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Puberty 1

Pubertal development and regulation

Ana Paula Abreu, Ursula B Kaiser

Puberty marks the end of childhood and is a period when individuals undergo physiological and psychological changes to achieve sexual maturation and fertility. The hypothalamic-pituitary-gonadal axis controls puberty and reproduction and is tightly regulated by a complex network of excitatory and inhibitory factors. This axis is active in the embryonic and early postnatal stages of life and is subsequently restrained during childhood, and its reactivation culminates in puberty initiation. The mechanisms underlying this reactivation are not completely known. The age of puberty onset varies between individuals and the timing of puberty initiation is associated with several health outcomes in adult life. In this Series paper, we discuss pubertal markers, epidemiological trends of puberty initiation over time, and the mechanisms whereby genetic, metabolic, and other factors control secretion of gonadotropin-releasing hormone to determine initiation of puberty.

Introduction

Puberty is the period of transition between childhood and adulthood, characterised by the development of secondary sexual characteristics, gonadal maturation, and attainment of reproductive capacity. Puberty and reproduction are controlled by the hypothalamic-pituitary-gonadal axis.1 Gonadotropin-releasing hormone (GnRH) is produced in the preoptic area of the hypothalamus and released from axon terminals in the median eminence in a pulsatile manner to stimulate the secretion of luteinising hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary, which in turn act on the gonads to promote gametogenesis and the production of sex steroids. In human beings, the hypothalamic-pituitary-gonadal axis is active in the mid-gestational fetus, but silenced towards the end of gestation. This restraint is removed at birth, leading to reactivation of the axis and an increase in gonadotropin concentrations.2 These concentrations then gradually decrease towards age 6 months, with the exception of FSH concentration in girls, which remains raised until age 3–4 years. In boys, testosterone concentration rises to a peak at age 1–3 months, but then falls in conjunction with the falling LH concentration.2

Prenatal and postnatal activation of the hypothalamic-pituitary-gonadal axis is associated with penile and testicular growth and testicular descent, and is therefore regarded as important for the development of male genitalia. In girls, raised concentrations of gonadotropins result in the maturation of ovarian follicles and an increase in oestradiol concentrations. This period of activity of the hypothalamic-pituitary-gonadal axis in the early stages of life is called minipuberty and has been proposed to lay the groundwork for pituitary LH and FSH responses to GnRH during the later reproductive phase of life.2

At about age 6 months in boys and 3–4 years in girls, there is an active inhibition of GnRH secretion, which persists throughout childhood. Puberty is initiated with a sustained increase in pulsatile release of GnRH from the hypothalamus after this quiescent period. The precise mechanisms that trigger the initiation of puberty remain elusive. Evidence suggests that the augmentation of activators of GnRH secretion together with the suppression of inhibitors of GnRH secretion culminates in puberty initiation. Whether the stimulatory tonus acting during minipuberty also contributes to puberty initiation is not yet clear. In this Series paper, we will discuss pubertal markers, epidemiological trends of puberty initiation, the currently known modulators of GnRH secretion, the hypothalamic-pituitary-gonadal axis, and the initiation of puberty.

Normal puberty and pubertal markers

The most visible changes during puberty are growth in stature and development of secondary sexual characteristics (figure 1). Equally profound are changes in body composition and the achievement of fertility. The first sign of puberty initiation is typically thelarche (breast development) in girls and testicular enlargement in boys. The landmark studies of Marshall and Tanner (1969), which classified puberty into five stages in girls and boys on the basis of somatic changes in breast, pubic hair, and genital development, have been widely used to assess puberty development.4 The ideal assessment of puberty initiation in girls is breast bud palpation. Commonly, self-description, visual assessment of breast development, or age of menarche (ie, the time of first menstrual bleeding) are used as markers of puberty in population studies, although these strategies are less accurate for assessment of the time of puberty initiation. Similarly, although testicular examination is a useful and key sign used in clinical practice when assessing boys for pubertal development, it is impractical to use in population studies of puberty. For these reasons and because of differences in study designs and methods used to assess puberty initiation, it can be difficult to determine the exact age of puberty onset in population studies. Although there is some physiological variation, pubertal development usually lasts 3–4 years and consists of a series of events that typically proceed in a predictable sequence. Menarche
is regarded as the final marker of puberty in girls. In girls with late initiation of puberty, there can be a decrease in the interval between thelarche and menarche. In boys, testicular enlargement is followed by an increase in growth velocity and subsequent spermatogenesis.

The development of axillary and pubic hair, a process termed pubarche, is also incorporated in the Tanner stages, but should not be used as a marker of the onset of puberty. In both sexes, pubarche is dependent on an increase in adrenal androgen production, a physiological event termed adrenarche. The serum concentration of dehydroepiandrosterone sulphate (DHEAS) is the best marker for the presence of adrenarche. This maturational process generally begins at about age 6 years, several years before activation of the hypothalamic-pituitary-gonadal axis (gonadarche). Although development of axillary and pubic hair requires adrenarchal concentrations of androgen, clinically evident pubarche does not usually appear until shortly after physical evidence of gonadarche (ie, breast development in girls and testicular enlargement in boys). Notably, adrenarche seems to be specific to human and some non-human primates, and the absence of adrenarche in human beings does not seem to prevent fertility nor to substantially affect the timing of gonadarche.

Adrenocorticotropic hormone has been suggested as a regulator of adrenarche because of its absence in children with hypopituitarism. However, the precise mechanisms underlying adrenarche are still unknown. Children with precocious puberty do not have a corresponding advance in the timing of adrenarche. Similarly, patients with hypogonadotropic hypogonadism and delayed puberty have no corresponding delay of adrenarche. In both cases, their adrenal androgen concentrations are appropriate for chronological age.

The age at puberty onset varies greatly between individuals, different ethnic populations, and between the sexes; girls on average experience the onset of puberty at younger ages than boys and are more likely to have idiopathic central precocious puberty, whereas boys are more predisposed to idiopathic delayed puberty. Puberty is deemed physiological when it begins between the ages of 8 and 12 years in girls and between 9 and 14 years in boys. These limits are selected to be 2.5–3 SDs below and above the mean age of onset of puberty, as defined by population studies. However, population studies have limitations and thus do the age limits used to define normal pubertal timing. The initiation of puberty at ages younger than these limits is regarded as precocious puberty and at ages beyond these limits, delayed puberty. The age of puberty onset is associated with subsequent health outcomes. Early age at menarche is a risk factor for breast cancer, cardiovascular disease, depression, behavioural disorders, diabetes, and increased all-cause mortality. Early puberty in boys is a risk factor for testicular cancer and delayed puberty can be a cause of being bullied, poor self-esteem, and psychosocial distress. A recent study showed that earlier or later timing of puberty in girls or boys was associated with increased risks for 48 adverse outcomes, across a range of cancer, cardiometabolic, gynaecological or obstetric, gastrointestinal, musculoskeletal, and neurocognitive categories. Most of these associations have been based on epidemiological studies and need to be validated and studied further.

**Epidemiology and trends**

Studies have shown a decrease in the average age of menarche between the mid-19th and the mid-20th centuries in the USA and in some countries in Europe. This change has been attributed to improvements in general health, nutrition, and other living conditions during this period. Although still controversial because of the limited comparability of the data from studies in different populations and the use of different methods, it has also been proposed that there was a trend towards an earlier onset of breast development and menarche in girls from 1940 to 1994. The amount of body fat and exposure to endocrine disruptors, particularly oestrogenic compounds and antiandrogens, have been suggested as important factors associated with this trend in pubertal timing. The effects of endocrine disruptors on GnRH secretion and therefore on the initiation of puberty and reproduction are still controversial and difficult to assess in human beings. Environmental factors might have effects on the neuroendocrine system, particularly during fetal and early postnatal life; however, we will not discuss the effects of specific compounds in this Series paper because of the controversies around this topic and the paucity of definitive data.
The characterisation of thelarche has varied from study to study. In some studies, breast development was characterised by visualisation only, making appropriate assessment difficult, especially in overweight or obese girls. A large cross-sectional study of about 17 000 girls done in an office setting in the USA (the Pediatric Research in Office Settings [PROS] study) used information obtained from mothers and photography, and the results suggested an advancement of pubertal development in the second half of the 20th century.\(^1\) By contrast, two European studies in which careful physical examination was used to ascertain breast development showed no substantial advance in age of puberty onset.\(^2\)\(^,\)\(^3\) However, in a more recent Danish study, 954 girls assessed in 2006–08 were compared with those studied in 1991–93, and substantially earlier breast development was noted in the girls born more recently.\(^4\)

The assessment of secular trends in male pubertal development is even more challenging because there are so few studies analysing puberty development in boys, and pubertal markers are even less reliable in boys than in girls. Similar to what has been reported in girls, results from some studies have suggested that age of puberty onset in boys might be occurring earlier than in the past, but additional studies are required to confirm this tendency.\(^5\)

There are substantial racial differences in sexual maturation; it is thought that non-Hispanic black and Mexican-American ethnic origins are independently associated with earlier pubertal development in girls.\(^6\)\(^,\)\(^7\) The age at menarche of non-Hispanic black girls is substantially earlier than that of non-Hispanic white and Mexican-American girls, and the mean age for onset of breast development in African-American girls is also earlier than that in white girls.\(^8\)

### Disorders of puberty

#### Classification of pubertal disorders

Puberty onset varies between normal individuals and there is a 4–5-year window judged to be the normal age of pubertal development. Changes in the limits of what is regarded as normal puberty initiation have occurred over the past few decades. The onset of puberty before or after these limits is deemed pathological. Precocious puberty generally has been defined as pubertal onset before age 8 years in girls and before age 9 years in boys. Because of the apparent advances in the age of onset of puberty in population studies, researchers have suggested that age 7 years in white girls, and age 6 years in African-American girls, should be used as the thresholds for classification of precocious puberty.\(^9\)\(^,\)\(^10\) However, other investigators have subsequently concluded that signs of puberty in girls aged 6–8 years should not be regarded as normal or benign as this might lead to underdiagnosis of endocrine disorders, so the appropriate thresholds for assessment have returned to the previously recommended parameters.\(^11\)

#### Precocious puberty

Precocious puberty can be a variant of normal development—eg, premature adrenarche or isolated premature thelarche—or can be attributable to pathological conditions (panel). GnRH-dependent or central precocious puberty is caused by early maturation of the hypothalamic-pituitary-gonadal axis, resulting in pulsatile secretion of GnRH and subsequent activation of the gonads. In these cases, the sexual characteristics are appropriate for the patient’s sex (isosexual). Several neurological disorders can cause central precocious puberty, such as tumours, trauma, or malformations (panel). Chronic exposure to sex steroids from external sources or as a result of some disorders with peripheral production of sex steroids have been proposed to result in early activation of the hypothalamic-pituitary-gonadal axis.\(^12\) Most cases of central precocious puberty in girls and up to 60% of cases in boys do not have a detectable CNS lesion and are described as idiopathic central precocious puberty. Some or most of these cases might have a genetic, metabolic, or environmental component, or a combination of these factors.

Production of sex steroids independent of the activation of the hypothalamic-pituitary-gonadal axis results in gonadotropin-independent or peripheral precocious puberty. In this form of puberty, sex hormones are usually derived from the gonads or adrenal glands, or from exogenous sources (panel). In patients with peripheral precocious puberty, the sexual characteristics can be appropriate for the child’s sex (isosexual) or inappropriate, with virilisation of girls or feminisation of boys (contrasexual).

#### Delayed puberty

Delayed puberty is defined clinically by the absence or incomplete development of secondary sexual characteristics by age 13 years in girls or age 14 years in boys. Delayed puberty can be a variant of normal development, known as constitutional delay of growth and puberty, when healthy teenagers spontaneously develop puberty after the upper age limit (panel). The absence or incomplete development of secondary sexual characteristics by age 18 years in both sexes is classified as hypogonadism. The absence of activation of the hypothalamic-pituitary-gonadal axis because of a defect in the CNS defines hypogonadotropic hypogonadism. This disorder can be caused by a deficiency in GnRH secretion, action, or both, also known as isolated hypogonadotropic hypogonadism. A genetic defect has been identified in approximately 40% of known cases. Alternatively, hypogonadotropic hypogonadism can have an organic cause such as a tumour, use of drugs, or inflammatory or systemic disorders (panel). Isolated hypogonadotropic hypogonadism can be classified as Kallmann’s syndrome when associated with absence of smell (anosmia), or isolated hypogonadotropic hypogonadism when there...
is no effect on olfaction. Kallmann’s syndrome typically results from abnormal fetal development of GnRH neurons; both GnRH and olfactory neurons originate from the olfactory placode and migrate together into the CNS during embryogenesis.32 impairment of the migration of these neurons is often associated with genetic defects.33–35 By contrast, isolated hypogonadotropic hypogonadism is more typically associated with normal olfactory and GnRH neuronal development, but impaired regulation of GnRH secretion.33,35 The absence of pubertal development caused by an intrinsic defect in the gonads results in hypergonadotropic hypogonadism, which can also be caused by genetic or organic disorders.

Normal physiological controls of the timing of onset and progression of puberty

Role of GnRH

In the 1970s, Belchetz and colleagues36 showed in animal models that intermittent release of GnRH is required for the activation of the pituitary-gonadal axis. although the mechanism that causes the pulsatility of the GnRH neuronal network can be intrinsic to the GnRH neurons themselves,37 lesions of the arcuate nucleus have been shown to abolish LH pulsatility, consistent with the idea that there is a higher order input into the network of GnRH neurons regulating GnRH secretion.38 Puberty is initiated with a sustained increase in pulsatile release of GnRH from the hypothalamus after a quiescent period during childhood. Studies of agonadal human beings

Panel: Variants of normal pubertal development and classification of pubertal disorders

<table>
<thead>
<tr>
<th>Variants of normal pubertal development</th>
<th>Classification of pubertal disorders</th>
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<tbody>
<tr>
<td>Isolated precocious thelarche</td>
<td>• Genetic causes</td>
</tr>
<tr>
<td>• Isolated breast development with no other signs of oestrogen action</td>
<td>• Testotoxicosis</td>
</tr>
<tr>
<td>Isolated precocious pubarche</td>
<td>• McCune-Albright syndrome</td>
</tr>
<tr>
<td>• Axillary hair, increased growth velocity, and slight advancement of bone age</td>
<td>• Prader-Willi syndrome, Williams syndrome, Temple syndrome</td>
</tr>
<tr>
<td>• Increase in adrenal hormones</td>
<td>• Others</td>
</tr>
<tr>
<td>Isolated precocious menarche</td>
<td></td>
</tr>
<tr>
<td>• Isolated vaginal bleeding without other pubertal signs or advancement of bone age</td>
<td></td>
</tr>
<tr>
<td>Constitutional delay of growth and puberty</td>
<td></td>
</tr>
<tr>
<td>• Puberty initiation between ages 14 and 18 years</td>
<td></td>
</tr>
<tr>
<td>• More frequent in boys with family history</td>
<td></td>
</tr>
<tr>
<td>Preocious puberty</td>
<td></td>
</tr>
<tr>
<td>Gonadotropin-dependent preocious puberty or central precocious puberty</td>
<td></td>
</tr>
<tr>
<td>• Without CNS abnormalities</td>
<td>• Hypogonadotropic hypogonadism</td>
</tr>
<tr>
<td>• Genetic causes</td>
<td>• Isolated congenital</td>
</tr>
<tr>
<td>• Secondary to previous chronic exposure to sex steroids (rare)</td>
<td>• Normal olfaction: isolated hypogonadotropic hypogonadism</td>
</tr>
<tr>
<td>• After exposure to endocrine disruptors (proposed mechanism)</td>
<td>• Functional hypogonadotropic hypogonadism</td>
</tr>
<tr>
<td>• Idiopathic</td>
<td>• Hypothalamic amenorrhoea</td>
</tr>
<tr>
<td>• CNS abnormalities</td>
<td>• Male functional hypogonadism</td>
</tr>
<tr>
<td>• Tumours</td>
<td>• Tumours</td>
</tr>
<tr>
<td>• Congenital malformations: hypothalamic hamartoma, arachnoid cyst, and others</td>
<td>• Adrenal, ovarian, or testicular tumours</td>
</tr>
<tr>
<td>• Acquired diseases: infections and inflammatory processes of the CNS</td>
<td>• Autonomous ovarian cysts</td>
</tr>
<tr>
<td>Gonadotropin-independent preocious puberty or peripheral precocious puberty</td>
<td>• Severe long-term untreated primary hypothyroidism</td>
</tr>
<tr>
<td>• Tumour</td>
<td></td>
</tr>
<tr>
<td>• Adrenal, ovarian, or testicular tumours</td>
<td></td>
</tr>
<tr>
<td>• Autonomous ovarian cysts</td>
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</tbody>
</table>

LH=luteinising hormone. FSH=follicle-stimulating hormone.
have shown that the prepubertal suppression of hypothalamic-pituitary-gonadal function occurs even in the absence of feedback by sex steroids, suggesting that the juvenile pause represents a state of neurally determined functional stasis rather than immaturity of the GnRH neurons. The exact mechanism that leads to the reinstatement of pulsatile GnRH secretion is not known, but evidence suggests that a complex interaction between nutritional, environmental, and genetic factors is involved in the regulation of puberty initiation.

Many neurotransmitters can modify the GnRH secretory pattern, with an excitatory or inhibitory effect on GnRH secretion. During childhood the predominant pathway is believed to be the inhibitory network, which decreases around the time of puberty initiation, along with augmentation of the excitatory tonus. Studies in animal models have identified several of the regulatory components of the GnRH system. Results for these studies suggest that catecholamines and glutamate are components of the excitatory input, whereas γ-aminobutyric acid (GABA) might be a component of the inhibitory network. Studies in patients with pubertal disorders have not identified genetic defects in the genes encoding these factors, which undermines the evidence for their potential roles, but does not rule them out entirely as important for puberty initiation.

**Genes involved in GnRH regulation**

**Overview**

Data showing an association between the ages at which a mother and her children attain pubertal milestones and between the timing of puberty within ethnic groups are suggestive of genetic modulation of the reproductive endocrine axis. These and other data suggest that around 50–80% of the variation in pubertal onset might be genetically determined.

Many studies have been done to investigate candidate mechanisms governing GnRH release; however, the most compelling evidence for specific GnRH neural inputs comes from genetic studies in patients with pubertal disorders. Two of the most important excitatory components of the GnRH network were identified with the demonstration that the genetic loss of such inputs results in failure to initiate the GnRH pulse generator at the expected time of puberty, resulting in isolated hypogonadotropic hypogonadism.

**Kisseptin system**

The discovery of the kisseptin system as a crucial component for pubertal activation of the hypothalamic-pituitary-gonadal axis occurred in 2003, when loss-of-function mutations of the KISS1R (previously known as GPR54) gene were identified in individuals with isolated hypogonadotropic hypogonadism, establishing KISS1R inactivation as a cause of this disorder (table, figure 2). Physiological and pharmacological studies have shown that kisseptin is an essential part of the complex excitatory network that regulates GnRH secretion. Administration of kisseptin results in increases in plasma LH concentrations in healthy men and in women kisseptin also induces LH release, although the response varies across the menstrual cycle. Kisseptin stimulates gonadotropin release less potently but in a more physiologically effective way than do current treatments with GnRH analogues. A non-constitutively activating mutation in KISS1R was the first identified genetic cause of central precocious puberty, reported in an adopted girl with breast development with slow progression that had first been noted at birth and that progressed at age 7 years. Mutations in KISS1R were also identified in patients with isolated hypogonadotropic hypogonadism and central precocious puberty, strengthening the evidence base for the association of this system with pubertal onset.

In animal models, the KISS1 neurons appear to mediate sex steroid feedback effects in the hypothalamus. These neurons express oestrogen receptor α and receive sex steroid signals from the gonads, which regulate KISS1 expression and control the release of GnRH. In many mammalian species, KISS1 expression is negatively regulated in the arcuate nucleus and positively regulated in the anteroven tral periventricular nucleus and preoptic area by sex steroids. The KISS1 positive regulation is believed to be important for the LH surge in females of many mammalian species.

**Tachykinins**

Neurokinin B (NKB) belongs to a family of closely related peptides called tachykinins. The role of NKB in the control of reproductive function was the subject of investigation for several years because of neuroanatomical evidence for potential NKB inputs to GnRH neurons. It was not until 2009 that Topaloglu and colleagues identified NKB as an important factor for human puberty initiation, studying ten consanguineous families with multiple affected members who had isolated hypogonadotropic hypogonadism. Homozygous loss-of-function mutations in the genes encoding the NKB receptor (TACR3) and neurokinin B (TAC3) were identified, providing compelling evidence that the NKB system is necessary for the activation of the hypothalamic-pituitary-gonadal axis in puberty (table, figure 2). The report by Gianetti and colleagues of reversibility of isolated hypogonadotropic hypogonadism in a high proportion of patients with TACR3 mutations suggested that the NKB system is not absolutely required for GnRH regulation after the maturation of the hypothalamic-pituitary-gonadal axis. However, other studies did not show a similar frequency of reversibility, and it is still not definitively known whether the NKB system is essential for the excitatory GnRH regulatory network after pubertal development.

Although the effect of NKB on GnRH secretion depends on the hormonal context, administration of NKB has been shown to elicit strong stimulation of LH
Receptors occurs at least partly through actions on KISS1 of gonadotropin release by the tachykinins and their production mediated by GnRH in animal models.64 Neurons co-expressing kisspeptin and NKB in the human mediobasal hypothalamus often co-express substance P.65 Substance P belongs to the tachykinin family along with neurokinin B and neurokinin A. Substance P and neurokinin B are known to stimulate the gonadotropic axis in men.66 The regulation of gonadotropin release by the tachykinins and their receptors occurs at least partly through actions on KISS1 neurons in mice.67

Makorin ring finger protein 3
The first gene with an inhibitory effect on GnRH secretion with mutations identified in human beings was MKRN3 (table, figure 2). The identification of this novel factor in the GnRH network arose from whole-exome sequencing analysis of families with central precocious puberty.52 MKRN3 is located in the Prader-Willi syndrome critical region on chromosome 15 and before the identification of its role in puberty, the few reports about this gene came from research into Prader-Willi syndrome.68,69 The maternal MKRN3 allele is imprinted and only the paternal allele is expressed.68–70 Following this inheritance pattern, all MKRN3 mutations identified in patients with central precocious puberty were inherited from their fathers.71 MKRN3 is the most common genetic defect associated with central precocious puberty so far; families with central precocious puberty from several different ethnic groups harbour mutations in this gene.72–76 There was no previous association of MKRN3 with the hypothalamic-pituitary-gonadal axis and the mechanism by which MKRN3 regulates puberty initiation is not yet completely understood. The mutations identified in patients with central precocious puberty are expected to disrupt protein function. MKRN3 expression in the arcuate nucleus of mice is high prepubertally and decreases before puberty initiation, reaching very low levels in adult life.77 Taken together, these findings suggest that the loss of function of MKRN3 results in early puberty, implying an inhibitory role of MKRN3 on GnRH secretion.78 The very low expression of MKRN3 in the adult mouse, the similarity of the phenotype of patients with central precocious puberty with and without MKRN3 mutations, and the absence of an identified phenotype beyond central

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<tbody>
<tr>
<td>GNRHR</td>
<td>GnRH receptor</td>
<td>Autosomal recessive</td>
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<td>35, 46</td>
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<td>KISS1</td>
<td>Kisspeptin</td>
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<td>Kisspeptin receptor</td>
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<td>TAC3</td>
<td>Neurokinin B</td>
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<td>TACR2</td>
<td>Neurokinin B receptor</td>
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<td>GNRH1</td>
<td>GnRH</td>
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**Stimulation of GnRH synthesis or secretion**

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<td>Anosmin-1</td>
<td>X-linked</td>
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<tr>
<td>FGR1</td>
<td>Fibroblast growth factor receptor 1</td>
<td>Autosomal dominant</td>
<td>Kallmann's syndrome or isolated hypogonadotropic hypogonadism</td>
<td>35, 46</td>
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<td>FGFR2</td>
<td>Fibroblast growth factor 8</td>
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<td>CHD7</td>
<td>Chromodomains helicase DNA binding protein 7</td>
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<td>WD repeat-containing protein 11</td>
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<td>Heparan sulphate 6-O-sulphotransferase 1</td>
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<td>FGFR17, IL17RD, DUSP6, SPRY4, and FLRT3</td>
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**Inhibition of GnRH synthesis or secretion**

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<th>Protein</th>
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<td>MKRN3</td>
<td>Makorin ring finger protein 3</td>
<td>Paternally inherited</td>
<td>Central precocious puberty</td>
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**GnRH migration**

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**References**

1. www.thelancet.com/diabetes-endocrinology

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**Table: Genes involved in the control of GnRH**

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<thead>
<tr>
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**Inhibition of GnRH synthesis or secretion**

<table>
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precocious puberty in patients with MKRN3 loss-of-function mutations imply that MKRN3 is important for puberty initiation, but not for the maintenance of pulsatile GnRH secretion later in life. However, further studies and larger samples of children with central precocious puberty and MKRN3 mutations are necessary to more fully clarify whether the clinical course of puberty might differ as compared with idiopathic central precocious puberty and whether there might be any other associated reproductive or non-reproductive phenotypes.

MKRN3 belongs to the Makorin family, a family of E3 ubiquitin ligases. Its protein structure has a ubiquitin ligase domain and it has been postulated that MKRN3 might inhibit stimulators of GnRH secretion (figure 2). Further research into the mechanism of action of MKRN3 will expand our knowledge of the GnRH inhibitory network.

In a recent meta-analysis of genome-wide association and custom-genotyping array studies of age at menarche in up to 182,416 women of European descent, a variant downstream of MKRN3 had the largest effect size for association with menarche when the variant was paternally inherited. This finding implicates common variation near MKRN3 as an important source of variation in pubertal timing in the general population.

**LIN28B**

In 2009, genome-wide association studies identified an association between the locus 6q21 (in or near the LIN28B gene) with age at menarche and height. LIN28B encodes a regulator of the let-7 class of microRNAs. Although several other human studies replicated the findings from the genome-wide association studies, the mechanism by which this gene is associated with the control of GnRH secretion is unknown. Mutations in LIN28B have not been identified in patients with central precocious puberty or constitutional delay of growth and puberty.

Many other genes have been identified in association with developmental defects in GnRH neurons. These genes affect GnRH neuronal migration and are associated with hypogonadotropic hypogonadism with anosmia, or Kallmann’s syndrome (table). Other genes have been identified in association with hypogonadotropic hypogonadism in conjunction with syndromic disorders, but are beyond the scope of this Series paper.

**Metabolic factors that affect GnRH secretion**

Animal and human studies in the 1960s and 1970s showed that the proper function of the hypothalamic-pituitary-gonadal axis is gated by metabolic and nutritional factors. A threshold bodyweight is necessary for pubertal development and reproduction. The identification of peripheral hormones (such as leptin, insulin, and ghrelin) that signal the metabolic status to the reproductive axis has expanded our knowledge about the neuroendocrine mechanisms linking metabolism and reproduction (figure 3). Nonetheless, our understanding of how such dynamic interplay occurs remains incomplete.

Leptin, an adipocyte-derived hormone, is a satiety factor secreted in proportion to the amount of body energy stores, and was one of the first factors linking metabolism with the reproductive axis. Serum leptin concentrations and leptin mRNA concentrations in adipose tissue are associated positively and very closely with fat mass. Leptin is a permissive metabolic signal to the reproductive system; its circulating concentrations inform of the actual size of energy reserves not only to the reproductive axis, but to several body systems. As has been shown for bodyweight, the attainment of appropriate leptin concentrations is indispensable for the maturation of the hypothalamic-pituitary-gonadal axis, normal pubertal progression, and maintenance of fertility.

Leptin acts through the leptin receptor, a single-transmembrane-domain receptor of the cytokine receptor family. The finding that homozygous mutations in...
genes encoding leptin (LEP) or leptin receptor (LEPR) cause hypogonadotropic hypogonadism in human beings emphasises the role of leptin in reproduction. In addition to the reproductive phenotype, individuals with homozygous mutations in LEPR presented with early-onset morbid obesity and reduced growth hormone and thyrotropin secretion. Leptin treatment of patients with hypogonadotropic hypogonadism and a homozygous mutation in LEP resulted in the induction of menstrual cycles, development of secondary sexual characteristics, and pulsatile gonadotropin secretion.

Moreover, studies show an increase in IGF-1 expression in reproductive and neuroendocrine function. IGF-1 is structurally homologous to insulin and findings suggest that it is also involved in GnRH regulation. IGF-1 is a major regulator of leptin production; therefore, some of the positive effects of insulin on the reproductive system might derive from its ability to stimulate leptin secretion. However, the precise site of action of insulin at the hypothalamic level in vivo remains in question.

Another systemic hormone with a major role in the regulation of reproduction is the pancreatic hormone insulin (figure 3). Studies have suggested that insulin acts on the hypothalamic-pituitary-gonadal axis at the level of the hypothalamus to directly or indirectly modulate GnRH secretion (possibly via kisspeptin), as well as at the level of the pituitary gonadotrope. Insulin is a major regulator of leptin production; therefore, some of the positive effects of insulin on the reproductive system might derive from its ability to stimulate leptin secretion. However, the precise site of action of insulin at the hypothalamic level in vivo remains in question.

IGF-1 is structurally homologous to insulin and findings suggest that it is also involved in GnRH regulation. Moreover, studies show an increase in IGF-1 expression during puberty in human beings.

Ghrelin, a peptide predominantly secreted by the stomach, has been postulated to be a peripheral signal for energy insufficiency, acting as a potent orexin (figure 3). Ghrelin has direct actions on the brain and the pituitary, where it has an inhibitory effect on gonadotropin pulsatility and decreases LH responsiveness to GnRH, as well as a stimulatory effect on prolactin secretion, probably involving direct action on somatotroph cells.

Conclusions

The triggers of puberty initiation have puzzled scientists for many years. Pubertal development culminates with reproductive competence. Because of its paramount evolutionary importance, the reproductive system is controlled by a complex regulatory network. In this system, a major hierarchical role is held by GnRH neurons. GnRH is secreted in a unique pulsatile manner by neurons located in the hypothalamus to stimulate the pituitary gonadotropins and gonadal sex steroids. The regulation of GnRH secretion is still not completely understood, but we have witnessed a substantial expansion of our understanding of the signals involved in this regulation in the past two decades. The decrease in the average age of puberty initiation between the mid-19th and mid-20th centuries suggested an association of nutrition and environmental factors with reproduction, which was substantiated by the identification of leptin as an important link between both systems, together with the characterisation of the effects of the metabolic hormones, insulin and ghrelin, on the reproductive axis. Although studies in animals have led to identification of regulators of GnRH such as catecholamines, GABA, and glutamate, genetic studies of patients with pubertal disorders have resulted in the identification of a major regulatory hub for the GnRH neurons—ie, the hypothalamic neurons expressing kisspeptin and neuropeptide B. These findings have important clinical implications, not only to aid in diagnosis and improve genetic counselling, but also to contribute to the development of new therapies for reproductive disorders. For example, kisspeptin has been used in clinical studies to induce ovulation in infertile women.
More recently, genetic studies have led to the identification of the most common known genetic cause of central precocious puberty, MKRN3.13 Although the mechanism by which MKRN3 regulates the hypothalamic-pituitary-gonadal axis is still to be elucidated, the identification of mutations in this gene in patients with different ethnic origins and its expression pattern in the hypothalamus of mice support the association of MKRN3 with GnRH regulation. Different from the other genetic factors regulating the hypothalamic-pituitary-gonadal axis, MKRN3 operates in an inhibitory mode. It is the first imprinted gene to be implicated in GnRH regulation. Genomic imprinting hypothetically evolved to control the dosage of developmentally important genes and occurs in about 100 mammalian genes.14 Increasing evidence implicates epigenetic mechanisms in timing of puberty.15 After identification of the association of MKRN3 with puberty regulation, single nucleotide polymorphisms in imprinted gene regions were linked to the age of menarche, supporting puberty timing as a selective trait.20 The availability of powerful new technologies has contributed to expanding our knowledge of the regulation of puberty and recognising the role of genetic imprinting in the control of the activation of GnRH secretion at the time of puberty initiation.21 An increased understanding of puberty regulation will help to improve the treatment of reproductive disorders.

Contributors
APA and UBK searched the scientific literature and wrote the report.

Declaration of interests
UBK has received personal fees from Takeda, unrelated to this work. APA declares no competing interests.

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AVALIAÇÃO DA PUBERDADE

Desvios fisiológicos da normalidade

SUZANA FIGUEIREDO

Bibliografia:


Novello L, Speiser P.W. Premature Adrenarche. Pediatric Annals, 2018; 47 (1): e7-e11


Premature Thelarche

Aditi Khokhar, MBBS; and Angela Mojica, MD

ABSTRACT

Premature thelarche is a benign condition that affects young girls and may be interpreted as a sign of central precocious puberty (CPP). Parental concern is common when breast development is noted in a young girl. It is important to differentiate premature thelarche from CPP, as the latter is a more serious disorder that may affect final adult height and menarcheal age, and may have psychological implications as well. Distinguishing between the two conditions clinically may help the patients avoid unnecessary testing. Pediatricians can play a pivotal role by providing reassurance to families and helping alleviate parental anxiety. This article reviews the clinical presentation of premature thelarche, its usual course, and implications. [Pediatr Ann. 2018;47(1):e12-e15.]

Wilkins1 first coined the term “premature thelarche” (PT) in 1957. He described it as an isolated breast development in the absence of any other clinical signs of pubertal maturation in girls younger than age 8 years, predominantly in the first 2 years of life.1 This was followed by multiple case reports on isolated premature thelarche that showed the condition was not associated with other features of puberty.2,3 Prevalence of premature thelarche has been reported to range from 2.2% to 4.7% among girls age 0 to 48 months.3,5

PATHOPHYSIOLOGY

The pathophysiology of isolated breast development is still elusive and various hypotheses have been proposed. It has been postulated to be a result of increased breast tissue sensitivity to normal circulating estrogen in prepubertal girls,5 transient estrogen secretion by follicular cysts of the ovary,7 increased production of estrogens from precursors of adrenal origin,8 increased dietary estrogen as a result of exogenous contamination of food,9 and transient partial activation of the hypothalamic-pituitary-gonadal axis with excessive secretion of follicle-stimulating hormone (FSH).10 Inhibin B secreted from granulosa cells is thought to be responsible for FSH increase in premature thelarche cases that start before age 2 years.11 A predominant FSH response to gonadotropin-releasing hormone stimulus is characteristically seen in these patients in contrast to a predominant luteinizing hormone (LH) response typically seen in patients with precocious puberty. The phenomenon has been termed “mini-puberty.”12

Exposure to certain environmental agents such as lavender oils and tea tree oils has been linked to premature breast development in both boys and girls.13 In vitro studies show that these oils possess weak estrogenic and antiandrogenic activities that may contribute to an imbalance in estrogen and androgen pathway signaling.13 Epidemics of isolated premature thelarche have been reported after consumption of contaminated food (eg, beef from cattle treated with hormones like diethylstilbestrol) from different parts of the world, raising the suspicion about the role of endocrine-disrupting chemicals.14,15 However, there is a lack of conclusive data and more research is needed in the field.

CLINICAL MANIFESTATIONS

Children with premature thelarche have isolated breast development without any other sign of sexual maturation. Breast development occurs most commonly during the first 2 years of life.2 Hypertrophy of the breast is usually bilateral but sometimes is unilateral. The enlargement is not excessive and no significant changes of nipples or areolae develop. It typically does not progress beyond Tanner stage 3; in unilateral cases it does not progress beyond Tanner stage 2.16

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Breast enlargement may be transient but in most cases it persists for several months or years. Time of onset is not understood to be a predictor of length of prevalence. Reported time of regression has been variable between studies. Mills et al. reported that the time from discovery to disappearance ranges from 3 to 60 months, with a mean of 23 months. de Vries et al. reported a mean regression time of 16 ± 11 months.

In contrast to patients with central precocious puberty (CPP), girls with premature thelarche do not have any other clinical evidence of sexual development besides breast development, and they have a normal growth pattern. Puberty onset occurs at the usual age and the pattern of sexual maturation is completely normal. Menarchal age correlates with maternal age of menarche in most of the cases. In some girls, age of menarche has been somewhat earlier but still within normal limits.

PREMATURE THELARCHE VERSUS CENTRAL PRECOCIOUS PUBERTY

Although the physiology of premature thelarche is not clear, CPP involves activation of the hypothalamic-pituitary axis. CPP is associated with progressive breast and pubic hair development, accelerated growth velocity, bone maturation, and early epiphyseal fusion. Children with premature thelarche have a normal pattern of growth. CPP may have a detrimental effect on final adult stature, but can be effectively treated with long-acting luteinizing hormone-releasing hormone agonists. Premature thelarche does not require treatment.

The consensus is that premature thelarche is a benign, self-limited condition that only rarely progresses to CPP. Prevalence of progression to precocious puberty has been reported to be from 9% to 14%. The authors did not suggest any predictive clinical signs or laboratory tests that may differentiate patients who are at risk of having precocious puberty. Bizzarri et al. indicated a positive association between older age at presentation (mostly after age 2 years) and progression to precocious puberty.

BIOCHEMICAL TESTING AND IMAGING

Differentiation between premature thelarche and CPP is crucial for both prognostic and treatment reasons. In isolated cases of premature thelarche, with no other concerning signs and with a reliable family that can monitor the situation closely, just close follow-up may be sufficient to differentiate the two. However, it may be difficult at times to distinguish between them simply based on clinical grounds, as both conditions initially present with isolated breast development (Figure 1).

In patients with clinical signs of potential puberty progression (progressive breast development, appearance of other secondary sexual characteristics, and growth acceleration), there are some laboratory and imaging studies that can help distinguish the two conditions. However, as mentioned above, none of the tests can predict which patients are at risk of developing precocious puberty and, therefore, do not replace close monitoring of sexual development and growth in these patients.

Bone Age

Bone age in patients with premature thelarche is consistent with chronological age. Patients with precocious puberty usually have advanced bone age.

Pelvic Ultrasound

Pelvic ultrasound is not indicated in most cases. However, if performed, uterine and ovarian volumes are in prepubertal range in patients with premature thelarche. Some studies have reported increased presence of ovarian microcysts in girls with premature thelarche, but other studies have not found this. In patients with CPP, pelvic ultrasound typically shows an increase in size of the uterus and ovaries consistent with pubertal volumes. An endometrial stripe may also be seen.

Basal Gonadotropins

The mean basal FSH levels are not significantly different between girls with premature thelarche and CPP. By contrast, girls with CPP have higher mean basal plasma LH levels than girls with isolated breast development. However, single basal LH value may not be sufficient to differentiate premature thelarche from CPP. This is because the basal LH value of >0.1 IU/L by immunochemiluminometric assay has variable sensitivity ranging from 56.4% to 94.7% and specificity ranging from 64% to 88.4% for detecting CPP. Therefore, a prepubertal LH value should not be used as a definitive diagnostic test to rule out precocious puberty in those children who have clinical signs of puberty progression.
Estradiol Levels
Estradiol levels are not elevated in patients with premature thelarche and have been shown to be lower than those seen in patients with precocious puberty. One study found estradiol levels to be higher in girls with premature thelarche compared to prepubertal girls, but there was large overlap in estradiol levels in the two groups and many girls with premature thelarche had estradiol levels in normal prepubertal range.25

Summary of Biochemical Tests
Biochemical tests should be considered in patients with clinical signs of pubertal progression as they may give clues to help differentiate between premature thelarche and CPP. However, there is a considerable overlap in the expected values for these laboratory tests in CPP and premature thelarche; hence, these tests do not replace close monitoring for sexual development and growth in these patients. Biochemical tests should be done using pediatric ultrasensitive assays for accurate interpretation. Imaging tests such as bone age and pelvic ultrasound can be challenging to interpret, especially in children younger than age 3 years; therefore, a pediatric endocrinologist should determine the clinical significance and indication for these tests. In cases with high clinical suspicion of precocious puberty, baseline biochemical or imaging tests may not be enough to rule out precocious puberty. These patients need to be referred to a pediatric endocrinologist, who may consider more specialized testing such as gonadotropin-releasing hormone stimulation test.

CONCLUSION
Even though the exact pathophysiological mechanism of premature thelarche is still unknown, the extensive literature surrounding the condition suggests its benign clinical course in most instances. There are no clinical or biochemical parameters that can help predict at time of onset which patients are at risk of having early or precocious puberty. However, this should not dissuade the pediatricians caring for these children from offering sympathetic reassurance to the families. Clinical follow-up every 3 to 6 months monitoring for pubertal progression and growth is recommended and helps differentiate premature thelarche from CPP in most cases, with laboratory and radiologic testing being reserved for selected patients.

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Premature Adrenarche
Laura Novello, MD; and Phyllis W. Speiser, MD

ABSTRACT

Adrenarche is when a child’s adrenal cortex starts to secrete adrenal androgen precursors. Dehydroepiandrosterone (DHEA) is the most abundant product of the adrenal cortex, and is a weak androgen agonist thought to be responsible for the clinical signs of pubarche by conversion to more potent androgens, testosterone, and dihydrotestosterone. DHEA’s extra-adrenal sulfation product, dehydroepiandrosterone sulfate, is a stable marker for adrenal androgenic activity. Pubarche is the physical manifestation of androgenic hormone production, and includes the development of pubic and axillary hair, adult body odor, and acne. This stage is usually considered premature if it commences before age 8 years in girls or age 9 years in boys. Premature adrenarche is a diagnosis of exclusion, as true centrally mediated precocious puberty, congenital adrenal hyperplasia, exogenous androgen exposure, and androgen-secreting tumors must be ruled out. Premature adrenarche may be associated with a history of an infant who was small for gestational age at birth who then gained weight rapidly thereafter or became obese. In some instances, premature adrenarche may predict functional ovarian hyperandrogenism in adolescence. Management of premature adrenarche is largely aimed at observation, lifestyle adjustments for weight concerns, and monitoring for future possible persistent androgen excess and insulin resistance.

ETIOLOGY

Premature adrenarche is more commonly seen in girls than boys, at a ratio of 9:1. Premature adrenarche is seen with increased frequency in children with certain medical histories or comorbidities. Low birth weight may be associated with increased DHEAS levels in childhood and early puberty. However, some studies have shown the incidence of premature adrenarche in children formerly small for gestational age to be equivalent to the general population. Children who were born preterm may continue...
have elevated DHEAS levels into their twenties.\(^2\) Children with a history of brain injury have an increased risk of premature adrenarche or precocious puberty.\(^9\)

Multiple studies have shown that children with premature adrenarche are often taller and heavier than their prepubertal peers.\(^2,3\) Overweight or obese children may have increased insulin-like growth factor-1 levels or advanced bone ages that do not portend abnormal stature or full-blown precocious puberty.\(^7\) Despite a reduced pubertal growth spurt, their adult height is within 2 standard deviations of their mid-parental height.\(^2\) The mean age of menarche for girls with premature adrenarche is no earlier than the general population.\(^7\)

According to studies of girls in Spain, those with a history of premature adrenarche were more likely to develop functional ovarian hyperandrogenism (FOH) once they reached adolescence.\(^2,8\) This may be more prominent in overweight or obese youngsters. Symptoms of FOH include oligomenorrhea, hirsutism, acne, anovulation, and hormonal evidence of hyperandrogenism.\(^2,7\) These girls may be diagnosed with polycystic ovary syndrome if these symptoms persist for longer than 3 years after onset of menarche.\(^7\)

PRESENTATION

The first symptom of premature adrenarche is typically the appearance of pubic or axillary hair.\(^2\) This is often accompanied by the development of apocrine body odor. Acne and increased skin sebum are also often noted.\(^2\) A step-by-step evaluation of a child presenting with these symptoms is summarized in Figure 1.

DIAGNOSIS/DIFFERENTIAL DIAGNOSIS

To be diagnosed with premature adrenarche, a child should not have signs of gonadarche, such as testicular enlargement or glandular breast development. Clinicians should perform an adrenal profile by the highly specific method of liquid chromatography/tandem mass spectroscopy (LC-MS/MS). The adrenal profile should consist of DHEAS, androstenedione, testosterone, and 17-hydroxyprogesterone (17-OHP) measured in an early morning (prior to 8 am) blood specimen, when these steroids are at their circadian peak. If the clinician is seeking to rule out central precocious puberty (CPP), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol levels may also be measured, and a bone age X-ray should be performed. Central puberty commences with pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, the rise of FSH and LH from the anterior pituitary, and subsequent rise of estradiol and testosterone from the gonads.\(^10\) Endocrine specialty laboratories can provide hormone reference ranges for prepubertal children, which should guide the diagnosis of premature adrenarche and puberty. CPP can be diagnosed in the setting of bilateral testicular enlargement in boys, glandular breast development in girls, and an accompanying pubertal growth spurt.\(^1\) A bone age X-ray can identify pubertal bone age advancement.\(^1\) Children with premature adrenarche do not typically have bone age advancement, growth spurt, or pubertal range FSH and LH levels.\(^9\)

It is critical to exclude other pathologic causes of androgen excess.\(^3\) These disorders include late-onset or nonclassic congenital adrenal hyperplasia (NCAH),\(^3\) genetic conditions such as familial male-limited precocious puberty, androgen-producing tumors in the gonads or adrenal glands, and exogenous androgen exposure.

Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) diagnosed outside the newborn period is most often caused by a mild or nonclassic form of 21-hydroxylase deficiency, caused by partial mutations in CYP21A2.\(^11\) NCAH, unlike the more severe or classic form of CAH, is not typically identified in the newborn period. The female patient with NCAH does not present with virilized external genitalia, or with an elevated 17-OHP on newborn screen in most cases. Patients with NCAH have residual 21-hydroxylase activity and do not present until later childhood, adolescence, or even until young adulthood.\(^11\) Among male and female patients with NCAH diagnosed before age 10 years, 92% present with complaints of premature pubarche.\(^11\) They have elevated adrenal steroid levels (specifically 17-OHP and androstenedione) associated with a variable degree of bone age advancement and slightly, but not significantly, shorter adult height compared with mid-parental height.\(^11,12\) In adolescence and adulthood, female patients may present with hirsutism, irregular menses, acne, or infertility.\(^11\) Male patients with NCAH may be identified due to premature pubarche, acne, an early growth spurt, or subfertility, but the majority are asymptomatic, and found to have NCAH during family screening.\(^11,13\)

To screen for NCAH caused by 21-hydroxylase deficiency, a serum 17-OHP should be measured by LC-MS/MS on an early morning (ie, prior to 8 am) specimen.\(^11\) Normal values are dependent on the child’s age, gender, and stage in puberty, and they vary by laboratory. Normal prepubertal children typically have 17-OHP levels of <90-100 ng/dL, whereas normal adolescents or young adults have 17-OHP levels of <200 ng/dL. If the child has a positive screen or a particular concern for NCAH, the next step in evaluation is to perform a cosyntropin (adrenocorticotropic 1-24) stimulation test. Cosyntropin at a dose of 250 mcg is given intramuscularly or intravenously, and serum 17-OHP is measured before and 60
Typically, a stimulated 17-OHP level of >1,000-1,200 ng/dL is consistent with NCAH. If the hormonal diagnosis is in doubt, or for purposes of genetic counseling, the diagnosis may be confirmed by genotyping \textit{CYP21A2}. Another rarer form of virilizing non-classic CAH is 11-beta-hydroxylase deficiency. CAH due to 11-beta-hydroxylase deficiency results in increased levels of deoxycorticosterone and 11-deoxycortisol, decreased aldosterone and cortisol, and increased adrenal androgens causing virilization. Deoxycorticosterone is a mineralocorticoid, causing patients with severe 11-beta-hydroxylase deficiency to develop low renin hypertension. These children are treated with hydrocortisone and may require antihypertensive treatment.
Unlike CAH caused by severe 21-
hydroxylase deficiency, in which pa-
tients require lifelong treatment with
glucocorticoids and mineralocorticoids
for survival, not all patients with NCAH
require treatment.\textsuperscript{11,14} Indications for hy-
drocortisone treatment include early
or rapidly progressive pubarche, bone
age advancement, or excessive viriliza-
tion.\textsuperscript{11,14} Hydrocortisone can ameliorate
signs of androgen excess. Once these
children reach adult height, or their
symptoms resolve, the hydrocortisone
may be tapered and discontinued.\textsuperscript{14}

**Tumors**

Certain adrenal or gonadal tumors
may produce androgens resulting in
premature virilization.\textsuperscript{1,4} Virilizing ad-
renal tumors include both adrenocorti-
cal adenomas and adrenocarcinomas.
Adenomas typically secrete cortisol but
may also produce mineralocorticoids or
androgens; adrenocarcinomas commonly
secrete multiple hormones.\textsuperscript{4} Testicular
Leydig cell tumors secrete high levels of
testosterone.\textsuperscript{1,15} Signs of virilizing tumors
in young boys include phallic enlarge-
ment, erections, body hair, increased
muscle mass, and voice deepening.\textsuperscript{4}
Gynecomastia may develop due to aro-
matization of testosterone to estradiol.\textsuperscript{1,6}
Boys with adrenal tumors will have no
testicular enlargement, whereas boys
with Leydig cell tumors may develop
asymmetric enlargement of a testis.\textsuperscript{1,15}

Ovarian Sertoli-Leydig cell tumors
also produce high levels of androgens
and androgen precursors. Girls with
virilizing tumors may experience cli-
toral growth, acne, body hair, voice
deepening, and oligo- or amenorrhea.\textsuperscript{17}
These signs tend to be rapidly progres-
sive relative to other causes of early
adrenarche.\textsuperscript{18} If Sertoli-Leydig tumors
are not hormone-secreting, abdominal
pain or swelling may be the presenting
sign.\textsuperscript{17,18}

Germ cells tumors secreting human
chorionic gonadotropin (hCG) can also
cause a child to virilize prematurely.\textsuperscript{10}
hCG cross-reacts with LH receptors, as
the two hormones share an identical al-
pha subunit.\textsuperscript{10} Activation of the LH recep-
tor in the testicle will cause the Leydig
cells to produce testosterone and virilize
a boy while only causing mild testicle
growth.\textsuperscript{10} Girls do not experience signs of
puberty, as they require FSH in addition
to LH stimulation to secrete estrogen.\textsuperscript{19}
hCG-secreting tumors include hepa-
tomas, hepatoblastomas, choriocarcino-
mas, and other germ cell tumors.\textsuperscript{10}

In the case of estrogen-, androgen-, or
hCG-producing tumors, gonadotropins
are suppressed, as the increased sex ste-
roid production is not due to central pre-
cocious puberty.\textsuperscript{15} In addition, midnight
salivary cortisol and 24-hour urinary-free
cortisol should be checked for Cushing
syndrome if suggestive clinical features
are observed.\textsuperscript{4} Ovarian Sertoli-Leydig
cells and some germ cell tumors may
produce elevated alpha fetoprotein lev-
els.\textsuperscript{10,20} Imaging should be focused on
the likely source of the sex hormone ex-
cess. Computed tomography or magnetic
resonance imaging of the adrenal gland
is recommended if there is no evidence
for a gonadal tumor or if the patient has
elevated cortisol or signs of Cushing
syndrome.\textsuperscript{4} If the child has an elevated
level of hCG, a liver ultrasound should
be performed to evaluate for nodules or a
mass.\textsuperscript{10} Other sources of pathologic hCG
secretion include the mediastinum and
brain.\textsuperscript{17} A testicular ultrasound would be
the appropriate step in a male with tes-
ticular asymmetry or mass, or if no other
source of androgen excess is detected. A
biopsy should be performed on the larger
of the asymmetric testes for pathology.
Initial treatment for nonhepatic tumor
types is surgical resection.\textsuperscript{4} Isolated es-
drogen production may be associated with
ovarian cysts, of which a small propor-
tion may represent malignancies. Surgical
resection is usually indicated, save for
those recurrent ovarian cysts attributable
to McCune-Albright syndrome caused by
Gs-alpha mutations associated with
gonadotropin-independent puberty.

**Other Differential Diagnoses**

Familial male-limited precocious pu-
berty (also known as testotoxicosis) is a
rare autosomal dominant condition.\textsuperscript{3} It is
carried by an activating mutation of the
LH receptor, causing the testicular Ley-
dig cells to secrete testosterone indepen-
dent of stimulation from gonadotropins.
This causes affected male patients who
are age 1 to 4 years to experience rapid
virilization, modest bilateral testicular
growth, growth acceleration, and bone
age advancement.\textsuperscript{10} They will have pu-
bertal range testosterone levels but prepu-
bertal LH levels.\textsuperscript{1,10} Treatment is aimed
at inhibiting steroidogenesis with keto-
conazole, blocking the androgen receptor
with bicalutamide, and/or preventing aro-
matization from testosterone to estrogen
with aromatase inhibitors to prevent bone
age advancement.\textsuperscript{10,21}

Testosterone preparations are pre-
scribed to male patients by pediatric en-
docrinologists and adult specialists for
various indications, including micrope-
nis, delayed puberty, and testosterone de-
iciency.\textsuperscript{22} Testosterone is available as an
intramuscular injection, a topical patch,
or in gel formulation. Women or children
in physical contact with patients using
transdermal testosterone (gel or patch)
may have high blood testosterone levels
with development of pubic hair, acne,
phallic or clitoral enlargement, and bone
age advancement.\textsuperscript{7,22} In 2009, the US
Food and Drug Administration issued a
black box warning for testosterone gels,
informing users of the risk of virilization
of young children and women who come
into contact with these gels.\textsuperscript{22} Users are
instructed to wash their hands immedi-

ately after applying the gel and cover the skin where the gel was applied.22 Similar signs of virilization have occurred from oral medications or vitamins contaminated with androgens.23

PROGNOSIS/NATURAL COURSE
Premature adrenarche in childhood can be associated with multiple medical conditions in adolescence and in adulthood; however, the majority of such children have no major health problems. Those children who have premature pubarche and adrenarche in the setting of overweight and obesity should be educated about incorporating a healthy diet and daily exercise into their routines.2

MANAGEMENT
Once other causes of prepubertal hyperandrogenism have been excluded, the patient can be diagnosed with benign premature adrenarche. Isolated premature adrenarche itself does not require any specific treatment.3 Parents should be informed about incorporating a healthy diet and daily exercise into their routines.2

REFERENCES
Premature adrenarche

Rachel M Williams, Caleb E Ward, Ieuan A Hughes

ABSTRACT
Premature adrenarche refers to the presence of secondary sexual hair in girls younger than 8 years old and boys younger than 9 years old. It is a relatively common presentation to paediatricians and is more frequent in girls than boys. It is a benign diagnosis, but other causes of androgen excess such as congenital adrenal hyperplasia or adrenal tumours should be excluded first. In conjunction with history and clinical examination, first line investigations should include determination of serum androgen concentrations, along with bone age, proceeding to synacthen stimulation test (for 17OH皮 levels) and adrenal ultrasound if indicated. The phenotype of premature adrenarche varies considerably between populations but may be associated with low birth weight, insulin resistance, adverse cardio-metabolic risk and progression to polycystic ovarian syndrome in some populations. In the majority of cases, no specific treatment is recommended, but where there is a history of low birth weight, with associated insulin resistance, intervention with the insulin sensitising agent metformin may be considered on a case by case basis.

BACKGROUND
Adrenarche
The adrenal cortex is divided into three zones, the zona fasciculata, zona glomerulosa and zona reticularis, secreting glucocorticoids, mineralocorticoids and adrenal androgens, respectively. The zona reticularis is predominantly responsible for the secretion of the adrenal androgens: dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS) and androstenedione (A4), hormones which have only weak androgenic activity. DHEA binds to the androgen receptor with an affinity of approximately 1260 nM and A4 with an affinity of 61 nM in comparison to dihydrotestosterone and testosterone which have affinities of 0.14 and 0.5 nM, respectively.1,2

The adrenal gland during fetal life is larger than the kidney both structurally and functionally. Concentrations of DHEA in cord blood are also high.3 After birth, the zona reticularis involutes and by early to mid-childhood is largely inactive, secreting only small amounts of DHEA and A4.4,5 However, from the age of 6 years, there is an increase in 17,20-lyase activity with an associated rise in the secretion of adrenal androgens from the zona reticularis. This is known as adrenarche (sometimes referred to as the puberty of the adrenal gland), which is unique to humans and higher primates. Activation of the zona reticularis occurs at a mean age of 6 and 8 years in boys and girls, respectively, and precedes central activation of the pituitary–gonadal axis with subsequent gonadarche by approximately 2 years.4

In the majority of children there are no signs to reflect a rise in adrenal androgens.

The step by step synthetic pathway for the adrenal androgens is pictured schematically in figure 1. The initial conversion of cholesterol to pregnenolone by cytochrome P450 sidechain cleavage enzyme is universally required for steroidogenesis and is rate determining.6 Subsequently, the P450c17 enzyme complex leads to DHEA production following the actions of 17α-hydroxylase and 17,20-lyase (steps 2 and 3). Thereafter, almost all DHEA undergoes sulfation, to form the inactive DHEAS (step 4). The precise mechanisms which regulate the onset of adrenarche via the induction of these synthetic pathways are not completely understood. It appears to be independent of central puberty and gonadarche as it persists in children with hypogonadotrophic hypogonadism and gonadal dysgenesis.7 A role for leptin has been postulated, as leptin deficient rodents and humans have hypogonadotrophic hypogonadism with complete absence of pubertal development.6 In healthy children, leptin concentrations rise before the onset of central puberty.9 In vitro work suggests that leptin may enhance the activity of 17,20-lyase with resultant increases in the production of A4 and DHEAS.10 Adrenocorticotropic hormone (ACTH) appears to play a facilitative rather than causal role in adrenarche, as patients with familial glucocorticoid deficiency have reduced concentrations of adrenal androgens.11 ACTH mediated activation of 17,20-lyase results in a gradual increase in DHEA, DHEAS and A4 production from the zona reticularis of the adrenal gland which predates the onset of central puberty, usually in the absence of any clinical manifestations.12

More recent studies using a human adrenocortical cell line and transfected cos-7 cells suggest that a rise in intra-adrenal cortisol probably leads to inhibition of 3β-HSD activity (step 5, figure 1) and increased DHEA, thereby initiating adrenarche.13 This observation supports a postulate made over 30 years ago when Anderson suggested that adrenarche is induced locally by high levels of cortisol.14 ‘Premature pubarche’, the development of pubic and axillary hair prior to the onset of true puberty, was first described by Silverman in 1952.15 Premature pubarche and premature adrenarche are used interchangeably within the literature. In this article, the term premature adrenarche will be used.

Adrenarche is a physiological process unique to humans and higher primates. The mechanism of onset and the significance of adrenarche remains a mystery, despite the fact that concentrations of DHEAS (micromolar) greatly exceed those of cortisol (nanomolar).
**Definitions**

*Pubarche* refers to the presence of pubic or axillary hair on clinical examination.

If this occurs before the age of 8 years in girls and 9 years in boys, it is referred to as *premature adrenarche* (also known as exaggerated adrenarche or premature pubarche).16

*Gonadarche* refers to the gonadotrophin-dependent activation of the gonads to produce sex steroids.

If this occurs before the age of 8 years in girls and 9 years in boys, it is referred to as *central precocious puberty*.

**PREMATURE ADRENARCHE**

**Clinical features**

The clinical features of premature adrenarche predominantly reflect the action of adrenal androgens on the development of secondary sexual hair (pubic and axillary), but there may be other features of androgen exposure such as greasiness of the skin and hair, acne and adult body odour. Parents may also report mood swings and behavioural changes. The diagnosis is benign but is one of exclusion, as other causes of androgen excess must be considered. Premature adrenarche occurs more frequently in girls with a female to male ratio of around 9:1.15

Table 1 summarises clinical features in 25 girls with a diagnosis of premature adrenarche seen in the Cambridge paediatric endocrinology clinic over a 2-year period.

The hallmarks of premature adrenarche are clinical evidence of androgen exposure (secondary sexual hair, acne and body odour) in the absence of breast development. Although children with premature adrenarche present with secondary sexual hair, the presence of marked growth acceleration, clitoromegaly in girls or genital maturation beyond Tanner stage 2 in boys (especially where testes remain prepubertal in size) should arouse suspicion.

**Evaluation of the child with premature adrenarche**

Children with clinical features of premature adrenarche should be investigated to exclude other pathologies, such as congenital adrenal hyperplasia (simple virilising), virilising adrenal or gonadal tumours, central precocious puberty, exogenous androgen administration (eg, testosterone gels) and other rarer causes such as Cushing’s syndrome. An algorithm depicting one approach to the assessment of a child presenting with early pubic and/or axillary hair development is illustrated in figure 2.

**History and examination**

The age at onset of signs, and the tempo of their change in manifestation should be asked about and recorded, as a rapid progression in symptoms might not be consistent with simple premature adrenarche. Birth weight and gestational age should be recorded, as premature adrenarche may be more prevalent in girls born with low birth weight (LBW) in some populations.16–18 A family history of premature adrenarche, polycystic ovarian syndrome (PCOS) and type 2 diabetes mellitus should be sought. Specific questions should be asked about recent acceleration in growth rate, mood swings, vaginal discharge, acne, greasiness of hair and skin, body odour and deodorant use.

Routine auxology including height and weight, and body mass index (BMI) should be performed (expressed as SD scores (SDS) using the appropriate normative reference data).19 Blood pressure should be measured (again considered with respect to age and sex specific reference data) as it may be elevated in conditions such as non-classical congenital adrenal hyperplasia or adrenal tumours.

Puberty staging should be performed with external assessment of genitalia for signs of androgen exposure (clitoromegaly in girls and penile enlargement in boys). The presence or absence of acne, hirsutism and acanthosis nigricans should be recorded. The presence of excessive generalised hirsutism would be concerning and it is important to ask about hair removal using creams or by shaving. In boys, the Prader orchidometer is used to record testicular volume. Where there is evidence of gonadarche (breast development in girls and testicular volumes greater than 3 ml in boys), central precocious puberty is more likely. It is advisable to review children with premature adrenarche soon after the first appointment (3 months is a pragmatic interval) in order to monitor clinical features and assess height velocity. After this appointment, if all investigations are within normal limits and there has been no progression in clinical features, discharge from follow-up could be considered.

**Investigations**

The initial investigations should include baseline measurements of serum DHEAS, 17-hydroxy progesterone (17-OHP), A4 and testosterone, together with radiological assessment of bone age. Baseline gonadotrophins and oestradiol (in girls) should be measured if clinical examination is consistent with gonadarche. Marked evidence of androgen exposure such as the presence of clitoromegaly in girls or genital maturation beyond Tanner stage 2 in boys merits an adrenal ultrasound.

**Table 1 Clinical features**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Mean ± SD or Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>7.2 ± 1.9</td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.45 ± 1.32</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>0.54 ± 1.17</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>0.44 ± 1.25</td>
</tr>
<tr>
<td>Pubic hair</td>
<td>19 of 25 (76%)</td>
</tr>
<tr>
<td>Axillary hair</td>
<td>8 of 25 (32%)</td>
</tr>
<tr>
<td>Body odour</td>
<td>13 of 25 (52%)</td>
</tr>
<tr>
<td>Acne</td>
<td>7 of 25 (28%)</td>
</tr>
<tr>
<td>Breast development</td>
<td>None (0%)</td>
</tr>
</tbody>
</table>

Clinical features of 25 girls presenting with premature adrenarche over a 2-year period to paediatric endocrinology services in Cambridge, UK.

Data are mean ± SD or number (%).

BMI, body mass index; SDS, SD score.
to exclude a tumour and a short Synacthen test measuring 17-OHP and cortisol to exclude non-classical congenital adrenal hyperplasia. A random 17-OHP below 5 nmol/l or a peak value below 30 nmol/l effectively excludes the diagnosis. Where there is strong suspicion of an adrenal tumour, CT may be required for definitive imaging of the adrenals. A bone age advance (of 1–2 years) is consistent with premature adrenarche. When there is bone age advancement beyond 2 years, causes may include non-classical congenital adrenal hyperplasia, adrenal tumour or central precocious puberty. The use of measurement of urinary steroid profile by specific chromatographic techniques in the investigation of premature adrenarche varies between centres and its use should be considered according to local practice. It can be helpful to exclude the presence of tumours (particularly ovarian) which may secrete androgens not detected by specific assays. The results of investigations undertaken in the Cambridge cohort of girls presenting with premature adrenarche are shown in table 2. Serum A4 may be a more specific androgen marker than DHEAS.

**ASSOCIATIONS**

**LBW and cardiometabolic risk**

A cohort of Catalan girls born with LBW (defined as SDS <−1) who presented with premature pubarche have been extensively studied and show evidence of associated features including earlier menarche and a reduced final height. The girls were more insulin resistant with evidence of increased cardiovascular risk (metabolic syndrome), visceral adiposity and an increased incidence of a polycystic ovarian phenotype in young adulthood. Intervention with the insulin sensitising agent metformin, either as monotherapy or in combination with the antiandrogen flutamide at low doses, appeared to have beneficial effects on abdominal adiposity, androgen levels and indices of insulin resistance. Furthermore, early treatment with metformin slowed the onset of puberty, delayed age at menarche and improved final height.

Similar findings have been reported in a population of Caribbean-Hispanic and African-American girls in the United States with LBW in girls with premature adrenarche. In this group, the presence of acanthosis nigricans and higher serum concentrations of 17-OHP had the strongest negative relationship with insulin sensitivity and predicted increased cardiometabolic risk. However, in other populations, the association with LBW, insulin resistance and premature adrenarche is less clear. In a study of 42 children in Scotland presenting with clinical features of premature adrenarche, there was no association with LBW, although the children were clinically overweight and had mildly elevated fasting insulin concentrations. Interestingly, girls with adrenarche...
had increased serum anti-Müllerian hormone concentrations compared to the control group, which the authors argue reflects a more advanced stage of follicular development. This may possibly provide a link with subsequent development of PCOS. From Italy, Ghizzoni and colleagues followed a cohort of 38 girls with premature adrenarche and found no association with LBW, and no effect on final height. Finally, 63 girls with premature adrenarche from Finland have been described. While there was no difference in birth weight SDS, girls with premature adrenarche had increases in BMI SDS, fasting insulin, post-glucose stimulated insulin secretion and increased prevalence of the metabolic syndrome (16% vs 5%).

Thus, while associations with LBW vary depending on the population, girls with premature adrenarche do seem to have increased cardiovascular risk when compared to control girls. However, it is not clear whether these described associations imply a lifelong increased risk as there is little by way of longitudinal data. The Catalan studies provide the most extensive longitudinal data where there appears to be a progression from premature adrenarche to PCOS in adulthood, and persistent markers of adverse cardiovascular risk. Importantly, intervention either before or after menarche seems to ameliorate this progression in this highly selected population.

A prediction model (the Premature Adrenarche Insulin Resistance Score) has been proposed for Hispanic and African-American girls in the USA. Using simple bivariate analysis, birth weight correlates with insulin resistance (coefficient of 0.524, p=0.0007) and with BMI (coefficient of 0.712, p<0.00001). Birth weight features in only one of the two proposed predictive models. Thus, while in the Catalan population, association with LBW and premature adrenarche does confer increased risk of insulin resistance, these relationships do not appear to be universally present across populations.

It should be noted that the Catalan cohort were slim, although with increased abdominal adiposity. In contrast, girls recruited in other studies were generally overweight. As well as phenotypic differences, there are a variety of methods by which insulin sensitivity and secretion may be expressed, with very few studies using gold standard (and expensive) methodology such as hyperinsulinaemic clamps or intravenous glucose tolerance tests. It is likely that differences in methodology and genetic diversity between populations may partly explain differences in results. When advising families, a pragmatic approach should be taken. The benefits of pursuing a healthy diet in conjunction with regular exercise should be stressed, especially where an individual child is overweight. There are insufficient data currently to advocate routine use of insulin sensitisers (such as metformin) in children presenting with premature adrenarche, but it should merit consideration on a case by case basis.

### Table 2 Investigations

<table>
<thead>
<tr>
<th>Result</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone age</td>
<td>+0.6±1.1</td>
</tr>
<tr>
<td>DHEAS</td>
<td>1.7 (0.8–2.6)</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>2.0 (1.0–2.7)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.2 (0.2–0.6)</td>
</tr>
<tr>
<td>17-OHP</td>
<td>1.1 (0.8–2.1)</td>
</tr>
</tbody>
</table>

Data presented as mean±SD or median (interquartile range).

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**Polycystic ovarian syndrome**

A progression from premature adrenarche to PCOS appears again to be a feature of the Catalan cohort. Both are associated with clinical and biochemical features of androgen excess, but associations with generalised and visceral adiposity and increased cardiovascular risk are less clear.

Insulin resistance may be the primary feature which links premature adrenarche and PCOS. Increased insulin may directly stimulate the ovary to result in ovarian hyperandrogenism, as insulin exerts a co-gonadotrophic effect on the ovarian theca cells. There is also evidence that insulin can enhance ACTH-mediated adrenal steroid precursors in hyperandrogenic women by way of 17,20-lyase deficiency. Insulin resistance itself is also an independent cardiometabolic risk factor as shown by the epidemiological studies of LBW and later cardiovascular disease in adult life. Data from the Avon Longitudinal Study of Parents and Children suggest that children born with LBW who exhibit catch-up growth in the first 2 years of life, have increased waist circumference and increased insulin resistance at age 8, related to concentrations of insulin-like growth factor I (IGF-I). Thus, LBW may be a marker for insulin resistance, linked to ovarian hyperandrogenism via a mechanism involving IGF-I. Reduced concentrations of insulin-like growth factor-binding protein 1 (IGFBP-1) have been reported in girls with premature adrenarche, but this is likely to reflect suppression of IGFBP-1 from increased insulin concentrations.

While premature adrenarche and PCOS share a number of clinical and biochemical features, there is insufficient evidence at present to counsel that girls presenting with premature adrenarche before puberty are at high risk of developing PCOS in adolescence or young adulthood.

### GENETICS

Polymorphic variation in a number of candidate genes has been explored in children presenting with premature adrenarche. As with all research of this nature, the sample sizes are often too small to conclusively exclude an association. Polymorphic variation in plausible candidates involved in either the regulation of insulin sensitivity (peroxisome proliferator-activated receptor-γ2), the regulation of androgen synthesis or low density lipoprotein receptor-related protein 5, have been studied in a Finnish cohort with negative results. However, the androgen receptor CAG repeat, the length of which correlates inversely with androgen sensitivity, has been shown to be shorter in girls with premature adrenarche from Finland. In the same cohort, the ACTH receptor (MC2R), -2 bp T/C diallelic promoter polymorphism was more frequently found in children with premature adrenarche than in control children and also seemed to correlate with a more severe phenotype within the premature adrenarche group. Genetic influences have also been explored in the Catalan cohort, with no association with variation in 17β-hydroxysteroid dehydrogenase. However, there was association with polymorphic variation in the aromatase gene with both clinical and biochemical hyperandrogenism. The results of these genetic studies may explain why some children display increased androgen sensitivity, manifesting as premature adrenarche, while others do not.

A girl presenting with severe adrenarche (bone age advance >5 years) but a low rather than an elevated DHEAS concentration, was found to have an inactivating mutation in the PAPS2 gene. This is a cofactor for DHEA sulfotransferase which results in sulfation of DHEA to an inactive form (figure 1, step 4). The girl had very low levels of DHEAS but high

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**AVALIAÇÃO DA PUBERDADE | DESVIOS FISIOLOGICOS DA NORMALIDADE | SUZANA FIGUEIREDO**
concentrations of the active androgen DHEA, leading to a virilised phenotype. It remains to be seen whether this genetic disorder of DHEAS metabolism is prevalent in the commoner, milder premature adrenarche phenotype.

**MANAGEMENT**

Once other pathologies have been excluded and a diagnosis of premature adrenarche has been reached, most children require no specific treatment but merit serial observation (potentially in primary care if local structures support this) to ensure central puberty proceeds in an orderly sequence with normal tempo. However, if there are features of insulin resistance and a history of LBW, the option of treatment with insulin sensitising agents such as metformin may be considered under the supervision of a paediatric endocrinologist.

**CONCLUSIONS**

Premature adrenarche is a common reason for children (particularly girls) to present either to the general paediatric or endocrine clinic. It is a benign condition but other causes of hyperandrogenism must be excluded. In some populations it may be associated with progression to a PCOS-like phenotype in conjunction with components of the metabolic syndrome. Some girls with more pronounced androgenic features in association with LBW may warrant more detailed assessment of insulin resistance and adverse cardiovascular risk factors. If found, treatment with metformin and/or an androgen antagonist such as low dose flutamide may be considered on a case by case basis to reduce the risk of PCOS developing later.

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**Competing interests** None.

**Provenance and peer review** Commissioned; externally peer reviewed.

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Management of adolescent gynecomastia: an update

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Summary. Gynecomastia refers to an enlargement of the male breast caused by benign proliferation of the glands ducts and stromal components including fat. It is the most common form of breast swelling seen in adolescent males. During pubertal development, gynecomastia can develop as a result of transient relative imbalances between androgens and estrogens. Pubertal gynecomastia is self-limited in 75 to 90% of adolescents and regresses over 1 to 3 years. However it may cause significant psychological stress and depression in adolescents. For boys with persistent gynecomastia that is causing substantial tenderness or embarrassment a tailored approach of close follow-up and use of anti-estrogen drugs may be recommended. These drugs block the effects of estrogens in the body and can reduce the size of the breasts somewhat. It appears that pharmacological therapy of persistent adolescent gynecomastia is reasonable effective if given early in the course of the disease and more successful in cases with small or moderate breast enlargement. However, neither of these drugs is universally approved for the treatment of gynecomastia because the risks and benefits have not been studied completely. Surgical approach may be needed under special conditions for cosmetic reasons. In this update, we review the different published trials for managing adolescent gynecomastia.

Key words: gynecomastia, adolescents, androgens, estrogens, anti-estrogen drugs, mammary adenectomy, liposuction

Introduction

Adolescent gynecomastia is defined as a benign glandular proliferation in the male breast and is derived from the Greek terms γυνή (female) and μαστός (breast). The condition may be unilateral or bilateral, acute or chronic, with or without tenderness on touch (mastalgia). It should be differentiated from pseudo gynecomastia (fatty breasts) which is commonly seen in obese males due to increased fat deposition without glandular proliferation. Gynecomastia may cause significant embarrassment and emotional distress in adolescent males. In this article, the authors focus on management of adolescent gynecomastia both from the medical and surgical lines (1, 2).

After the neonatal period, the adolescent age represents a peak incidence of gynecomastia. The reported prevalence in the literature varies widely, due to different methods of assessment and the assessments of different ages and groups of individuals. The incidence differs in many reports and ranges between 4 to 69% of palpable breast tissue. It may present as early as age 10, with a peak onset between the ages of 13 and 14 years (Figure 1). The typical onset occurs at 13 to 14 years of age, or Tanner stage 3 or 4 (Figure 2). This is followed by a decline of incidence in late teenage years.
Management of adolescent gynecomastia

Only 10% of boys at the age of 17 years have persistent gynecomastia (3-7).

Etiologies and pathogenesis

Most cases of adolescent gynecomastia (physiologic gynecomastia) have no known cause. An imbalance in the ratio of estrogen to androgens tissue levels is postulated as a major cause in the development of gynecomastia. In general, this imbalance between estrogen and androgen action occurs if there is an absolute increase in free estrogens, a decrease in endogenous production of free androgens, an increase in the free estrogen-to-free androgen ratio, androgen insensitivity, or an estrogen-like effect of drugs.

In adolescents with gynecomastia, endocrine investigations may include the measurement of serum concentrations of testosterone, estradiol, gonadotrophin, prolactin and thyroid function tests. Moore et al. (8) studied plasma profiles of 8 hormones over the course of prepuberty and puberty in adolescent males who developed gynecomastia and who did not. Throughout puberty, ratios of delta 4-androstenedione and dehydroepiandrosterone-sulfate to estrone (E1) and estradiol (E2) were significantly lower in the gynecomastia group than in the control group. In contrast, ratios of plasma testosterone to E1 and E2 as well as plasma progesterone and prolactin (PRL) concentrations, were similar in both groups. Because of the adrenal origin of dehydroepiandrosterone and its sulfate, and of peripheral conversion of adrenal androgens to E1 and to E2, it is suggested that either decreased adrenal production of androgens and/or increased conversion of dehydroepiandrosterone-sulfate and delta 4-androstenedione to estrogens cause transient gynecomastia in adolescent boys. Therefore, it appears to be a local imbalance between estrogen stimulation and the inhibitory action of androgens on breast tissue proliferation, although the majority of adolescents with gynecomastia have normal estrogen levels (9-15). This may be due to enhanced sensitivity of the breast tissue to normal circulating levels of estrogen even in the presence of normal circulating androgen concentrations. An increased aromatization of androgens to estrogens in the breast tissue itself as increased aromatase activity has been found in pubic skin fibroblasts of patients with gynecomastia (16).

Secondary causes of gynecomastia in adolescents are relatively rare (less than 5%) and may arise from a broad array of uncommon pathological conditions (17-19). These are well described elsewhere and may include conditions such as: congenital anorchia, Klinefelter’s syndrome, testicular feminization, hermaphroditism, adrenal carcinoma, chronic liver disease, primary hypogonadism, secondary hypogonadism (Figure 3), testicular tumors, hyperthyroidism, renal disease and malnutrition (5,17-19). On the other
hand, several drugs may induce proliferation of male breast tissue (20).

Clinical assessment and diagnostic procedures are usually successful to differentiate these conditions from the common physiologic gynecomastia (17-19).

**Does obesity play a role in the adolescent gynecomastia?**

The prevalence of gynecomastia appears to be greater in obese adolescents. A study screened the database for young male “breast” specimens between 1997-2008. Sixty-nine patients were identified. By body mass index criteria (BMI), 51% were obese, 16% overweight and 33% normal-weighted (9).

In some studies, BMI is positively correlated with both breast diameter and the presence of gynecomastia in both adolescents and adults. Breast adipose tissue contains the aromatase enzyme complex that converts testosterone and androstenedione to E2 and E1, respectively. Peripheral androgen aromatization is enhanced with increased body mass index. Obese males show significantly increased plasma estradiol concentrations and low testosterone concentrations. Testosterone levels also improve on weight loss, which is the intervention of choice for obese men with or without low testosterone levels. In three small studies, letrozole or testolactone has been administered to morbidly obese men to improve their testosterone levels (21-23). Treatment resulted in normalization of testosterone levels in all subjects, with a concomitant suppression of the originally increased levels of estradiol. In addition, the increase in breast fat with weight gain may lead to pseudogynecomastia, which may or may not be associated with true gynecomastia (17-26).

**Classification and grades of severity of gynecomastia**

Simon et al. (9) identified four grades of gynecomastia:
- Grade I: Small enlargement without skin excess
- Grade IIa: Moderate enlargement without skin excess
- Grade IIb: Moderate enlargement with minor skin excess
- Grade III: Marked enlargement with excess skin, mimicking female breast ptosis (Figure 4).

Rohrich et al. (10) have proposed a similar classification of gynecomastia with four grades of severity:
- Grade I: Minimal hypertrophy (<250 g) without ptosis
- Grade II: Moderate hypertrophy (250-500 g) without ptosis
- Grade III: Severe hypertrophy (>500 g) with grade I ptosis
- Grade IV: Severe hypertrophy with grade II or grade III ptosis

Bannayan et al. (27) have described three histological types of gynecomastia: florid, fibrous, and intermediate. The florid type is characterized by ductal hyperplasia and proliferation, with loose and edematous stroma. The fibrous type contains more stromal fibrosis and fewer ducts. The intermediate type of gynecomas-
Management of adolescent gynecomastia

tia presents features of the two. In the majority of cases, if the duration of gynecomastia is greater than one year, the fibrous type is more prevalent and irreversible, which may limit success of medical treatments.

The course of the disease in relation to pubertal progression and severity

Pubertal gynecomastia is self-limited in 75 to 90% of adolescents and regresses over 1 to 3 years. Observation and reassurance are widely regarded as the safest and most reasonable treatment in mild cases. However, because gynecomastia in adolescents occurs at a delicate time when boys are increasingly conscious about self-image, the role of pharmacological or surgical therapies is always in question.

Zosi et al. (28) evaluated the progression with time in children with pubertal gynecomastia for a mean period of five years. Children were divided into 3 groups. Group A comprised 15 children with breast enlargement of less than 6 cm (mild gynecomastia), group B: 5 children with 6-11 cm (moderate) and group C: 5 children with breast tissue of more than 11 cm (severe). This study denoted that mild gynecomastia regresses entirely in all patients, while moderate in only 20% after a 3 year duration while severe gynecomastia usually persists and requires management in 40% of cases. Pain is more common in patients with gynecomastia that is rapidly progressive or of recent onset.

In our experience, a regression of gynecomastia was observed, after a mean period of 3 years, in 84%, 47% and 20% of 220 adolescents with mild, moderate or severe gynecomastia (De Sanctis V, personal observations - unpublished data).

Investigating and managing cases of adolescent gynecomastia

The suggested diagnostic approach and treatment strategies for gynecomastia consist of expert opinion, case series, and observational studies. Therefore, the evidence is considered to be of low to very low quality. By acknowledging this low quality of evidence when discussing testing and treatment options with patients, physicians allow room in the process of decision making for consideration of other factors, such as resources, availability of services, and patients’ values and preferences (29).
For managing adolescent gynecomastia, a tailored therapy is advised.

Attentive waiting with biannual follow-up is appropriate for those with physiologic gynecomastia who are untroubled by symptoms and who have no features that suggest underlying disease. Few patients with adolescent gynecomastia need treatment for cosmesis or analgesia.

Early treatment can maximize benefit in adolescents with significant physical symptoms or emotional distress. Medical treatment appears to be more effective if used early in the course of the disease possibly after symptoms are first noted, whereas surgery can be performed at any time with similar results (30, 31).

For patients with non-physiologic gynecomastia, treatment is directed toward improving the underlying illness or discontinuing use of the contributing.

Mammography appears unnecessary in most men particularly in young male patients and should not be used as a routine imaging procedure.

**Pharmacological treatment**

Medical treatment of gynecomastia aims to correct the estrogen-androgen imbalance by three possible pathways: (a) blocking the effects of estrogens on the breast (e.g., clomiphene, tamoxifen, raloxifene); (b) administering androgens (e.g., danazol), and (c) inhibiting estrogen production (e.g., anastrozole, testolactone) (32-49).

Data on efficacy of pharmacological treatment of gynecomastia in adolescents is mostly limited to small case series and case reports without control groups, which makes conclusions difficult to draw. A number of medications have been used to treat gynecomastia.

Tamoxifen is an antiestrogen. Its principal mechanism of action is mediated by its binding to the estrogen receptor and the blocking of the proliferative actions of estrogen on mammary epithelium. It is thought to be an effective and safe treatment for physiologic, persistent pubertal or idiopathic gynecomastia.

A systematic review to assess the efficacy of tamoxifen in the management of idiopathic pubertal gynecomastia was performed by Lapid et al. (33) fifty nine studies were selected. There were no randomized controlled studies. The studies found have methodological flaws but show promising results.

No clinical side-effects were reported or observed. They concluded that tamoxifen may be effective for the treatment of pubertal gynecomastia and it seems safe to use and randomized controlled studies are necessary to confirm this indication.

A retrospective chart review of men presenting to a breast clinic for gynecomastia found that only 13 of 220 patients required medication for treatment. Patients were treated with 10 mg of tamoxifen per day for three months, and 10 of the 13 had resolution of pain and breast enlargement (34). A randomized controlled trial of 80 participants also found that 20 mg of tamoxifen once per week is as effective as 20 mg once per day (35).

Derman et al. (36) evaluated the efficacy of the tamoxifen treatment in 37 patients with pubertal gynecomastia with a diameter of over three cm. Pain and size reduction was seen in all patients with tamoxifen treatment. No long-term side effects of tamoxifen were observed. The dose of tamoxifen was increased in three patients due to poor response. Two of the treatment group had recurrence problem at follow-up. No patient required breast surgery.

Devoto et al. (37) studied 43 patients with gynecomastia, aged 12 to 62 years. Twenty seven patients had a pubertal physiological gynecomastia. Twenty patients had mastodynia and in 33, gynecomastia had a diameter over 4 cm. All were treated with tamoxifen 20 mg/day for 6 months. Mastodynia disappeared in all patients at three months. At six months gynecomastia disappeared in 26 patients (62%), but relapsed in 27%. Fifty two percent of gynecomastias over 4 cm and 90% of those of less than 4 cm in diameter disappeared (p<0.05). Fifty six percent of gynecomastias lasting more than two years and 70% of those of a shorter duration disappeared (p=NS). They concluded that tamoxifen is safe and effective for the treatment of gynecomastia and larger lesions have a lower response to treatment.

Another two small double-blind, crossover trials found only modest benefit when compared with placebo (38,39).

Histologically, the therapeutic effect of tamoxifen appears to be satisfactory.
In three cases who reported dissatisfaction with the results of tamoxifen treatment and were therefore submitted to adenectomy preceded by liposuction. Pathology results showed adipose tissue alone, with no evidence of intraductal epithelial proliferation. The lack of residual glandular breast tissue after treatment with tamoxifen proves that it is effective in histopathologically eliminating pubertal gynecomastia (40).

The long-term safety of tamoxifen was evaluated in 10 pubertal boys treated with tamoxifen for gynecomastia for more than 3 months. They were evaluated after 2.5-7 years (mean 4.6 years) to determine the side effects of this therapy. Authors did not find any serious side effects of tamoxifen in these patients (41).

Raloxifene is a second-generation selective estrogen receptor modulators (SERM). In 34 healthy males at the dose of 60 mg/day for one month it increased serum testosterone by 20%, and slightly decreased serum estradiol. When used in eugonadal patients with gynecomastia, 75% had a reduction in the size of breast tissue of at least 50% (average, 73%) in the first two months of treatment. However, the study was small and the males have wide range of ages (42).

In thirty eight patients with persistent pubertal gynecomastia who received reassurance alone or a 3- to 9-month course of an estrogen receptor modifier (tamoxifen or raloxifene) there was a mean reduction in breast nodule diameter by 2.1 cm after treatment with tamoxifen and 2.5 cm with raloxifene. Some improvement was seen in 86% of patients receiving tamoxifen and in 91% receiving raloxifene, but a greater proportion had a significant decrease (>50%) with raloxifene (86%) than tamoxifen (41%). No side effects were seen in any patients. Authors suggested that raloxifene may be safe and effective in reducing persistent pubertal gynecomastia, with a better response compared to tamoxifen (43).

Anastrozole, an aromatase enzyme, was used in a controlled trial of 80 patients with gynecomastia. However, the study demonstrated no statistically significant difference between anastrozole and placebo in the percentage of patients with greater than 50 percent breast volume reduction at three months (44).

Danazol is a synthetic steroid with antigonadotropic and anti-estrogenic activities that acts as an anterior pituitary suppressant by inhibiting the pituitary output of gonadotropins. It possesses some androgenic properties. Buckle et al. (45) treated 42 patients with gynaecomastia with danazol. There were 31 adults and 11 cases of pubertal gynaecomastia. Dosage schedules in adults were 300-600 mg a day and in adolescents 200-300 mg a day. In the 31 adults, marked regression of gynaecomastia occurred in 18 and a moderate regression in 10, whilst in the 11 cases of puberty gynaecomastia, there was a marked regression in 7 and a moderate regression in three patients. Plasma testosterone, FSH, and LH concentration fell in most patients.

Beck et al. (46) evaluated the effect of danazol treatment in pubertal boys with marked gynaecomastia (breast size more than 6 cm in diameter). During danazol treatment (200 mg daily for 6 months) gonadotropin levels in response to luteinizing-hormone releasing hormone (LHRH) stimulation were blunted and testosterone values decreased. After 6 months of therapy, four of 5 boys treated so far, demonstrated a reduction of gynaecomastia to a maximum of 3 cm in diameter. There were no signs of a recurrence of the marked pubertal gynaecomastia in all boys after stopping danazol. These results indicate that danazol is a specific gonadotropin inhibitor acting at the hypothalamo-pituitary level. No side effects on other hormones or on the development of secondary sex characteristics were noted during and after danazol treatment.

Ting et al. (47) compared the efficacy of tamoxifen versus danazol in the treatment of 68 patients with idiopathic gynaecomastia at different ages (range: 13-82 years). Medical treatment with either tamoxifen (20 mg/d) or danazol (400 mg/d) was offered and continued until a static response was achieved. Twenty-three patients were treated with tamoxifen and 20 with danazol. Complete resolution of the gynaecomastia was recorded in 18 patients (78.2%) treated with tamoxifen, whereas only 8 patients (40%) in the danazol group had complete resolution. Five patients, all from the tamoxifen group, developed recurrence of breast mass. It was concluded that although the effect is more marked for tamoxifen compared with danazol, the relapse rate is higher for tamoxifen.

Plourde et al. (48) studied the effect of clomiphene citrate in 12 boys, aged 12 to 19 years, with persistent gynaecomastia, at a dose of 50 mg/day by mouth for one to three months. The mean breast size
decreased by 0% to 36%, with only five boys experiencing a reduction of greater than 20%. Five boys subsequently required reduction mammoplasty. Levels of urinary gonadotropins, serum testosterone, and estradiol increased significantly during therapy. The antiestrogen effects were achieved primarily at the level of breast tissue because the ratio of testosterone to estradiol remained unchanged during treatment. Authors concluded that clomiphene citrate in a dose of 50 mg/day resulted in only small decreases in persistent pubertal gynecomastia and was not a satisfactory medical therapy for the condition.

Testolactone is a non-selective, irreversible, steroidal aromatase inhibitor, thereby preventing the formation of estrogen from adrenal androstenedione and reducing endogenous estrogen levels.

Zachmann et al. (49) treated 22 boys with pubertal gynaecomastia (age 15.9±1.9 years) with testolactone (450 mg daily by mouth) for 2 to 6 months without side-effects. The mean breast gland diameter regressed from 4.4 to 3.3, 3.2 cm and 1.7 cm at 2, 4, and 6 months respectively, while pubic hair and testicular volume progressed normally. It was concluded that testolactone, an inhibitor of steroid aromatization, is an effective and safe medical treatment for pubertal gynaecomastia.

In conclusion, it appears that pharmacological therapy of persistent adolescent gynecomastia is reasonable effective if given early in the course of the disease and more successful in cases with small or moderate breast enlargement. In cases with marked enlargement with excess skin, mimicking female breast, surgery may be considered if no regression is observed after a period of observation of at least one year.

Surgical treatment

Surgical management of pubertal gynecomastia may be considered in non-obese male adolescents who present persistent breast enlargement after a period of observation of at least 12 months, breast pain or tenderness, and/or significant psychosocial distress. Obesity is not a contraindication to surgical approach. Liposuction techniques are helpful in those patients with considerable fat deposition in the breast during the removal of the glandular component. The aim of surgical treatment is to achieve a normal appearance of the masculine thorax with the smallest possible scar. Surgical treatment of gynecomastia requires an individualized approach.

The most commonly used technique is subcutaneous mastectomy that involves the direct resection of the glandular tissue using a periareolar or transareolar approach with or without associated liposuction. Skin resection is needed for more advanced cases (50, 51).

In grade I, the enlargement is caused solely by glandular proliferation without adipose accumulation. Surgical correction involves mammary adenectomy performed by a semicircular inferior periareolar incision. Liposuction is not required. Grade II is characterized by excessive glandular tissue and local adiposity. In these cases, liposuction and surgical excision must be combined in the same operation. Mammary adenectomy without liposuction leads to unsatisfactory outcomes, with an uneven surface or asymmetry. In grade III, the operation begins with liposuction and is followed by glandular excision with periareolar removal of the tissue. It is necessary to detach the excess skin to obtain a good chest silhouette. The hallmarks of grade IV are severe ptosis and a large amount of redundant skin. One of the techniques for reduction mastoplasty is used to remove gland and skin and flatten the chest outline (30).

The most frequent early complication following surgical management of gynecomastia is hematoma.
Seroma, overresection with saucer-type deformity, underresection, unappealing scarring and infections are also observed. Patients and their parents or guardians should be well informed about possible risks, as some complications are managed surgically.

Ultrasound-assisted liposuction (UAL) is another modified method that may facilitate the removal of tougher sub-areola glandular tissue at the time of liposuction. Care is needed with this technique to avoid the potential complication of thermal injury to the overlying skin. Standard liposuction or UAL in combination with gland resection through a minimal caudal semicircular periareolar incision and conventional liposuction effectively corrects most grades of gynecomastia (52).

An alternative modification to the simple liposuction is the power-assisted liposuction technique. This is performed to contour the breast tissue without having to exert as much physical force as standard liposuction with a syringe and cannula. The cannula reciprocates at a controlled but surgeon adjustable rate, with separate precise control of the suction pressure. This technique works very effectively in combination with a tumescent and super tumescent approach.

The aspirate volume from liposuction can range from 50 to over 1,000 mL. In contrast the excision of the fibroglandular tissue can range from a few grams to over 1,000 g. This illustrates the different degrees of gynecomastia for which surgery is used (53-55).

The use of mammotome is a minimal invasive tool that appears safe and ensures reasonable cosmetic and patient satisfaction rates, although there are only limited reports of its use in gynecomastia and no long-term follow-up data (56). The potential risk of skin injury and hemorrhage may limit the use of mammotome.

In general, surgical treatment produces good cosmetic and is well tolerated. Nevertheless invasive techniques that require minimal surgical incision have recently emerged and may offer faster recovery and lower rates of local complications. Histologic analysis is recommended in true gynecomastia corrections to rule out unexpected histologic findings.

In conclusion, adolescent gynecomastia has a favorable prognosis with spontaneous complete or partial resolution. Small percent have persistent gynecomastia after the end of pubertal development and some adolescents have concerns about the cosmetic correction. Therefore, the decision to perform surgery depends on the degree to which this condition has affected the quality of life and on their desire for cosmetic correction (57-59).

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AVALIAÇÃO DA PUBERDADE

Avaliação clínica, imagiológica e laboratorial da puberdade

ANA LUÍSA LEITE

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Normal Pubertal Development: 
Part I: The Endocrine Basis of Puberty

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Objectives  After completing this article, readers should be able to:

1. Explain how puberty is regulated by the hypothalamic-pituitary-gonadal axis.
2. Describe the hormonal interactions involved in pubertal development in boys and girls.

Introduction
Puberty is a defining developmental stage of every child’s life, both physically and psychosocially. Concerns about the normalcy of pubertal development and menstrual patterns are among the most common questions posed to every physician caring for children. This article reviews the primary physiologic changes in the hypothalamic-pituitary-gonadal (HPG) axis and in adrenal androgen and growth hormone (GH) production that underlie the normal pubertal milestones. Understanding of these changes allows interpretation of laboratory data in children suspected of having pubertal abnormalities.

Puberty is the developmental stage during which a child becomes a young adult, characterized by the maturation of gametogenesis, secretion of gonadal hormones, and development of secondary sexual characteristics and reproductive functions. Adolescents is used widely as a generally synonymous term for puberty, but the term often is used to convey an added connotation of cognitive, psychological, and social change.

Thelarche denotes the onset of breast development, an estrogen effect. Pubarche denotes the onset of sexual hair growth, an androgen effect. Menarche indicates the onset of menses and spermatarche the appearance of spermatozoa in seminal fluid. Gonadarche refers to the onset of pubertal function of the gonads, which produce most of the sex hormones that underlie the pubertal changes in secondary sex characteristics. Adrenarche refers to the onset of the adrenal androgen production that contributes to pubarche.

The Hormonal Axes Underlying Puberty
The Hypothalamic-Pituitary-Gonadal Axis
Normal puberty results from sustained, mature activity of the HPG axis. (1). The major hormones of the HPG axis are shown in Figure 1. In response to a single gonadotropin-releasing hormone (GnRH), the pituitary gland releases two gonadotropins: luteinizing hormone (LH) and follicle-stimulating hormone (FSH). GnRH is secreted by specialized neurons of the hypothalamus in a pulsatile fashion. Pituitary LH and FSH secretion consequently is pulsatile and can be sustained only in response to pulsatile GnRH signals. LH acts primarily on the specialized interstitial cells of the gonads to stimulate formation of androgens, and FSH acts primarily on the follicular/tubular compartment to stimulate formation of estrogen from androgen precursors, inhibin, and androgens. The function of the two compartments of the gonad is coordinated by paracrine regulatory mechanisms.

The HPG axis is active during three phases of development: fetal, neonatal, and adult, with puberty being the period of transition to mature function. Changes in GnRH secretion underlie the changing activity of the HPG axis. The sexually dimorphic patterns of sex hormone secretion during the prenatal and neonatal periods of HPG activity appear to play a role in programming sexually dimorphic patterns of behavior, metabolism, and neuroendocrine function in later life.
The HPG axis is established during the first trimester. Its activity in the second trimester contributes to the establishment of normal penile size and the inguinal-scrotal phase of testicular descent. (2) (3) In the latter half of pregnancy, activity is suppressed by the high estrogens elaborated by the fetoplacental unit.

The HPG axis promptly functions at a pubertal level in the newborn after withdrawal from maternal estrogens. This "minipuberty of the newborn" is subclinical, except for contributing to genital growth, acne, and transient thalcrea in the neonate.

HPG function subsequently comes under gradual central nervous system restraint at the end of the neonatal period. The axis is relatively, but not absolutely, schemes throughout childhood, particularly in girls, who have a higher hGh serum concentrations than boys and a few ultrasonographic visible ovaries follicles as evidence of this effect. The HPG axis becomes increasingly active again in the late prepubertal period, as central nervous system restraint recedes, followed by an increasing tempo throughout puberty.

The gonads account for the most important circulating estrogen (estradiol) and androgen (testosterone). Gonadal function accounts for more than 90% of estradiol production in the female (50% in the male) and more than 90% of testosterone production in the male (50% in the female) (Fig. 2). (4)(5)
Adrenarche, the “Puberty” of the Adrenal Gland

Adrenarche is actually a re-onset of adrenal androgen production. The fetal zone of the adrenal cortex elaborates large amounts of dehydroepiandrosterone sulfate (DHEAS), which is important as the major substrate for placental estrogen formation during pregnancy. This zone then regresses over the first several postnatal months.

Adrenarche is the pseudopuberty of the adrenal gland that begins in mid-childhood as the zona reticularis of the adrenal cortex develops. (1) This zone has the capacity to form 17-ketosteroids, but not cortisol, in response to adrenocorticotropic hormone (ACTH), and DHEAS is the primary endpoint of this biosynthetic pathway. Consequently, although cortisol concentrations and the cortisol response to ACTH do not change from childhood to adulthood, DHEAS values gradually rise from mid-childhood until adulthood. This timeframe coincides approximately with the gonadal androgen production of true puberty, but adrenarche is an incomplete aspect of puberty that is independent of pubertal maturation of the HPG axis. The adrenal gland secretes more than 90% of DHEAS in children and women and more than 70% in adult men, while 50% of testosterone in the female and less than 10% of testosterone in the male is produced by the adrenal. (6) Adrenal androgen concentrations increase to a point sufficient to stimulate apocrine odor and mild acne after about 5 years of age and pubic hair growth after about 10 years of age (Table).

Interactions Between Pubertal Hormones and the Growth Hormone/Insulin-like Growth Factor-1 Axis

Pituitary GH secretion increases during puberty in response to sex steroids. (1) This rise in GH causes a rise in insulin-like growth factor-I concentrations to peaks in late puberty that are above those of adults, sometimes in the adult acromegalic range. Half of the characteristic pubertal growth spurt is due to the direct effect of sex steroids on epiphyseal growth and half to GH stimulation. Conversely, in accord with the general principle that everything grows better with GH, GH is necessary for optimal gonadotropin effects on gonadal growth and sex steroid effects on secondary sex characteristics. For example, selective GH resistance is characterized by small testes and micropenis, poor breast and sexual hair development, and absence of a pubertal growth spurt. (12)

Regulation of the Onset and Progression of Puberty

There is no single “trigger” for puberty, rather, puberty results from a gradual increase in GnRH pulsatility that arises from maturation of central nervous system developmental programs that send inhibitory and stimulatory signals to GnRH neurons. (1) Puberty is associated with changing sensitivity of the neuroendocrine system to negative feedback by gonadal hormones. When GnRH secretory activity is low due to central nervous system inhibition in mid-childhood, it is inhibited by trace amounts of sex steroids. Increasing central activation during puberty permits sex steroids to rise to adult concentrations before exerting negative feedback effects.

The major GnRH-inhibitory systems are GABAergic and opioidergic; the major excitatory systems involve glutamate and kisspeptin, with glial cells facilitating GnRH secretion. Kisspeptin is a hypothalamic neuropeptide discovered in the search for the molecular basis of hypogonadotropic hypogonadism; it acts as an important signal for pubertal GnRH release via GPR54, a G-protein coupled receptor located on GnRH neurons. It has been estimated that at least half of the varia-

Table. Typical Early Morning Pubertal Hormone Blood Concentrations

<table>
<thead>
<tr>
<th>Age Group</th>
<th>LH (mIU/mL)</th>
<th>FSH (mIU/mL)</th>
<th>Estradiol (pg/mL)</th>
<th>Testosterone (ng/dL)</th>
<th>DHEAS (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepubertal 1 to 5 yr</td>
<td>&lt;0.3</td>
<td>&lt;4.0</td>
<td>&lt;10</td>
<td>&lt;20</td>
<td>5 to 40</td>
</tr>
<tr>
<td>Prepubertal males</td>
<td>≤12</td>
<td>1.0 to 12</td>
<td>20 to 85</td>
<td>15 to 25</td>
<td>75 to 250</td>
</tr>
<tr>
<td>Postpubertal males</td>
<td>2.0 to 11</td>
<td>1.0 to 12</td>
<td>20 to 85</td>
<td>15 to 25</td>
<td>75 to 250</td>
</tr>
<tr>
<td>Adult men</td>
<td>1.4 to 9.0</td>
<td>1.0 to 12</td>
<td>20 to 85</td>
<td>15 to 25</td>
<td>75 to 250</td>
</tr>
</tbody>
</table>

DHEAS = dehydroepiandrosterone sulfate, FSH = follicle stimulating hormone, LH = luteinizing hormone, TT = total testosterone. Conversion to SI units: LH: 5 x 10^-3, FSH: 5 x 10^-3, Estradiol: 10^3, Testosterone: 10^-6, DHEAS: 10^0. Values given are mean ± SD.

Data from: 
- Borsini et al. (11) 
- Montemar et al. (23) 
- Zettel et al. (8) 
- Mayo Clinical Laboratories (10) 
- Esoterix Laboratory Services (11)
tion in the timing of puberty is genetically determined, and ethnicity is one such factor. (1)(13) Sex hormones, hormonally active environmental chemicals (“environmental disruptors”), (14) diverse somatic stimuli (including nutrition, the growth hormone/insulin-like growth factor system, thyroid hormones), and general health all affect the pubertal process.

Pubertal and skeletal maturation appear to have common somatic determinants. Children generally enter puberty when they achieve a pubertal bone age. Pubertal stage normally correlates better with the bone age than with chronologic age. (15) Thus, for example, the onset of breast development normally occurs at a bone age of about 10 years and menarche occurs at a bone age of about 12.5 years, whether the child is 9 or 14 years of age.

Optimal nutrition is necessary for initiation and maintenance of normal reproductive function. The hypothesis that a critical amount of body fat is the weight-related trigger for pubertal development originated with the discovery by Frisch and coworkers that weight correlated with pubertal growth and menarche better than it did with chronologic age or height. (16) Early to mid-childhood may be a critical period for weight to influence the onset of puberty. (17) Suboptimal nutrition related to socioeconomic conditions is an important factor in the late onset of puberty in underdeveloped countries. Conversely, obesity is an important factor in advancing the onset of puberty in United States girls. (13)

Leptin, a hormone secreted by fat cells, appears to be an important link between nutrition and the attainment and maintenance of reproductive competence. (1) Leptin acts on the hypothalamus to reduce appetite and stimulate gonadotropin secretion. Leptin deficiency causes obesity and gonadotropin deficiency. Blood leptin concentrations rise throughout childhood and puberty to reach higher values in girls than boys. Attainment of a critical threshold appears to signal that nutritional stores are sufficient for mature function of the GnRH pulse generator and, thus, permits puberty.

Although production and vaginal gland hormones affect puberty in lower animals and can cause pubertal disorders in humans, neither has a clear role in normal human puberty.

**Hormonal Changes of Normal Puberty**

The first hormonal change of puberty is a sleep-related increase in the pulsatile release of LHRH by the pituitary gonadotropes. FSH is secreted in parallel but increases relatively less. At the beginning of puberty, a unique diurnal variation of pubertal hormones occurs, with little LH secretion during the day and a significant increase in pulsatile secretion during sleep (Fig. 3). (18)(19) In response to nocturnal LH secretion, the pattern of gonadal sex steroid secretion differs between the sexes: ovarian secretion of estradiol peaks in mid-day and testicular secretion of testosterone peaks promptly during sleep. In addition, girls’ pubertal hormone secretion is subclinically cyclic from early puberty. As puberty progresses, LH secretion persists further into the daytime. After menarche, this diurnal variation no longer exists. Adult sex steroid concentrations, however, have a mild diurnal variation, being highest on awakening.

The two gonadotropins each act primarily on specific gonadal cell types. LH stimulates the interstitial cells of the ovaries (theca cells) to form androgenic precursors of estradiol and those of the testes (Leydig cells) to secrete testosterone itself. FSH acts on the sex cord derivatives of the ovary (granulosa cells) and testes (Sertoli cells) to stimulate gametogenesis and gonadal growth. In granulosa cells, FSH strongly stimulates aromatase to form estradiol from thecal androgens.

As the gonads become increasingly sensitized to gonadotropin stimulation, they grow and secrete sex hormones at steadily increased rates. Within 3 years of rising above the prepubertal range, estradiol increases an average of 20 pg/mL (73.4 pmol/L) yearly to reach the mid-adult range and testosterone increases an average of 100 ng/dL (3.47 nmol/L) yearly to reach the lower adult range (Table). (20) These concentrations then gradually induce their effects. The hormonal increases culminate in positive feedback in girls, which refers to the female neuroendocrine system becoming capable of secreting a mid-cycle surge of LH when the ovary signals that it is prepared for ovulation via a critical and sustained level of estrogen secretion.

Estrogen stimulates the classic female target tissues: the female genital tract (e.g., endometrial growth, cervical mucus secretion) and breasts. Androgen stimulates the classic male target tissues (e.g., sexual hair and sebaceous glands) but stimulates sex steroid account for the pubertal growth spurt, directly and indirectly through sex hormone. Both directly stimulate epiphyseal growth and epiphyseal maturation, which is indicated by bone age radiographs and peak bone mass accrual. (21) However, they differ in some of their effects on skeletal growth. Androgen is responsible for the wider bones (the laryngeal enlargement accounting for the pubertal voice change), while estrogen is necessarily required for epiphyseal fusion and is the more potent inhibitor of bone resorption. They also affect growth of wide variety of other somatic tissues. During puberty, estrogen promotes lipogenesis and lowers body
fat distribution. In contrast, androgens generally are lipolytic, although they favor the development of visceral fat stores, and promote muscular development. The similar increase of body mass index during puberty in girls and boys, thus, is due to differences in body composition, with a higher percent being body fat in girls and lean body mass in boys. (22)

The menstrual cycle arises from cyclic maturation of ovarian follicles that result in cyclic changes in sex hormones, particularly estradiol and progesterone, which entrain cyclic changes in gonadotropin concentrations (Fig. 4). The biologic goal of this monthly variation is to select and nurture one dominant follicle to the point of ovulation for potential fertilization. A normal average 28-day cycle consists of two phases: the follicular phase (variable in duration, averaging 14 days at maturity) and the luteal phase (14 ± 5D days), with the latter occurring only in ovulatory cycles. The follicular phase begins with the onset of menses and culminates in the mid-cycle LH surge, which induces ovulation from the follicle. The empty follicle forms the corpus luteum, initiating the luteal phase. Progesterone increases steadily to be sustained at very high levels for several days, along with lesser but substantial increases in estradiol. Progesterone and estradiol secretion from the corpus luteum maintain the endometrial layer of the uterus in preparation for potential pregnancy. If pregnancy does not occur, with its resultant increase in human chorionic gonadotropin.

Figure 4. Diagram of average gonadotropin and sex steroid concentrations during the normal menstrual cycle. The data are centered in reference to days before (−) or after (+) the day of the mid-cycle surge of luteinizing hormone (LH). The gonadotropin concentrations are typical of polyclonal radioimmunoassay, and the baseline values are about twice as high as those obtained by current monoclonal assays. M=menses begin, E2=estradiol, PROG=progesterone. Reprinted with permission from Rosenfield et al. (1)
the corpus luteum life span is exhausted, which results in withdrawal of female sex steroids, followed by endometrial sloughing and menstrual flow.

Assessment of pubertal hormone concentrations requires reliable hormone assays in addition to consideration of the diurnal changes of early puberty and cyclic changes in girls. Although early pubertal children have greater average hormone concentrations than prepubertal children, their values still are much less than those of adults (Table). (7)(8)(9)(10)(11) The widely available, older generation of polyclonal antibody-based radioimmunoassays for gonadotropins do not possess sufficient sensitivity and specificity for optimal diagnosis of pubertal disorders. The modern multichannel platform assays available in many community hospitals are generally adequate for these purposes, as indicated by sensitivities of 0.1 to 0.15 U/L for LH and FSH. These platform assays are also reliable for DHEAS assays. On the other hand, platform assays are very unreliable for measuring testosterone and estradiol at the relatively low values that are normal for pubertal children and women. The practitioner should not order these tests unless provision can be made to assay them by accurate methodology, preferably in consultation with a pediatric endocrinologist. (23)

Daytime pubertal hormone concentrations may not indicate the early stages of puberty accurately because of diurnal and cyclic variations (Fig. 3). For this reason, GnRH-stimulated values may be necessary to diagnose pubertal disorders. A peak LH value greater than approximately 4.0 U/L in response to GnRH or GnRH agonist testing has been suggested as indicative of the onset of puberty. (24)(25)

Part II of this article, which deals with the clinical aspects of puberty, will be published in the July issue of Pediatrics in Review.

References
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PIR Quiz

Quiz questions also available online at http://pedsinreview.aappublications.org.

1. Which of the following statements about normal puberty in children is true?
   A. Bone age correlates better with pubertal development than chronologic age.
   B. Gonadotropin–releasing hormone (GnRH) secretion in response to negative feedback from sex steroids is constant throughout life.
   C. Growth hormone secretion is the sole determinant of the pubertal growth spurt.
   D. Menarche is the first stage of puberty in girls.
   E. Normal pubertal development is unrelated to nutritional status.

2. Which of the following statements best describes adrenarche?
   A. Breast development becomes evident in girls.
   B. Hypothalamic production of adrenocorticotropic hormone increases.
   C. Maternal estrogens are withdrawn, causing neonatal acne.
   D. Spermatozoa begin to appear in seminal fluid.
   E. The adrenal gland increases production of dehydroepiandrosterone sulfate.

3. Which of the following is the primary action of luteinizing hormone?
   A. Secretion of follicle-stimulating hormone.
   B. Secretion of GnRH from the pituitary gland.
   C. Stimulation of gametogenesis in the testes.
   D. Stimulation of the gonads to produce androgens.
   E. Stimulation of the ovarian follicle to produce estrogen.

4. At which of the following phases of the menstrual cycle is the concentration of progesterone the highest?
   A. The beginning of the follicular phase.
   B. The beginning of the luteal phase.
   C. The end of the luteal phase.
   D. The middle of the follicular phase.
   E. The middle of the luteal phase.
Yu Ding, Juan Li, Yongguo Yu, Peirong Yang, Huaiyuan Li, Yongnian Shen, Xiaodong Huang* and Shijian Liu*

Evaluation of basal sex hormone levels for activation of the hypothalamic–pituitary–gonadal axis

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Abstract

Background: This study aimed to identify the predictive value of basal sex hormone levels for activation of the hypothalamic–pituitary–gonadal (HPG) axis in girls.

Methods: Gonadotropin-releasing hormone (GnRH) stimulation tests were performed and evaluated in a total of 1750 girls with development of secondary sex characteristics. Correlation analyses were conducted between basal sex hormones and peak luteinizing hormone (LH) levels ≥5 IU/L during the GnRH stimulation test. Receiver operating characteristic (ROC) curves for basal levels of LH, follicle-stimulating hormone (FSH), LH/FSH, and estradiol (E2) before the GnRH stimulation test were plotted. The area under the curve (AUC) and 95% confidence intervals (CIs) were measured for each curve.

Results: The maximum AUC value was observed for basal LH levels (0.77, 95% CI: 0.74–0.79), followed by basal FSH levels (0.73, 95% CI: 0.70–0.75), the basal LH/FSH ratio (0.68, 95% CI: 0.65–0.71), and basal E2 levels (0.61, 95% CI: 0.59–0.64). The appropriate cutoff value of basal LH levels associated with a positive response of the GnRH stimulation test was 0.35 IU/L, with a sensitivity of 63.96% and specificity of 76.3% from the ROC curves when Youden’s index showed the maximum value. When 100% of patients had peak LH levels ≥5 IU/L, basal LH values >2.72 IU/L, but the specificity was only 5.45%.

Conclusions: Increased basal LH levels are a significant predictor of a positive response during the GnRH stimulation test for assessing activation of the HPG axis in most girls with early pubertal signs.

Keywords: diagnosis; gonadotropin-releasing hormone stimulation test; hypothalamic–pituitary–gonadal axis; precocious puberty; sex hormones.

Introduction

Precocious puberty is a common disease in the field of pediatric endocrinology [1]. Most patients with precocious puberty suffer from inappropriate activation of the hypothalamic–pituitary–gonadal axis (HPG), which results in idiopathic central precocious puberty (CPP). Activation of the HPG is important in the diagnosis of CPP and is based on progressive sexual development, accelerated growth rate, and advanced bone maturation. In cases where the gonadal axis is not activated, peripheral precocious puberty (PPP) is considered. PPP can show similar clinical manifestations to CPP, although the pathogenesis, clinical outcomes, and treatment methods for PPP differ from those of CPP.

Measurements of peak luteinizing hormone (LH) following the gonadotropin-releasing hormone (GnRH) stimulation test is the gold standard for assessing early activation of the HPG axis in cases with clinical symptoms and signs of puberty [2, 3]. However, the GnRH stimulation test requires several blood samples over long time periods, with relevant technicians and facilities. This highlights the need for a simple measure that can be used as a screening test for assessing early activation of the HPG axis. Early in the activation of the HPG axis, amplitude and pulse frequency of serum LH and follicle-stimulating hormone
(FSH) secretion are significantly increased. However, pre-pubertal and pubertal gonadotropin baseline values have some overlap [4]. With use of a third-generation gonadal hormone detection method, which uses an immunochromoluminometric assay, detection sensitivity has significantly improved compared with traditional detection methods. This has helped to differentiate between prepubertal and pubertal hormone levels [5]. Children with CPP were proposed to have higher basal FSH and LH levels than children with PPP in a cross-sectional study [6]. However, further studies are required to determine a more sensitive index for detecting gonadal axis activation and how to select the appropriate cutoff value.

Therefore, the present study aimed to identify the predictive value for activation of the HPG axis in girls. We analyzed basal LH and FSH levels, the ratio between LH and FSH (LH/FSH), and estradiol (E2) levels prior to the GnRH stimulation test.

Subjects and methods

Subjects

We studied a total of 1750 girls with breast enlargement before 8 years of age, who had a physical examination and breast ultrasound indicating breast development, with a breast of Tanner stage 2 or higher. These girls were diagnosed and treated in the Endocrinology Department of Shanghai Children’s Medical Center from January 2010 to June 2015. Patients with precocious puberty as a result of another etiology, such as a central tumor, infection, or cranial irradiation, were excluded from the study. Measurements included height, weight, bone age by X-ray photography, and ultrasonography of the uterus and ovaries. Bone age was measured by the Greulich-Pyle method [7]. The volumes of the uterus and ovary were calculated by multiplying the length by the width, thickness, and 3.14, and then dividing by six according to ultrasonography. GnRH stimulation tests were performed and evaluated. The girls were divided into two groups according to GnRH stimulation test results. Girls with peak LH values ≥5 IU/L were considered to have pubertal activation of the HPG axis. These girls were categorized into the positive GnRH stimulation test group. Girls with peak LH values <5 IU/L were considered to have inactivation of the HPG axis and were categorized into the negative GnRH stimulation test group. Informed consent was obtained from the participating children and their parents, and the study was approved by the Institutional Review Board of the Shanghai Children’s Medical Center. This study was in accordance with the tenets of the Helsinki Declaration.

Methods

The GnRH stimulation test was performed in the early morning after fasting for 10 h. Gonadorelin (AnhuiFengyuan Pharmaceutical Co., Anhui, China) was injected at a dose of 2.5 µg/kg, with a maximum dosage of 100 µg. Blood samples were drawn from an inserted intravenous cannula before and 30 and 60 min after GnRH injection. Previous studies have suggested that LH levels between 30 and 60 min are sufficient for diagnosis of activation of the HPG axis [8–10]. All samples were analyzed for LH and FSH levels. The maximal LH and FSH levels achieved at any time point of testing were considered to be the peak levels. Additionally, E2 levels were determined prior to GnRH administration. An electrochemiluminescence immunoassay (Dxi800 automated chemiluminescence assay and commercial kit; Beckman Coulter, Inc., CA, USA) was used to determine hormone levels. The intra-assay coefficient of variation for LH was 3.6%–5.4%, with an inter-assay imprecision of 4.3%–6.4% and sensitivity of 0.2 IU/L. The calibration range of the assay was up to 250 IU/L. The intra-assay coefficient of variation for FSH was 3.1%–4.3% and inter-assay imprecision was 4.3%–5.6%. The sensitivity was 0.2 IU/L and the calibration range of the assay was up to 200 IU/L. E2 assay sensitivity was 20 pg/mL. The calibration range of the assay was up to 4800 pg/mL. The intra-assay coefficient of variation was 12%–21%.

Statistical analysis

The Student’s t-test was performed to compare the mean of subjects’ characteristics between groups, and normal distribution transformation was conducted on a 1/square. Correlation analyses were conducted between sex hormones and puberty status. The odds ratio was calculated according to the data test value between the positive GnRH test group and negative GnRH test group. Because the cutoff value was LHmax = 5.0, the continuous variable LHmax was converted into a binary variable. The LHmax values were categorized as 0 or 1 if LHmax values were <5.0 or ≥5.0. Receiver operating characteristic (ROC) curves for basal levels of LH, FSH, LH/FSH, and E2 were plotted. The area under the curve (AUC) and 95% confidence intervals (CIs) were measured for each curve. Youden’s index (sensitivity + specificity – 1) was used to determine the optimal gonadotropin cutoff point from the ROC. The test of equality of ROC areas was performed to compare the AUC between groups. ROC analysis for multiple comparisons was performed using different AUCs using the DeLong method [11]. If multiple comparisons were significant, every two AUCs were further compared. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using Stata 13.0 (Stata Corporation, College Station, TX, USA).

Results

Clinical and hormonal characteristics in the patients

There were 1138 patients in the positive GnRH test group (mean age± standard deviation: 7.95±1.25 years) and 612 patients (7.16 ± 1.59 years) in the negative GnRH test group. The difference between chronological age and bone age was 1.38 years in the negative GnRH test group and 1.43 years in the positive GnRH test group (p = 0.671). There
Discussion

Our study showed that the basal LH level was useful for predicting gonadal axis activation. Activation of the

Logistic regression analysis

The biochemical parameters that were considered to be related to the GnRH stimulation test results were adjusted using binary logistic regression analysis (Table 2). After regression analysis, basal LH levels were the most significantly and positively related to a positive response in the GnRH stimulation test (p < 0.05).

ROC analysis

ROC curves were plotted for basal LH levels, FSH levels, the LH/FSH ratio, and E2 levels (Figure 1). The AUC was measured for each curve (Table 3). A larger AUC represented a more positive rate of excitation. The maximum AUC was observed for basal LH levels, followed by basal FSH levels, the basal LH/FSH ratio, and basal E2 levels. This finding suggested that the basal LH value was best for predicting activation of the gonadal axis. The p-values of AUC comparisons was <0.05 between each AUC of basal hormone (Table 3). The appropriate cutoff value of basal LH levels associated with a positive response was 0.35 IU/L when Youden’s J index reached the maximum value. The sensitivity was 63.96% and specificity was 76.35% from the ROC curves. Therefore, a basal LH value that reached 0.35 IU/L suggested that gonadal axis activation was relatively high and further GnRH stimulation testing was required. A basal LH value that reached 2.72 IU/L (specificity was 100%, but sensitivity decreased to 54.5%) suggested a 100% peak LH level of ≥5 IU/L (Table 4).

Table 1: Hormonal and clinical characteristics of participants.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean±SD</th>
<th>Median</th>
<th>Distribution</th>
<th>P-Value a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>12.0±1.5</td>
<td>12.5</td>
<td>Normal</td>
<td>0.08</td>
</tr>
<tr>
<td>Bone age (chronological age, year)</td>
<td>0.0±5.0</td>
<td>0.0</td>
<td>Normal</td>
<td>0.86</td>
</tr>
<tr>
<td>Height, cm</td>
<td>130.0±10.0</td>
<td>130.0</td>
<td>Normal</td>
<td>0.89</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>45.0±10.0</td>
<td>45.0</td>
<td>Normal</td>
<td>0.83</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>17.0±2.0</td>
<td>17.0</td>
<td>Normal</td>
<td>0.88</td>
</tr>
<tr>
<td>Uterine volume, ml</td>
<td>1000.0±500.0</td>
<td>1000.0</td>
<td>Normal</td>
<td>0.89</td>
</tr>
<tr>
<td>Mean ovarian volume, ml</td>
<td>1000.0±500.0</td>
<td>1000.0</td>
<td>Normal</td>
<td>0.89</td>
</tr>
<tr>
<td>Uterine size, cm³</td>
<td>1000.0±500.0</td>
<td>1000.0</td>
<td>Normal</td>
<td>0.89</td>
</tr>
<tr>
<td>Mean ovarian volume, cm³</td>
<td>1000.0±500.0</td>
<td>1000.0</td>
<td>Normal</td>
<td>0.89</td>
</tr>
</tbody>
</table>

One-tailed t-test, n=1138

- Basal LH, IU/L: 32.0±27.0, n=46, p<0.001
- Basal FSH, IU/L: 13.1±26.1, n=46, p=0.017
- Basal LH/FSH ratio: 18.0±27.0, n=46, p<0.001
- Basal E2, pg/ml: 27.1±10.3, n=46, p<0.001
- Peak LH, IU/L: 5.0±12.0, n=46, p=0.017
- Peak FSH, IU/L: 13.5±5.5, n=46, p=0.017
- Peak LH/FSH ratio: 3.7±2.7, n=46, p=0.017

**Table 2: Hormonal and clinical characteristics of participants.**
gonadal axis is important for diagnosing CPP, which can accelerate bone maturation, result in impaired adult height and early menstruation, and can greatly affect the patient’s psychological health [12]. Previous studies have shown that early menstruation is related to adverse health outcomes in later life [13–15]. Therefore, timely diagnosis and appropriate treatment can help to improve the prognosis of these patients. Diagnosing CPP is difficult and should include clinical manifestations and correct and timely assessment of activation of the HPG axis. However, clinically diagnosing activation of the HPG axis by a simple examination and clinical data is challenging.

Currently, the biochemical criteria for diagnostic confirmation of gonadal axis activation are primarily based on the LH response during a standard GnRH stimulation test. A stimulated LH value ≥5 IU/L and/or a stimulated peak LH/FSH ratio >0.6 are considered pubertal responses during GnRH testing [2, 16, 17]. Pubertal LH secretion is characterized by high levels of peak LH secretion, which leads to higher levels of sex hormones in pubertal compared with prepubertal subjects. This eventually leads to the appearance of pubertal physical signs and accelerated growth [18]. With the development of newer and more sensitive immunoassays that measure serum gonadotropins, measurement of basal gonadotropins is hypothesized to allow discrimination between activated and inactivated values in HPG axis maturity.

In our single-center study, we investigated 1750 girls with early breast development. We found that basal LH values that were obtained during the GnRH stimulation test were significantly correlated with stimulated LH values. Additionally, LH values were useful as a screening test for predicting a positive response during the GnRH test. The highest Youden’s J index (0.40) was used to determine the appropriate cutoff LH value for diagnosing activation of the HPG axis. The basal LH cutoff point was 0.35 IU/L, with a sensitivity of 63.96% and specificity of 76.35%. When the basal LH value was 2.72 IU/L, the specificity reached 100%, although sensitivity decreased to 5.45%, which is higher than the value reported by Houk et al. [19]. They evaluated basal LH levels for discriminating activation of the HPG axis using two different chemiluminescent third-generation immunoassays (Wallac DELFIA and Architect) in 55 girls. Using the Architect assay, the LH cutoff point was 0.83 IU/L, with a

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**Table 2:** Binary logistic regression analysis of hormones compared between GnRH test (+) group and GnRH test (−) group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>Z-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal LH</td>
<td>12.11</td>
<td>8.29–17.71</td>
<td>12.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Basal FSH</td>
<td>1.73</td>
<td>1.60–1.87</td>
<td>13.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Basal LH/FSH ratio</td>
<td>1.90</td>
<td>1.19–3.04</td>
<td>2.68</td>
<td>0.007</td>
</tr>
<tr>
<td>Basal E2</td>
<td>1.02</td>
<td>1.01–1.02</td>
<td>5.10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>


**Table 3:** Comparison of AUCs for each basal hormone.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC (LH)</th>
<th>95% CI</th>
<th>p-Value</th>
<th>AUC (FSH)</th>
<th>95% CI</th>
<th>p-Value</th>
<th>AUC (LH/FSH)</th>
<th>95% CI</th>
<th>p-Value</th>
<th>AUC (E2)</th>
<th>95% CI</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;LH&lt;/sub&gt;</td>
<td>0.77</td>
<td>0.74–0.79</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;FSH&lt;/sub&gt;</td>
<td>0.73</td>
<td>0.70–0.75</td>
<td>0.002</td>
<td>0.21</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;LH/FSH&lt;/sub&gt;</td>
<td>0.68</td>
<td>0.65–0.71</td>
<td>&lt;0.001</td>
<td>0.021</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;E2&lt;/sub&gt;</td>
<td>0.61</td>
<td>0.59–0.64</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</tr>
</tbody>
</table>

AUC, area under curve, –, Data is not available. *p-Values of AUC comparisons between each AUC of basal hormone. *p-values of AUC<sub>LH</sub> vs. AUC<sub>FSH</sub>.

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**Figure 1:** ROC curves of basal LH and FSH, basal LH/FSH ratio, and E2 value for predicting positive results following GnRH stimulation testing.
sensitivity of 100% and a specificity of 100%. However, in the current study the basal LH cutoff level for evaluating activation of the HPG axis is different from previous studies. Pasternak et al. [20] measured basal serum LH and FSH levels using a chemiluminescent immunometric assay. They showed that low basal serum LH levels (≤0.1 IU/L) were sufficient for ruling out a positive response in the GnRH test in 94.7% of 38 prepubertal girls, with a sensitivity of 64%. Additionally, Suh et al. [21] reported cutoff values of basal LH (0.22 IU/L) that were measured using the sequential two-step immunoenzymatic assay (Access hLH, FSH Reagent Pack; Beckman Coulter, Inc., Brea, CA, USA). They detected a positive response of the GnRH stimulation test with 87.8% sensitivity and 20.9% specificity in 540 girls with clinical signs. They also demonstrated that basal FSH levels, basal E2 levels, and the basal LH/FSH ratio did not have predictive values for the diagnosis of CPP [21]. Mogensen et al. [22] showed that basal LH levels were superior in predicting the maximal LH level during GnRH testing compared with FSH, E2, and inhibin B levels. In another study, a total of 803 girls were included, and serum LH and FSH levels were measured using the immunoradiometric assay [23]. Based on the ROC curve, the optimal cutoff point for the basal LH level that was related to a pubertal response was 1.1 IU/L, which was associated with a sensitivity of 69.1% and specificity of 50.5%. Because of these different results among studies, clinicians must first determine the local cutoff before GnRH stimulation in patients with precocious puberty when applying this method.

In the present study, the AUC for LH was greater than that for FSH, the LH/FSH ratio, and E2. This finding indicated that basal LH levels were superior to FSH, the LH/FSH ratio, and E2 levels for determining activation of the HPG axis. Moreover, some researchers believe that determination of the LH/FSH ratio is helpful for improving the diagnostic accuracy of CPP [24]. However, FSH levels overlap between prepubertal and pubertal girls, which can affect the LH/FSH ratio and limit its application in evaluating activation of the gonadal axis. Our study showed that, when the cutoff value of basal LH levels was 0.35 IU/L, the sensitivity and specificity were 63.96% and 76.35%, respectively, which were relatively low. A basal LH value that reached 2.72 IU/L showed a specificity of 100%, but sensitivity decreased to 5.45%. These data indicated that increased basal LH levels were associated with a positive response to the GnRH test. Therefore, physicians should pay attention to basal LH testing in patients with early breast development. However, the appropriate cutoff value depends on sensitive measurement of basal gonadotropins and clinical manifestations. Therefore,
conducting further GnRH stimulation tests might be necessary. Notably, evaluation of HPG axis activation based on LH cutoff values is not consistent between research centers. This could be due to hormone testing methods, apparatus, and GnRH stimulation test methods.

In conclusion, measurement of basal LH levels could be better than FSH levels, the LH/FSH ratio, and E2 levels for initial evaluation of HPG axis activation with clinically suspected early puberty. Increased basal LH values are a significant predictor of a positive response during the GnRH stimulation test for assessing activation of the HPG axis.

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