Physiology of pubertal development in females

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Abstract: Puberty is the critical time when children develop into sexually mature adults. The span of time over which puberty occurs is often referred to interchangeably as adolescence. Adolescence can be an emotionally and physically challenging time for not only the adolescent, but also for their family. The onset and tempo of puberty is controlled by many factors, and the step-wise progression through pubertal milestones in females is best described as the Tanner stages of pubertal development. Puberty in females normally begins with breast development (thelarche) and then progresses to pubarche (onset of pubic hair), peak growth velocity (also known as the growth spurt), and menarche. The onset of puberty is controlled by the hypothalamic-pituitary-gonadal (HPG) axis. Another hormonal cascade, known as the hypothalamic-pituitary-adrenal (HPA) axis, is responsible for stimulating other supporting (secondary) sexual characteristics. An understanding of normal physiology of puberty is essential in order to recognize deviations from the norm and the disorders associated with pubertal development. This article reviews various aspects of the physiology of pubertal development in females.

Keywords: Puberty; menarche; menstrual cycle; hypothalamic-pituitary-gonadal axis (HPG axis); hypothalamic-pituitary-adrenal axis (HPA axis)

Introduction

Puberty is defined as the developmental time period during which secondary sexual characteristics appear and a child progresses from a sexually immature to sexually mature state. The start of puberty is controlled by the hypothalamic-pituitary-gonadal (HPG) axis, which is responsible for initiating the hormonal and physical changes seen with normal puberty. Both the HPG and hypothalamic-pituitary-adrenal (HPA) axes are the neuroendocrine systems that play a key role in normal puberty. The HPG axis, controlled by feedback mechanisms (which evolve early in life), is responsible for the onset of true puberty via pulsatile GnRH release, subsequent rise in luteinizing hormone (LH) and follicle stimulation hormone (FSH), and gonadal (ovarian) maturation. The HPA axis controls adrenarche, the increase in adrenal androgen production beginning in mid- to late-childhood. Adrenarche, though independently not true puberty, triggers the development of secondary sexual characteristics such as pubic and axillary hair in females (1-5).

Onset and tempo of puberty

In females, the first clinical manifestation to indicate the start of puberty is thelarche. As many as 1 in 5 girls may exhibit signs of pubarche prior to thelarche (*Table 1*) (1). The clinical progression through normal puberty is best classified using the Tanner Stages of sexual development for breast and pubic hair (5). The pubertal growth spurt

Table 1 Common terms associated with description of pubertal features

Term	Description
Thelarche	The onset of breast development
Adrenarche	The increase in production of androgens by the adrenal cortex, hormonally indicated by rise in DHEA-S
Menarche	The onset of menstrual cycles
Pubarche	The appearance of pubic hair, a response to adrenal androgens
Gonadarche	The activation of the gonads (ovaries) by the pituitary hormones LH and FSH
Gonadotropins	LH, FSH
Sex steroids	Androgens, estrogens
Androgens	DHEA, DHEA-S, androstenedione, testosterone, DHT

DHEA-S, dehydroepiandrosterone sulfate; LH, luteinizing hormone; FSH, follicle stimulation hormone; DHT, dihydrotestosterone.
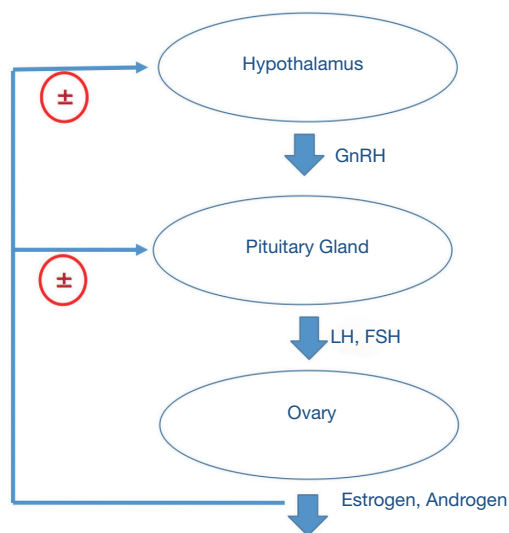
Figure 1 Hypothalamic-pituitary-gonadal axis.

in females occurs early in puberty, due to both the direct stimulation of sex steroids on bone as well as indirectly via the growth hormone-insulin-like growth factor (GH-IGF) axis (6,7). There has been considerable debate on the age at which reaching pubertal milestones is considered normal, though it is still not confirmed with certainty. This is best estimated by the National Health and Nutrition Examination Survey (NHANES III), which provides predicted normative reference ages of sexual maturity ratings for the general population of children over 8 years of age in the United States (4). According to NHANES III, in the female population with a body mass index (BMI) between 10–85th percentile (3), thelarche (classified as Tanner stage 2) presents at an average age of 10.2 years and can occur as

early as 8.25 years (5th percentile). Pubarche (classified as Tanner stage 2 for pubic hair) presents at an average age of 11.6 years and as early as 9.25 years, and menarche presents at an average age of 12.6 years and as early as 11 years (3,4). Studies have looked at growth in all stages of life including puberty. The 1998 study by Abbassi noted that females have a peak height velocity at a mean of 11.5 years of age, and average peak growth velocity of 8.3 cm/year (8).

Pubertal milestones in females with a normal BMI can be further deduced based on ethniticy. Thelarche is considered premature prior to 8 years of age in non-Hispanic white females, but can be normal in non-Hispanic black and Mexican American females as early as 7 years of age. Pubarche prior to 8 years of age is considered premature in all ethnic groups. Ethnic differences in age at menarche are minor. Menarche occurs normally around 11.3 years of age in non-Hispanic white females, and as early as 10.5 years of age in non-Hispanic black and 10.7 years of age in Mexican American females (3). In addition to ethnic differences, increased BMI appears to have an independent correlation with earlier pubertal onset (3). In the overweight and obese females (BMI >85th percentile), signs of puberty may present earlier independent of ethnicity. However, caution must be taken when evaluating such patients as the presence of lipomastia can be mistaken for true breast development (3).

Development and regulation of the HPG axis

Fetal period

The development of the HPG axis begins during fetal development (*Figure 1*). During the first trimester Gonadotropin-releasing hormone (GnRH) neurons migrate

from the olfactory area to their permanent secretory region in the hypothalamus, and the pituitary gland differentiates into an anterior lobe (adenohypophysis) and posterior lobe (neurohypophysis). The GnRH neuronal migration stimulates a pulsatile release of GnRH and subsequent rise in the secretion of gonadotropins (LH and FSH) by the anterior pituitary gland. LH and FSH levels reach their peak by the middle of the second trimester, when sex steroid production begins to rise and GnRH release is subsequently inhibited by negative feedback mechanisms. This causes a decrease in LH and FSH levels. During the third trimester GnRH and gonadotropin levels are fully suppressed due to this negative feedback by the high sex steroid levels produced by the fetoplacental unit (5,9,10).

Neonatal, infantile, and childhood period

The HPG axis temporarily operates on a pubertal level shortly after birth, so-called the "minipuberty of infancy". The clinical manifestations are brief and its regulation is unknown (5). In the first week of life, LH and FSH levels rise in pulsatile fashion in response to a fall in serum fetoplacental estrogen levels. LH and FSH quickly reach pubertal levels, stimulating increased production of gonadal sex steroids. By four months of life, however, increased sensitivity of the hypothalamic sex steroid receptor leads to decreases in LH and FSH to prepubertal levels, at which they remain for the remainder of childhood. This increased sensitivity to negative feedback accounts for the inhibitory effect on estrogen and testosterone levels throughout childhood (5).

The HPG axis remains relatively quiescent throughout childhood, and by 6 years of age, a nadir in LH, FSH, and GnRH is seen. Despite this absolute decrease, FSH remains higher comparative to LH throughout childhood. FSH levels are a reflection of GnRH secretion, as well as the relatively higher concentrations of activin-A (which promotes stimulation of gonadal, pituitary and extra gonadal tissue hormones) and lower concentrations of gonadal inhibin-B (which inhibits FSH release) (11). Between 7 and 10 years of age, GnRH levels begin to rise in pulsatile fashion and there is a subsequent increase in gonadotropin levels, representing the advancement of puberty (5).

Adolescence

Changes in hormone secretion occur approximately one year before the physical signs of puberty become manifest.

These hormonal changes begin with increasing frequency and amplitude of pulsatile GnRH, which first occurs at night and then progressively throughout the day. The associated rise in LH and FSH is disproportionate, with LH rising higher than FSH during puberty due to increased sensitivity to GnRH stimulation. This provokes ovarian follicular maturation in a cyclic manner (5). The major circulating estrogens include estrone, estradiol, and estriol. More than 95% of estradiol and 50% of estrone is produced and secreted by the ovaries. Estriol is the major estrogen produced during pregnancy by the placenta (10). Ovarian estradiol is produced in a pulsatile manner early in puberty, reflective of LH and FSH secretory patterns, indicating that random measurements are an insensitive marker of pubertal onset. Once estradiol levels increase to those high enough to stimulate a withdrawal menstrual bleed, menarche occurs. Menarche itself does not indicate full maturation of the HPG axis, as initially it is a sign of estrogen-withdrawal bleeding and not ovulation (5). Thelarche correlates well with rising concentrations of estradiol, and pubarche corresponds with an associated rise in serum testosterone (12). Staging of breast and pubic hair development is best characterized by the Tanner stages of breast and pubic hair development, respectively (12).

Regulation

The stimulus for the onset of puberty is multifactorial. Although genetic factors may contribute upwards of 75% of the variability of pubertal timing, it is also important to recognize that other factors such as neuroendocrine hormones, ethnicity, endocrine-disrupting chemicals (hormonally active chemicals), and nutrition, all play a role in the rate of pubertal progression (8,13,14).

It is well established that the onset of puberty begins with pulsatile release of GnRH from the hypothalamus. This is controlled by a balance between central nervous system excitatory and inhibitory signals, and as shifts in these signals occur, GnRH is released and the pubertal cascade ensues. During childhood, the major inhibitory systems preventing GnRH release are gamma-aminobutyric acid (or GABAergic) and opiodergic. Excitatory systems stimulating GnRH release include neuronal kisspeptin, glutamate, as well as glial cells that use growth factors to mediate GnRH secretion (15).

The mechanisms by which these excitatory systems stimulate GnRH release differ. Kisspeptin is a neuropeptide released by the hypothalamus which acts via GPR54, a G-protein-coupled receptor on GnRH neurons, as a signal
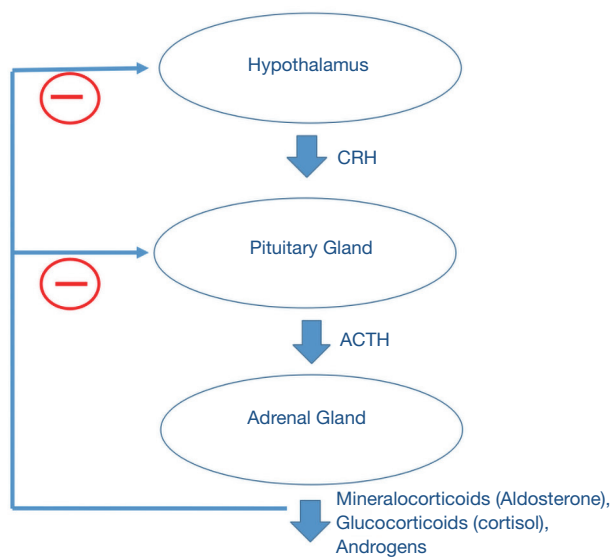
Figure 2 Hypothalamic-pituitary-adrenal axis.

for pubertal GnRH release. Upregulated signaling of glutamate receptors stimulates GnRH release; glial cells facilitate GnRH release via interplay between enzymes that control concentration of glutamate, prostaglandin E2, and transforming growth factors (5,15).

Development of the HPA axis: adrenarche

The HPA axis controls adrenarche, the increase in adrenal androgen production in response to heightened sensitivity of the zona reticularis to adreno-corticotrophic hormone (ACTH) stimulation (*Figure 2*). It begins in mid-childhood independent of pubertal maturation of the HPG axis, years prior to the onset of physical changes. These changes are notable for the development of secondary sexual characteristics: pubic and axillary hair, sweat gland maturation, and acne (5,10,16).

Prior to the onset of adrenarche, ACTH stimulates adrenal cortisol secretion alone, and has very little effect on adrenal 17-ketosteroid production. As girls approach 6–7 years of age, the zona reticularis of the adrenal gland becomes increasingly sensitive to ACTH levels with a resultant increase in androgen production without rise in cortisol secretion. The hallmark of adrenarche is a rise in adrenal dehydroepiandrosterone sulfate (DHEA-S) levels. Serum DHEA-S greater than 40 g/dL is indicative of adrenarche (5,10,16). The major circulating androgens are testosterone, DHEA-S, and androstenedione. During

puberty the ovaries are responsible for producing 50% of serum testosterone (the other 50% produced by the adrenal glands), and the adrenal glands are responsible for producing 90% of DHEA-S (10).

Development of the female reproductive system

Embryology and histology

In the absence of the sex-determining region of the Y gene (SRY), the undifferentiated gonad is stimulated toward ovarian development. This begins in the first few weeks of gestation, during which time primordial germ cells are noted to be located within the yolk sac endoderm. By eight weeks of gestation, the primordial germ cells relocate to the ovary and once there continue multiplying via mitosis. Now called oogonia, the mitotic replication continues and is maximal by twelve weeks' gestation and ending by about the third trimester (5,10). During the first trimester, some oogonia are noted to exit mitosis and begin meiosis, where they arrest in prophase 1 and are then called primary oocytes. The nearly 7 million primary oocytes are individually contained within a primordial follicle, and these resting primordial follicles remain the source of germ cells throughout childhood.

Primordial follicles continue to undergo atresia throughout childhood at a tempo unique to the female. At the time of puberty, these are decreased to nearly 300,000 in number (10,17). An estimated 400–500 primordial follicles undergo maturation between menarche and menopause. A large portion of the remaining follicles undergo atresia throughout childhood; at the time of menopause there is an estimated 1,000 primordial follicles remaining inside the ovaries (10,17). It is important to note that ovarian primordial follicles do not generate sex steroid production (5,10,17-21). After puberty begins, small numbers of primordial follicles are stimulated to grow into primary and then secondary follicles in waves. Primary follicles are distinguished histologically from primordial follicles by the appearance of granulosa cells. As the granulosa cells proliferate, they eventually form a multilayer barrier around the follicle and it becomes known as a secondary follicle. Secondary follicles continue to mature, and once they begin producing thecal cells surrounding the outer granulosa cell layer, they are then considered mature preantral follicles. Granulosa cells display the FSH receptor (these cells do not have hormonal production at this stage, though will later be responsible for converting ovarian androgens into estrogen),
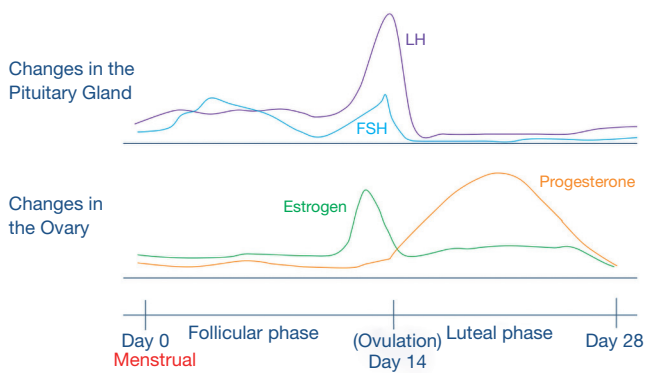
Figure 3 The menstrual cycle (22).

Figure 4 Female reproductive system.

and thecal cells display the LH receptor (responsible for minimal to no androgen production at this stage, however later in puberty produce both androstenedione and testosterone). Preantral follicles develop into a dominant antral follicle over a span of nearly one month. Once puberty begins, antral follicles are released in clusters in a cyclic pattern (with the menstrual cycle), however only one antral follicle becomes the dominant follicle to undergo ovulation (*Figure 3*) (10,21). After the LH surge leading to release of a follicle from the ovary, luteinization occurs in that the dominant follicle (known as the corpus luteum) begins its production of estrogen and progesterone (21).

Anatomy

The female reproductive system consists internally of the ovaries, fallopian tubes, uterus, cervix, and vagina, and externally of the labia majora and labia minora, clitoris, vaginal opening, and urethra (*Figure 4*). The ovaries themselves are not directly connected to the reproductive tract. The ovaries are located within a fold of peritoneum, called the broad ligament, which suspends each ovary on opposite sides of the abdomen near the opening of the fallopian tubes. The ovary is composed of an outer cortex and an inner medulla (21,23). Ovarian follicles grow and mature from the outer cortex, and as ovulation occurs, the process includes a mechanism by which the outer wall of the ovary is destroyed and the follicle is released. Rapid mitosis of ovarian epithelial cells leads to repair of the ovarian wall. Released follicles remain briefly suspended within the broad ligament before entering the fallopian tubes (23). Fallopian tubes are open-ended structures with fingerlike projections called fimbriae extending outward. Medial to the fallopian
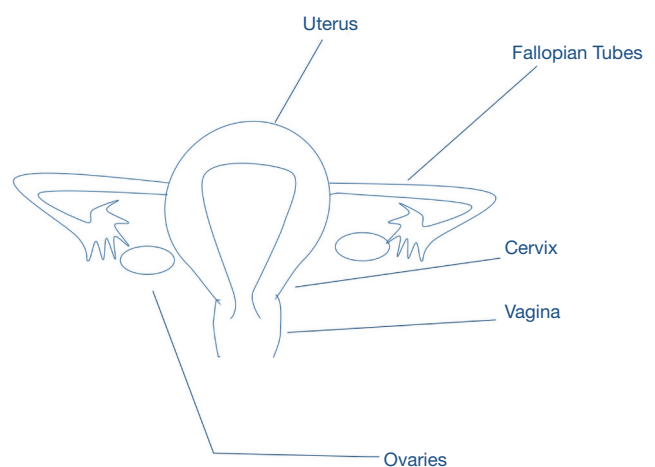
tubes is the uterus, which is positioned between the bladder and rectum. The uterus is divided into a fundus superiorly, a body, an isthmus inferiorly, and extends further to become the cervix. The cervix continues into the vagina. Externally, the labia majora and labia minora, clitoris, vaginal opening, and external urethral opening can collectively be referred to as the vulva. The vaginal opening is shielded by a partial covering termed a hymen; there are normal variations in its appearance (5,21).

The menstrual cycle

The menstrual cycle averages 28 days in length and is divided into two phases: the follicular phase and luteal phase (*Figure 3*) (8,22,24). The follicular phase begins on day 0 with onset of menses and ends the day before the LH surge and may be 14 to 21 days long. Early in the cycle, FSH is the dominant gonadotropin secreted by the pituitary gland. As estradiol levels gradually begin to rise mid-phase, there is a mutual decrease in the levels of FSH as LH secretion begins. Peak estradiol levels stimulate progesterone to rise, and this in turn causes maximal LH levels, known as the LH surge (5,18,21).

The luteal phase begins on the day of the LH surge and ends at the onset of the next menstrual bleed, with a total length of 14 days. The LH surge is responsible for stimulating ovulation and as the corpus luteum forms, it secretes both estradiol and progesterone (progesterone being the primary hormone secreted by the corpus luteum). Estrogen and progesterone aid in maintaining the uterine endometrium in preparation for pregnancy. If fertilization

Table 2 Tanner stages for breast development

Stage	Description
Tanner 1 (B1)	Pre-pubertal or infantile appearance
Tanner 2 (B2)	Breast buds* appear. The breast and papilla are elevated and the diameter of the areola is increased
Tanner 3 (B3)	Breast and areolar enlargement as a rounded contour
Tanner 4 (B4)	Areolae and papillae form a secondary mound projected above the level of the contour of the breast
Tanner 5 (B5)	Mature breasts. Recession of areolar secondary mount but continued projection of papillae. No secondary mound noted

*, use caution in assessing overweight females who may have signs of lipomastia, which may interfere with accurate assessment.

Table 3 Tanner stages for pubic hair development

Tanner 1 (PH1)	Pre-pubertal. No hair or may have fine, vellus, downy hair
Tanner 2 (PH2)	Straight, sparse hair located along labia majora
Tanner 3 (PH3)	Hair becomes darker and curlier, spreads over the mons pubis
Tanner 4 (PH4)	Adult-like pubic hair with increased coarseness and curliness, without spread to medial thighs
Tanner 5 (PH5)	Adult pubic hair quality with spread to medial thigh

does not occur and therefore there is a lack of human chorionic gonadotropin (hCG), the corpus luteum involutes and both progesterone and estrogen levels decline. The withdrawal of these hormones leads to sloughing of the endometrial lining, leading to a menstrual bleed. As this happens FSH levels rise in a greater proportion to LH, stimulating growth of another follicle such that another cycle begins (5,18,21).

Hormonal changes and the development of secondary sexual characteristics

Soon after the pulsatile increase in GnRH levels stimulate the onset of puberty, a corresponding rise in LH is seen primarily at night as the first detectable biochemical sign of puberty. FSH rises in concordance with LH but in a lower concentration. Though LH levels are initially highest at night, as puberty progresses there is a shift and LH levels are present in higher concentrations during the day. After menarche, however, the diurnal variation of LH is modified and takes on a morning predominance (5,10,16). Ovarian estradiol level production corresponds to levels of FSH production throughout childhood. In the neonatal period when FSH production is high, estradiol levels are similar to those seen in puberty but as FSH levels decrease after a few months of age, estradiol levels also fall. In mid- to late-childhood when LH and FSH

levels begin to rise again, ovarian estradiol levels also correspondingly rise (5).

LH stimulates theca cells of the ovary to form androgens. FSH stimulates granulosa cells to form estrogens via aromatization of thecal androgenic precursors. Estrogen stimulates endometrial growth, cervical mucus secretion and breast development. The endometrium and cervix undergo cyclical changes concordant with menstrual cycles. Progesterone antagonizes estrogen effects on the vaginal epithelium and cervix (5). Androgens stimulate development of pubic and axillary hair, sweat gland maturation, and acne as noted previously in this chapter (5,10,16). Sex steroids stimulate overall somatic growth directly and indirectly via increased GH secretion (1). Both stimulate sexual drive and function.

Estradiol is the key hormone stimulating skeletal maturity in both males and females (1). Estrogen and testosterone both play a role in epiphyseal growth and maturation. However, estrogen is the key driver behind epiphyseal plate closure as well as inhibition of bone resorption. Estrogen also promotes lipogenesis and causes redistribution of adipose to the lower body, however testosterone is lipolytic and supports muscular development (18).

Schematized in 1969 by Drs. Marshall and Tanner from the University of London, the Tanner staging for breast and pubic hair development is widely used for evaluation of pubertal milestones (*Tables 2,3*) (20).

Growth pattern during puberty

The onset of puberty and puberty stages best correlate with the level of skeletal maturation versus chronological age. This indicates that the onset of breast development normally occurs at a bone age of approximately 10 years and menarche occurs at a bone age of approximately 12.5 years, irrespective of the chronological age (18).

As puberty begins and there is a resultant rise in sex steroid hormones, linear height is increased via direct and indirect stimulation of growth hormone production. The indirect mechanism by which sex steroids stimulate linear growth is via stimulation of the GH-IGF axis. In females, the peak height velocity occurs at Tanner stage 2 to 3 for breast development (23). Approximately 1 year prior to menarche, the average peak height velocity is 8 cm/year, which corresponds to Tanner stage 3 for breast and pubic hair. Menarche occurs approximately 6 months after the pubertal growth spurt has occurred (24). Most girls begin puberty by the time their skeletal age is 12.5 years, and menarche typically occurs by the time their skeletal age reaches 14 years (5). Growth is approximately 99% complete at bone age of 15 years (5,18). Estrogen plays an important role in inhibiting bone resorption. A decreased exposure to estrogen, as in cases of delayed menarche, has been shown to be associated with an increase in fracture risk. This risk increases with increasing age of menarche (5,19).

Conclusions

Puberty involves a complex interplay of biochemical changes and the resultant physical maturation spanning several years' length of time. The age range during which all females are expected to normally progress through puberty is not accurately known, and therefore there exists a range of acknowledged ages at which females can achieve pubertal milestones normally. There are many factors that can affect the normal progression through puberty, and one should be familiar with not only the physiology of normal female puberty but also with potential causes of abnormal pubertal development.

Maturation of the HPG axis begins in utero and this system is key in controlling the onset of puberty, while the HPA axis plays an independent role in the development of secondary sexual characteristics. Menarche is the culmination of pubertal development, ultimately leading to reproductive abilities. The secretion of pubertal levels of sex steroids triggers many physical changes, which are commonly assessed using the Tanner staging system. Important among the effects of sex steroid production is the pubertal growth spurt, which is controlled by elevations in these hormones. Estrogen has protective effects on maintenance of bone integrity, however excess estrogen exposure may lead to known adverse effects on endometrial health.

Puberty can be a stressful psychological time for both adolescents and their parents. A healthy and engaging support system at home can mitigate many of these stressors. As a medical practitioner taking care of adolescents, this time can also be an encouraging one that establishes great rapport with one's patients. The ability to provide reassurance on normal pubertal development and promote balance between mental and physical health can be one of the most rewarding aspects in one's career.

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