3 therefore serves as a biomarker of Leydig cell functional capacity.

### *Neurodevelopmental aspects of puberty*

Whilst physical growth is largely complete at the end of puberty the changes in central nervous system biology continue into the third decade. Neuroimaging studies have shown that cortical and subcortical grey matter decrease in puberty whereas white matter increases into adulthood.

During adolescence there is a gradual increase and then reduction in grey matter. The sensory and motor regions mature first followed by the remainder of the cortex. There is then a posterior to anterior loss of grey matter. Histological and imaging studies in animals and humans show there is large synaptic proliferation in the pre-frontal area in early adolescence, followed by a plateau phase and subsequent reduction and reorganisation that may reflect the regression of rarely used synaptic connections leading to a more efficient and specialized brain. The grey matter changes in the same sequence in boys and girls but girls' grey matter peaks about one year before that of boys suggesting a link with hormonal status. The behavioural changes of adolescence correspond to the timing of puberty, not chronological age. Larger grey matter volumes in limbic system structures in both sexes are associated with later stages of puberty and higher levels of circulating testosterone [65].

## **Summary**

By seeing and assessing children referred to endocrine clinics on a regular basis the paediatrician will quickly appreciate the nuances of normal growth and development. Whilst many components of gonadal function and pubertal development can appear quite logical and straightforward this is not necessarily the case. It is, for example, surprising to learn that testicular androgen secretion is so substantial in early infancy and to learn that both categories of sex steroid – androgens and oestrogens – have such a fundamental role in the development and function of both sexes. An understanding of normality will mean that abnormal growth and abnormal puberty can be recognised and an appropriate set of investigations instigated.

## **Conflicts of interest**

The authors have no conflicts of interest to declare.

### **Practice points**

- Understanding the normal variation in growth and pubertal development is a key foundation for any scientist or clinician researching or practicing in this area of paediatric medicine.
- Whilst the changes in physical form at puberty are profound this is not the first time that the hypothalamo-pituitary axis has had a role to play in normal development.
- Understanding the strengths and limitations of local biochemical assays and associated reference ranges is important when deciding what constitutes normality versus abnormality.

## Research agenda

- What is the role of gonadotrophin production in infancy?
- What are the mechanisms behind the slow growth in children with abnormal muscle function?
- What is the role of inhibin B during puberty, particularly in females?
- How best to optimise growth and development in childhood whilst at the same time reducing the risk of disease development in later life?

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