The Pathophysiology of Amenorrhea in the Adolescent

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Menstrual irregularity is a common occurrence during adolescence, especially within the first 2–3 years after menarche. Prolonged amenorrhea, however, is not normal and can be associated with significant medical morbidity, which differs depending on whether the adolescent is estrogen-deficient or estrogen-replete. Estrogen-deficient amenorrhea is associated with reduced bone mineral density and increased fracture risk, while estrogen-replete amenorrhea can lead to dysfunctional uterine bleeding in the short term and predispose to endometrial carcinoma in the long term. In both situations, appropriate intervention can reduce morbidity. Old paradigms of whom to evaluate for amenorrhea have been challenged by recent research that provides a better understanding of the normal menstrual cycle and its variability. Hypothalamic amenorrhea is the most prevalent cause of amenorrhea in the adolescent age group, followed by polycystic ovary syndrome. In anorexia nervosa, exercise-induced amenorrhea, and amenorrhea associated with chronic illness, an energy deficit results in suppression of hypothalamic secretion of GnRH, mediated in part by leptin. Administration of recombinant leptin to women with hypothalamic amenorrhea has been shown to restore LH pulsatility and ovulatory menstrual cycles. The use of recombinant leptin may improve our understanding of the pathophysiology of hypothalamic amenorrhea in adolescents and may also have therapeutic possibilities.

Key words: **amenorrhea; adolescents**

Menstrual irregularity is a common occurrence during adolescence, especially within the first 2 to 3 years after menarche. Prolonged amenorrhea, however, is not normal and can be associated with significant medical morbidity, which differs depending on whether the adolescent is estrogen-deficient or estrogen-replete. In both situations, appropriate intervention can reduce morbidity. Old paradigms of whom to evaluate for amenorrhea have been challenged by recent research that provides a better understanding of the normal menstrual cycle and its variability. The aim of this chapter is to review the pathophysiology of amenorrhea in adolescents and to serve as an overview for the more detailed discussions of specific etiologic conditions in the chapters to follow. Priorities for future research will be proposed on the basis of review of the recent literature.
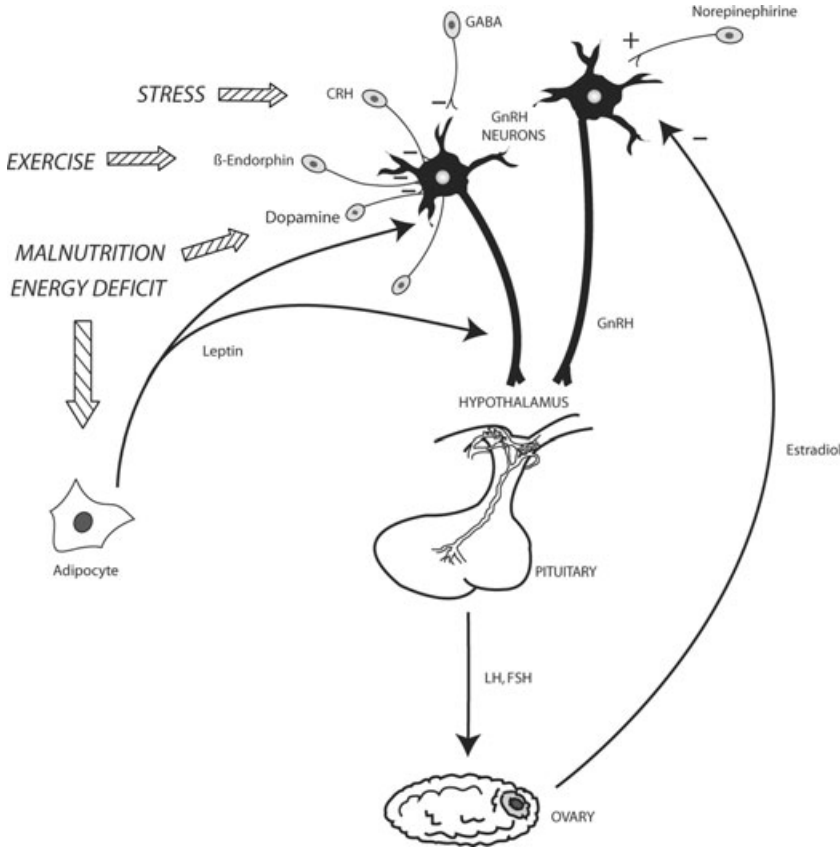
The Normal Menstrual Cycle

Menarche

In the United States and Europe, the median age of menarche declined from 16 to 17 years in the mid-1800s to approximately 12.5 years in the mid-1900s, presumably because of better nutrition and improved socioeconomic living conditions. Over the past 30 years, however, the median age of menarche in the United States has remained relatively stable. Age of menarche varies in different countries and tends to be higher in less-developed countries and lower in well-developed countries. Using a national probability sample of 2,510 girls aged 8.0 to 20.0 years, Chumlea *et al.* found that the median age of menarche in the United States is 12.43 years and 80% of all girls begin to menstruate between 11.0 and 13.75 years. Fewer than 10% are menstruating by age 11 and 90% are doing so by age of 13.75 years.[1] By the age of 15 years, 98% of all girls have reached menarche.[2] Both pubertal development and age at menarche vary by race, with black girls entering puberty earlier than their white counterparts and reaching menarche before them.[3] In the United States, mean age of menarche is 12.06 years in blacks, 12.25 years of age in Mexican Americans,

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FIGURE 1. Regulation of pulsatile GnRH secretion by leptin and neurotransmitters.

and 12.55 years of age in non-Hispanic whites.[1] There is a known relationship between age of menarche and BMI, with early menarche associated with higher BMI.[4,5] What is not known, however, is the direction of the association—whether increased body fat leads to early menarche or whether early sexual maturation is the reason for an increase in the amount of body fat and a change in its distribution.

Physiology of the Normal Menstrual Cycle

An understanding of the physiology of the normal menstrual cycle (see the chapter by Dr. Hillard in this volume) is essential in order to understand the variability of menstrual cycles that occurs during adolescence. Gonadotropin-releasing hormone (GnRH), a decapeptide produced by the neurosecretory neurons of the preoptic area of the hypothalamus, is released at the axon terminals of the neurosecretory cell at the median eminence of the hypothalamus. The hormone is secreted into the capillaries of the hypophysial portal system and is transported to the anterior pituitary, where it stimulates the synthesis and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The GnRH is released in pulses in response to serum levels of gonadal steroids. Secretion of GnRH is also regulated by a number of neurotransmitters, including dopamine, endogenous opioids, norepinephrine, gamma amino butyric acid (GABA), and corticotropin- releasing hormone (CRH). Some of these neurotransmitters (e.g., dopamine) are released by the tuberoinfundibular neurons which abut the neurosecretory cells of the median eminence.[6] The modulation of GnRH release by the neurotransmitters is complex. with interactions between the different regulatory systems (FIG. 1). For example, infusion of dopamine inhibits the LH pulsatile release induced by naloxone, an opioid receptor antagonist.[7] Other neurotransmitters (e.g., serotonin, neuropeptide Y, and neurotensin) may be involved in the regulation of GnRH secretion, but their exact role is not clear.

The GnRH pulses increase in amplitude and frequency during puberty. Each GnRH pulse is followed

by a corresponding LH pulse. Follicle-stimulating hormone is also secreted in a pulsatile manner, but the FSH peaks may be obscured because of the long half-life of FSH (about 3 hours). The hypothalamus is exquisitely sensitive to circulating levels of estrogen, which inhibit GnRH secretion by the hypothalamus (the "negative feedback loop"). The negative feedback loop is present in the fetus and is active throughout childhood and adolescence. However, the "positive feedback loop," in which a critical level of estrogen stimulates pulses of GnRH, which triggers the LH surge and ovulation, only develops later in puberty. In the follicular phase of the normal menstrual cycle, rising levels of FSH stimulate the emergence of a dominant ovarian follicle and estrogen production. When a critical estrogen level is reached, GnRH pulses trigger the LH surge and ovulation. After ovulation, a corpus luteum is formed, which produces progesterone to prepare the endometrium for implantation of a fertilized ovum. After approximately 14 days, if implantation has not occurred, the corpus luteum regresses and progesterone levels drop. The endometrium can no longer be sustained and menstrual shedding occurs. The spiral arterioles linking the functional and basal layers of the endometrium are able to clamp down, causing the menstrual bleeding to stop, usually within 7 days. The luteal phase of the menstrual cycle is fairly constant at 14.0 ± 2.0 days.

Withdrawal of progesterone from an estrogen-primed endometrium is necessary for normal menstruation to occur and for the bleeding to stop in a timely fashion. In the absence of adequate estrogen priming, there will be no positive feedback and ovulation will not occur, resulting in estrogen-deficient amenorrhea. In the presence of adequate estrogen levels, but the absence of ovulation, there will be no progesterone withdrawal and once again amenorrhea will occur. In this situation, the patient is estrogen-replete and because of the effect of unopposed estrogen on the endometrium, the patient will be at risk for dysfunctional uterine bleeding (DUB) in the short term and endometrial carcinoma in the long term.

Maturation of the
Hypothalamic-Pituitary-Ovarian Axis

The hypothalamic-pituitary-ovarian (HPO) axis becomes active in the second trimester of pregnancy. Gonadotropin levels peak at mid gestation and decline at term because of the negative feedback from placental hormones. There is a mild secondary peak in gonadotropin levels after withdrawal of placental steroids after birth, but after 1–2 years of age, levels of gonadotropins remain low until puberty begins. They are suppressed by circulating sex hormones primarily produced by the adrenals and mediated via the negative feedback loop.[8]

The mechanism for initiating puberty in late childhood is incompletely understood. What is known is that for some reason, the hypothalamus becomes less sensitive to circulating gonadal hormones. Pulses of GnRH increase in amplitude and frequency and are followed by pulses of LH and FSH. Augmented secretion of both LH and FSH is more prominent during sleep.[8,9] With the onset of puberty, the amplitude of the pulsatile LH and FSH secretion increases markedly, particularly during wake-time.[8] In response to rising LH and FSH levels, the ovary produces estrogen, which initiates sexual maturation, heralded by breast development (thelarche).

There is a normal progression of pubertal development in both boys and girls. In girls, menarche usually occurs during Tanner stage 4 of breast development and usually within two and a half to three years after thelarche. Once menarche has occurred, it takes approximately 5–7 years for the HPO axis to mature and for the establishment of regular menstrual cycles. The interval from the first menstrual period to the second period can be quite long, but subsequent cycles usually vary from 21 to 45 days, with very few cycles falling out of this range.[2] In the first year after menarche, approximately 50% of cycles are anovulatory, but 80% still fall within the range of 21–45 days' duration. By the third year after menarche, 95% of menstrual cycles fall into this range.[10] In general, anovulatory cycles occur most frequently during the first two years after menarche.

Primary and Secondary Amenorrhea
Definitions

Amenorrhea is the absence of spontaneous menses in a woman of reproductive age. In adolescents, amenorrhea traditionally has been divided into primary amenorrhea and secondary amenorrhea.

Primary amenorrhea is amenorrhea in a patient otherwise expected to have regular periods.

Secondary amenorrhea is amenorrhea in a patient who has already established regular menstrual cycles. Traditionally this term has only been used in those with amenorrhea of greater than 3 months' duration.

The distinction between primary amenorrhea and secondary amenorrhea is somewhat arbitrary and there is a great deal of overlap. Any cause of secondary amenorrhea (including pregnancy) can also be a cause of primary amenorrhea. In general, however, for a patient with primary amenorrhea the suspicion should

TABLE 1. Indications for evaluation of an adolescent with primary amenorrhea[a]

1. An adolescent who has not had menarche by age 15 years
2. An adolescent who has not had menarche and more than 3 years have elapsed since thelarche
3. An adolescent who has not had a menarche by age 13 years and no secondary sexual development
4. An adolescent who has not had menarche by age 14 years and:
   (i) there is a suspicion of an eating disorder or excessive exercise, or
   (ii) there are signs of hirsutism, or
   (iii) there is suspicion of genital outflow obstruction

[a]Adapted from Diaz *et al.*[2]

be high for the possibility of a chromosomal or structural abnormality.

Who Should Be Evaluated for Amenorrhea?

In the light of what is now known about normal menstrual physiology in adolescents, the American Academy of Pediatrics recently recommended that an evaluation for primary amenorrhea should be considered in the conditions listed in TABLE 1.[2]

Given that 98% of girls have reached menarche by the age of 15 years, it is reasonable to evaluate any adolescent female who is 15 years or older and has not yet begun to menstruate. This recommendation replaces older recommendations using a cut-off of 16 years of age. Recent longitudinal data show that the median time interval from thelarche to menarche is 2.7 years for black girls and 2.5 years for white girls.[11] If more than 3 years have elapsed since thelarche, and menarche has not yet occurred, an evaluation is indicated. National probability data demonstrate that, depending on race, 75% of girls have begun breast development by age 11.0 years and pubic hair development by age 11.5 years.[12] A cross-sectional study of young girls in pediatric practices further showed that by the age of 12 years, more than 95% of girls have begun breast development and over 90% have begun pubic hair development.[3] Lack of secondary sexual development and no menarche by the age of 13 years is therefore another reasonable cause for evaluation. Disordered eating and eating disorders are prevalent in adolescent females. If an adolescent is 14 years of age, has primary amenorrhea, and has features suggestive of an eating disorder, appropriate evaluation and referral is warranted. Similarly, if a patient has signs of hirsutism, a work-up should be performed to rule out the possibility of late-onset congenital adrenal hyperplasia or polycystic ovary syndrome (PCOS). Finally, if an adolescent is 14 years old,

is fully developed, has primary amenorrhea, and has lower abdominal pain occurring at monthly intervals, the patient should be assessed for genital outflow tract obstruction.

Etiology of Amenorrhea

A classification of the major causes of amenorrhea in adolescents by organ system and by estrogen status is shown in TABLE 2. In clinical practice, hypothalamic amenorrhea and PCOS are the most prevalent causes of amenorrhea in adolescents. In some cases, a teenager presumed to have amenorrhea secondary to immaturity of the HPO axis may subsequently turn out to have PCOS. In a study of 203 adolescent girls presenting to a youth clinic in Sweden for menstrual disorders, hypothalamic inhibition of the HPO axis was the most frequent cause and PCOS was the second most frequent. An eating disorder was diagnosed in 68% of those presenting with amenorrhea and PCOS was identified in 55% of those presenting with oligomenorrhea.[13]

Hypothalamic Causes of Amenorrhea

Hypothalamic amenorrhea is the most prevalent cause of amenorrhea in the adolescent age group. Patients with hypothalamic amenorrhea have low LH, FSH, and estrogen levels with preserved LH and FSH responsiveness to GnRH. As previously described, within the first 2 to 3 years after menarche, immaturity of the HPO axis can lead to anovulation and amenorrhea, but prolonged amenorrhea secondary to immaturity of the HPO axis is not as common as once thought. The most common causes of hypothalamic amenorrhea in those who are at least 2–3 years post menarche are eating disorders, excessive exercise, medications, and psychosocial stress.[13,14]

The discovery of leptin in 1994 has markedly improved our understanding of the pathophysiology of hypothalamic amenorrhea and its relationship to energy balance. Leptin, a protein produced by the adipocyte, acts on the hypothalamus to regulate food intake, energy expenditure, and body weight. In addition to its role in regulating energy homeostasis, leptin also plays a role in sexual maturation and reproductive functioning in rodents. Leptin receptors have been identified in the hypothalamus in close proximity to the median eminence and could theoretically influence GnRH secretion by the hypothalamus. The current thought is that in both anorexia nervosa and in exercise-induced amenorrhea, amenorrhea is an adaptive response to an energy deficit, mediated in part by leptin. Leptin levels in are low in anorexia nervosa

TABLE 2. Etiology of amenorrhea in adolescents

Type	Estrogen-deficient	Estrogen-replete
Hypothalamic		
	Eating disorders	Immaturity of the HPO axis
	Exercise-induced amenorrhea	
	Medication-induced amenorrhea	
	Chronic illness	
	Stress-induced amenorrhea	
	Kallmann syndrome	
Pituitary		
	Hyperprolactinemia	
	Prolactinoma	
	Craniopharyngioma	
	Isolated gonadotropin deficiency	
Thyroid		
		Hypothyroidism
		Hyperthyroidism
Adrenal		
		Congenital adrenal hyperplasia
		Cushing syndrome
Ovarian		
		Polycystic ovary syndrome
	Gonadal dysgenesis (Turner syndrome)	
	Premature ovarian failure	
		Ovarian tumor
	Chemotherapy, irradiation	
Uterine		
		Pregnancy
		Androgen insensitivity
		Uterine adhesions (Asherman syndrome)
		Müllerian agenesis
		Cervical agenesis
Vaginal		
		Imperforate hymen
		Transverse vaginal septum
		Vaginal agenesis

and increase with refeeding.[15–18] After weight restoration, leptin levels remain lower in those who remain amenorrheic compared with those who are menstruating regularly.[18,19] It is felt by some that leptin acts as a metabolic gate, allowing increased gonadotropin secretion when leptin concentration exceeds a critical level.[20] In amenorrheic athletes, leptin levels are also lower than in weight- matched controls or in athletes who have regular menses.[21,22] In a pilot study of 14 women with hypothalamic amenorrhea (who had either lost weight or were exercising excessively but reportedly did not have an eating disorder), administration of recombinant leptin for 3 months increased LH, FSH, and estradiol levels in the 8 women receiving leptin and led to ovulatory menstrual cycles in three of them.[23]

Eating Disorders

The prevalence of *anorexia nervosa* in young women is 0.3–0.5%,[24] with the highest incidence in adolescent girls between the ages of 15 and 19 years.[25] While amenorrhea is one of the features necessary for the diagnosis of anorexia nervosa, dietary restraint, even in the presence of a normal body weight, can lead to hypothalamic amenorrhea. The etiology of amenorrhea in anorexia nervosa is thought to be a disturbance in the neurotransmitter regulation of pulsatile GnRH release. Levels of LH, FSH, and estradiol are low, pulsatile secretion of LH reverts to a prepubertal pattern, and the uterus and ovaries shrink in size.[6]

Contributing factors include low body weight, excessive exercise, stress-induced activation of the hypothalamic-pituitary-adrenal axis, and caloric restriction with a negative energy balance. While there clearly is a relationship between low body weight and amenorrhea in a number of different conditions, earlier theories proposed by Frisch and McArthur[26] of a critical percentage of body fat being necessary for the onset or maintenance of regular menses have been challenged because they are not based on experimental data. Some women with a low percentage of body fat have regular menses and, both in athletes and in those with anorexia nervosa, studies have demonstrated no difference in percentage of body fat between those who are amenorrheic compared with those who are menstruating regularly.[27] Approximately 20% of patients with anorexia nervosa develop amenorrhea before significant weight loss.[6,27] The reason for this is not clear, but it is possible that the amenorrhea is mediated by leptin's response to caloric restriction and a negative energy balance prior to significant weight loss.

The prevalence of *bulimia nervosa* in young women is approximately 5%. In contrast to anorexia nervosa, patients with bulimia nervosa are usually of normal weight and usually have regular menses. However, they may also have irregular menses as a result of repeated episodes of dietary restriction. They do not usually have prolonged amenorrhea though, unless

they are also of low weight, in which case, they would meet diagnostic criteria for anorexia nervosa, binge-eating/purging type. Most adolescents with an eating disorder do not meet strict criteria for either anorexia nervosa or bulimia nervosa and are classified as having an *eating disorder not otherwise specified* (EDNOS).[28] Some of these patients may also have amenorrhea.

In anorexia nervosa, nutritional rehabilitation and weight restoration are associated with resumption of menses. After one year of treatment, approximately two-thirds of patients resume menses and after two years, approximately 95% do so. Resumption of menses usually occurs at a weight that is approximately 5 lbs greater than the weight at which menses were lost. The average weight at resumption of menses is 90% of median weight for age and height and 86% of patients will resume menses within 6 months of achieving this weight.[27]

Exercise-Induced Amenorrhea

As more women become active in sports and physical activities, more research is being accrued on effects of physical activity on the menstrual cycle. It has been noted that female athletes often have a delay in menarche and/or develop irregular menses during their training.[29] In the early 1990s the syndrome of Female Athlete Triad (irregular menses, disordered eating, and osteoporosis/osteopenia) was described to make physicians and coaches aware of the interrelationships among these three conditions. Although the prevalence of menstrual irregularities differs between various sports, the rates are often quite high, affecting from 12 to 79% of athletes.[30] In a recent study of female high-school athletes, 23.5% of those surveyed had menstrual irregularity.[31]

Generally, exercise-induced amenorrhea results from suppression of the GnRH pulsatility leading to hypoestrogenism, although the mechanisms by which this occurs may vary. In sports that emphasize leanness, amenorrhea results from hypoestrogenism secondary to suppression of the HPO axis. Several hypotheses have been advanced to explain the mechanism of this suppression, including the body-composition hypothesis and the exercise-stress hypothesis.[26,32] More recent evidence, however, suggests that for athletes participating in lean sports, the energy-drain hypothesis is the most likely explanation for the menstrual irregularity.[33] Athletes with amenorrhea may be consuming too few calories to meet their energy needs, thus resulting in a state of chronic caloric deprivation. As described above, the effects of this negative energy balance on the hypothalamus may be mediated by leptin.[34]

In sports where strength rather than low body weight is the emphasis, another mechanism may explain the hypothalamic disruption of the reproductive axis. These athletes often have elevated androgen levels, elevated LH levels, and elevated LH/FSH ratios rather than the aforementioned hypoestrogenism.[35] It has been hypothesized that the elevated androgens are secondary to stimulation of the hypothalamic-pituitary-adrenal axis, though it is unclear what initiates this stimulation. The increased androgens may impair follicular development at the ovarian level, thus causing anovulation and amenorrhea.

Medication-Induced Amenorrhea

Approximately 50% of patients who are taking antipsychotic medication develop menstrual disturbances and 12% develop amenorrhea.[36] Many of the antipsychotic drugs block pituitary dopamine D2 receptors, thereby removing the inhibitory effect of dopamine on prolactin secretion by the pituitary. Prolactin levels can increase five- to tenfold. High prolactin concentrations inhibit the HPO axis by suppressing pulsatile GnRH release, by inhibiting the effect of GnRH on the pituitary, and by blocking the positive feedback effect of estradiol on the hypothalamus.[37] In contrast to the conventional antipsychotic medications, most of the "atypical antipsychotic agents" (e.g., clozapine, olanzapine, quetiapine, ziprasidone, and zotepine) usually have a minimal effect on prolactin secretion. Two exceptions to this rule, however, are risperidone and amisulpride, both of which can cause marked hyperproloactinemeia. Other dopamine antagonists such as methyldopa and metoclopramide may also cause hyperprolactinemia and amenorrhea.

Antidepressants with increased serotonergic activity (e.g., the selective serotonin receptor inhibitors, the monoamine oxidase inhibitors and some tricyclics) may also increase prolactin levels, but to a lesser degree. Discontinuing the offending drug usually results in resolution of the problem. Another group of medications frequently used in the adolescent age group and that can cause amenorrhea is injectable contraceptives. After one year of treatment with medroxyprogesterone acetate (DMPA) approximately 50% of users develop amenorrhea and after two years the number increases to 75%.

Stress-Induced Amenorrhea

Physical and psychosocial stress interrupts homeostasis and redirects energy and other resources away from nonessential functions. such as reproduction, to the central nervous system and cardiovascular systems. This adaptive response results in improved

alertness and arousal, with increases in pulse, blood pressure, and respiratory rate. There is activation of the hypothalamic-pituitary-adrenal axis, with increased secretion of corticotropin-releasing hormone (CRH), and stimulation of the sympathetic nervous system, with release of epinephrine and norepinephrine. Many of the central nervous system, metabolic, and cardiovascular responses are mediated via CRH release from the paraventricular nucleus of the hypothalamus. Secretion of CRH is responsible for activation of central endogenous opioid activity, in particular, that of beta-endorphin. The HPO axis is inhibited at a number of levels by complex interrelated mechanisms. Both CRH and endogenous opioids directly inhibit GnRH release by the hypothalamus. In addition, glucocorticoids inhibit pituitary LH secretion as well as ovarian estrogen and progesterone production.[38] The effect of beta endorphin appears to be modulated by estrogen. For example, both in postmenopausal women and in ovariectomized rats, naloxone, an opioid receptor antagonist, does not induce the expected rise in plasma LH levels, whereas after hormonal replacement therapy, the LH response to naloxone is restored.[39] Stress-induced inhibitory effects on the HPO axis may be responsible, in part, for the amenorrhea associated with depression, eating disorders, and excessive exercise.

Chronic Illness

Chronic illness can lead to pubertal delay and cessation of menses through a variety of mechanisms. Because chronic illnesses may affect nutritional, behavioral, metabolic, and hormonal aspects, different causes of the amenorrhea exist. The pathways may be similar to those for hypothalamic amenorrhea associated with eating disorders, stress, and medications. In particular, illnesses such as renal disease, liver disease, immunodeficiencies, inflammatory bowel disease, and uncontrolled diabetes have been associated with anovulation and amenorrhea.

In multiple illnesses, a delay in sexual maturation has been shown.[40,41] Malnutrition may be a leading factor in both pubertal delay and secondary amenorrhea. Many diseases cause an increased caloric need: intestinal diseases leading to malabsorption and protein wasting, the chronic inflammation of juvenile arthritis, and increased cardiac expenditures in congenital heart disease. Despite the increased demand, many patients have decreased intake either from suppressed appetite or from a concomitant eating disorder that is found with increased prevalence in certain diseases such as type 1 diabetes mellitus.[42–44] The resulting caloric discrepancy may lead to chronic malnutrition. In a study of patients with cystic fibrosis, it was shown that 23 of 45 patients had irregular menstrual cycles and that lower weight was a major determining factor.[45]

Factors due to specific disease states may also contribute to delay of puberty and secondary amenorrhea. For example, ovarian autoantibodies have been found in patients with type 1 diabetes mellitus.[46] In peripubertal patients with chronic renal failure, an end-organ hyporesponsiveness to GH was identified.[47] Additionally, medications used on a chronic basis for treatment and management of certain illnesses may contribute to menstrual dysfunction.

Kallmann Syndrome

Kallmann syndrome, the association of isolated hypogonadotropic hypogonadism and anosmia, is a genetic disorder caused by one or more mutations of the KAL gene at Xp22.3.[48] The syndrome affects approximately 1 in 10,000 males and 1 in 50,000 females.

The olfactory and GnRH neurons have a common origin outside the central nervous system in an area called the olfactory placode. The GnRH neurons subsequently migrate from this region to their final destination in the preoptic area of the hypothalamus. In Kallmann syndrome, there is a defect in the process of migration of the olfactory and GnRH neurons. Girls with Kallmann syndrome typically are not identified until adolescence, when they present with failure of sexual development and primary amenorrhea.

Pituitary Causes of Amenorrhea
Hyperprolactinemia

Approximately 10–40% of women with hyperprolactinemia present to their physician with amenorrhea. Hyperprolactinemia's effect on the menstrual status is via its effect on the pulsatility of GnRH. With interference of the normal GnRH pulsatility, gonadotropins are suppressed, and a subsequent decrease in estrogen levels is seen. Multiple causes of hyperprolactinemia exist, including: physiological states such as pregnancy and lactation, certain medications, endocrinopathies such as primary hypothyroidism and PCOS, systemic diseases such as systemic lupus erythematosus or rheumatoid arthritis, and chronic renal failure. Additionally, tumors of both the pituitary gland, as well as other organs, can cause hyperprolactinemia.[49]

Prolactinoma

Prolactinomas are tumors of the anterior pituitary gland that are typically classified as microadenomas (<10 mm) or macroadenomas (>10 mm).[49] Adenomas are the most common cause of anterior pituitary dysfunction[50] and are thought to be present in 50–60% of women with hyperprolactinemia.[51] Prolactinomas

result in a estrogen-deficient state via the inhibitory action of elevated prolactin levels on GnRH pulsatility and a subsequent decrease in pituitary release of LH and FSH.

Isolated Gonadotropin Deficiency

Congenital isolated gonadotropin deficiency is defined by complete or partial absence of GnRH-induced gonadotropin secretion, normal anatomy of the hypothalamus and pituitary, normal baseline functioning of the remaining HP axis, and a normal sense of smell. When problems with olfactory function are present, this disorder is referred to as Kallman syndrome. A genetic basis for this disorder has been found in fewer than 20% of cases, but mutations in the GnRH receptor (*GnRH-R*), G protein–coupled receptor-54 (*GPR54*) and its ligand, kisspeptin, have been implicated.[52]

Craniopharyngioma

Craniopharyngiomas are epithelial tumors arising from the craniopharyngeal duct in the sellar or parasellar region. They represent 2–5% of all intracranial neoplasms and 5.6–15% of intracranial tumors diagnosed in children.[53] They have an uncertain pathogenesis, and present with a variety of symptoms—endocrine, visual, behavioral, and cognitive. Hormonal disruption is prevalent, and a deficiency of LH/FSH is found in approximately 38–82% of patients.[53] This disruption is the likely mechanism for menstrual dysfunction.

Thyroid Causes of Amenorrhea
Hypothyroidism

Thryoid disease is more prevalent in females than males and often presents in adolescence. Previous studies have cited menstrual disturbances in hypothyroid patients ranging from 20% to 70%,[54] with decreasing incidence as thyroid disorders are diagnosed earlier. Although hypermenorrhea or oligomenorrhea are the more prevalent menstrual disorders, amenorrhea can also be seen. One proposed mechanism for the amenorrhea is through the effect of TSH-releasing hormone on prolactin levels. This releasing hormone acts on the thyrotrophs to release TSH and on lactotrophs to release prolactin. Secondary to the increased levels of TSH-releasing hormone found in hypothyroidism, increased levels of prolactin are released, leading to functional hyperprolactinemia.[55] The estimated incidence of hyperprolactinemia in hypothyroidism ranges from 0–40%.[56]

Hyperthryoidism

Patients with hyperthyroidism have rates of menstrual irregularities ranging from 20% to 60%, with rates of amenorrhea reaching up to 20%.[54] The exact mechanism for the amenorrhea is not clear, but appears to be a combination of hormonal abnormalities, nutritional deficiencies, and emotional stress that may accompany hyperthyroidism.

Hyperthyroidism results in increased levels of sex hormone-binding globulins, which may cause an increased level of plasma estrogen. Levels of circulating androgens, particularly testosterone and androstenedione, are also elevated, as is their conversion to their estrogen counterpart, estradiol or estrone.[54] While mean LH levels are generally higher in hyperthyroid women, LH peaks may be completely absent in women with amenorrhea.

Adrenal Causes of Amenorrhea
Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia refers to a group of autosomal recessive disorders of steroidogenesis. A number of enzyme deficiencies have been found, but more than 90% of cases are caused by deficiency of 21-hydroxylase, which catalyses the conversion of progesterone to deoxycorticosterone and 17-OH progesterone to 11-deoxycortisol.[57,58] There is an interruption in the pathway leading to synthesis of mineralocorticoids and glucocorticoids. Consequently, progesterone, 17-OH progesterone, and its precursors are, instead, shunted to the androgen pathway. Hypothalamic secretion of CRH increases to overcome the effects of the block. As a result, ACTH secretion is increased, further increasing androgen production. The two other major enzyme deficiencies are deficiencies of 11-beta-hydroxylase and 3-beta-hydroxysteroid dehydrogenase.

There are two major clinical forms of congenital adrenal hyperplasia, depending on whether the enzyme deficiency is complete or partial—the classic form and the nonclassic form. The classic form usually presents in infancy with salt wasting or ambiguous genitalia. It occurs in approximately one in 16,000 births. The nonclassic form is one of the most frequently seen autosomal genetic disorders and occurs in approximately 0.2% of the general population.[58] This nonclassic form of congenital adrenal hyperplasia usually presents in childhood and is characterized by premature pubarche and in adolescence by hirsutism or amenorrhea.[59] Hyperandrogenism has a direct effect on the hypothalamus, suppressing pituitary–ovarian function. The suppression of the HPO axis can be reversed after adequate treatment of the excess adrenal androgen production.[60]

Cushing Syndrome

Cushing syndrome is caused by high circulating levels of cortisol. In adolescents, Cushing syndrome is

most frequently caused by iatrogenic exogenous administration of corticosteroids. Other causes include hypersecretion of corticotropin by a microadenoma of the anterior pituitary (Cushing Disease); secretion of corticotropin by an adrenal tumor, or, occasionally, ectopic production of corticotropin by a nonpituitary tumor such as carcinoma of the lung.

The clinical findings are usually self-evident. Oligomenorrhea and amenorrhea may be part of the clinical picture. The pathophysiology is direct suppression of the HPO axis.

Ovarian Causes of Amenorrhea
Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is an endocrine disorder that may affect 5 to 10% of 18–25-year-old women worldwide.[61,62] Although characterized by hyperandrogenism (either biochemical or clinical signs), chronic oligo- or anovulation, and polycystic ovaries seen on ultrasonography, the criteria for diagnosis are still under debate: the 1990 National Institutes of Health criteria require evidence of hyperandrogenism and oligo- or anovulation for diagnosis, while the 2003 Rotterdam criteria require two of the three aforementioned characteristics.[63] There are no formal diagnostic criteria for adolescents with PCOS, though the syndrome may present as early as the first decade of life. PCOS can be extremely varied in its presentation, which may reflect the different pathologies leading to the syndrome.

The cause of PCOS is unclear, but it does appear to be a combination of defects in insulin resistance and ovarian and/or adrenal hypersensitivity. The inherent metabolic feature is insulin resistance and hyperinsulinemia that is independent of obesity.[64] The insulin resistance also appears to be tissue-selective; the adrenal and ovary remain highly insulin-sensitive, whereas skeletal muscle is resistant. Through different mechanisms, hyperinsulinemia stimulates increased androgen production. It directly activates ovarian insulin receptors to cause activation of the P450c17 enzyme, and it activates IGF-1 receptors. Elevated insulin levels reduce the circulating levels of sex hormone–binding globulin (SHBG), which, in turn, increases the levels of circulating free testosterone. The longer-term sequelae of this insulin derangement include dyslipidemia, glucose intolerance, and central adiposity, which can increase the risk of premature cardiovascular disease.

Typical hormonal findings in PCOS include elevated free testosterone, normal or low total testosterone, and elevated levels of DHEAS, androstenedione, LH, and the LH/FSH ratio. The elevated androgen levels are secondary to defects in ovarian and/or adrenal steroidogenesis; approximately 80% of patients have a form of functional ovarian hyperandrogenism and 50% have functional adrenal hyperandrogenism. At the level of the ovary, there is an increased response in 17-OHP to GnRH analogue stimulation.[65] Additionally, there appears to be ovarian hypersensitivity of cytochrome P450c17, which plays a key role in the biosynthesis of androgens.[66] Within the adrenal gland, there is an exaggerated steroid response with corticotropin analogue stimulation that results in augmented production of 17-OHP and androstenedione.[67] The typical inhibitory feedback of estrogen and progesterone on the hypothalamus is interrupted, likely because of these increased levels of androgens.[68] The result is an increase in GnRH pulsatility, which leads to excessive LH secretion and the abnormal LH/FSH ratio.

Gonadal Dysgenesis

Gonadal dysgenesis refers to a number of conditions in which gonadal development is abnormal, leading to streak gonads. Estrogen levels are low and levels of LH and FSH are markedly elevated. The most common form is Turner syndrome (45,X karyotype), but other forms do exist, such as pure gonadal dysgenesis (where the karyotype is normal) and Swyer syndrome (XY gonadal dysgenesis).

Turner syndrome occurs in approximately 1 in every 2,500–3,000 live births in females. In approximately 50% of cases it is associated with complete or partial absence of an X chromosome (45,X karyotype). Other forms include mosaics (a combination of 45,X and a normal 45,XX cell line, as well as several other karyotype variations). In addition to delayed puberty and primary amenorrhea, other clinical features include short stature, webbed neck, low hairline, widely spaced nipples, cubitus valgus, cardiac anomalies (coarctation of the aorta), and a horseshoe kidney.

Premature Ovarian Failure

Premature ovarian failure (better termed "premature ovarian insufficiency") can occur at any age and is characterized by elevated gonadotropins in the presence of estrogen deficiency. It occurs in a number of conditions including autoimmune oophoritis, mumps oophoritis, chemotherapy, irradiation as well in less common conditions such as galactosemia, Trisomy 21, female fragile X carriers, and sarcoidosis. The mechanism of premature ovarian failure in galactosemia is not clear, but it appears that galactose or galactose-1-phosphate may be toxic to the ovary during the neonatal period through adulthood, leading to ovarian atrophy or failure.[69,70]

Autoimmune oophoritis may be associated with other autoimmune conditions such as thyroiditis, Addison's disease, hypoparathyroidism, diabetes mellitus, myasthenia gravis, and vitiligo. The course is variable, and levels of gonadotropins and estradiol may fluctuate for months or years. Usually there is progression, but there may be spontaneous remission.

Uterine Causes of Amenorrhea
Androgen Insensitivity (Testicular Feminization Syndrome)

Androgen insensitivity has an estimated incidence of 1/20,000 to 1/99,000 and is due to an X-linked recessive inheritance of a single gene.[71] Androgen insensitivity is most commonly due to a complete defect in the binding of androgens at androgen receptor sites, though there can be varying degrees of androgen receptor dysfunction. Testosterone levels are normal. Patients appear phenotypically female in their external habitus, but have a chromosomal pattern that is XY. Typically, patients will have normal breast development, minimal axillary and pubic hair, a short vagina, and an absent uterus and cervix. Testes are present, but are cryptorchid, without spermatogenesis. Androgen insensitivity will often be diagnosed in adolescence, when a patient presents with primary amenorrhea.

Uterine Adhesions (Asherman Syndrome)

In secondary amenorrhea, the most common cause of outflow obstruction is Asherman syndrome. Asherman syndrome is the presence of intrauterine synechiae or scarring, typically from a previous infection or from a curettage procedure following a postpartum or postabortal endometritis. In addition to amenorrhea, patients with Asherman syndrome may present with recurrent abortions or infertility. Generally, the extent of menstrual cycle disruption is indicative of the severity of intrauterine scarring.

Müllerian Agenesis

Müllerian agenesis is the congenital malformation of the genital tract, resulting in absence of the vagina, an abnormal or absent uterus, and normal ovaries. In addition, skeletal, ear, and renal deformities may be present. The syndrome, often referred to as Mayer–Rokitansky–Kuster–Hauser syndrome, is the second most common cause of primary amenorrhea.[72] It is has been estimated to have a prevalence varying from 1 in 4,000 to 5,000 female births. Patients with müllerian agenesis will typically have normal secondary sexual characteristics and ovarian function, but present with primary amenorrhea.

The etiology of müllerian agenesis in unknown, though it has been hypothesized to result from the activation of anti-müllerian hormone or the anti-müllerian hormone receptor through a genetic mutation. This activation results in increased secretion of the hormone or the receptor, which acts on the developing female fetus, to cause regression of the müllerian duct.

Cervical Agenesis

Rarely, isolated cervical agenesis with a normal uterus can be found. Typically, patients will present with amenorrhea, cyclic abdominal pain, and a distended uterus on examination. Due to obstruction of the outflow outlet, hematosalpinx and endometriosis are likely sequelae.

Vaginal Causes of Amenorrhea
Imperforate Hymen

Imperforate hymen occurs in 1 in 1,000 women. It may be diagnosed in childhood, but may also be missed and may present in adolescence with cyclic abdominal pain and primary amenorrhea. The typical physical finding is a bulging, bluish hymen, behind which is a blood-filled mass in the distended vagina (hematocolpos). If the mass becomes large enough, it may cause acute urinary retention.

Transverse Vaginal Septum

A complete transverse septum occurs in approximately 1 in 80,000 women and is due to incomplete fusion of the müllerian duct portion of the vagina and the urogential sinus component. The thickness and placement of the septum may vary: lower vagina, middle vagina, or upper vagina. Eighty to 90% occur in the middle or upper vagina, and the external genital examination appears normal.[73] Though a perforation in the septum is often present, patients may still present with amenorrhea and hematocolpos. Additionally, other malformations of the urological tract or rectum may be associated.

Vaginal Agenesis—Isolated

Vaginal agenesis is typically associated with uterine agenesis; however, there have been rare cases of isolated vaginal agenesis with a normal uterus.

Clinical Evaluation

An algorithm describing the clinical approach to amenorrhea in adolescents, based on whether the patient has primary amenorrhea or secondary amenorrhea and whether she is estrogen-deficient or estrogen-replete, is shown in FIGURES 2 and 3.

Both for primary amenorrhea and for secondary amenorrhea, the key is the history and physical examination, together with some basic tests to rule out

FIGURE 2. Algorithm for management of an adolescent with primary amenorrhea.
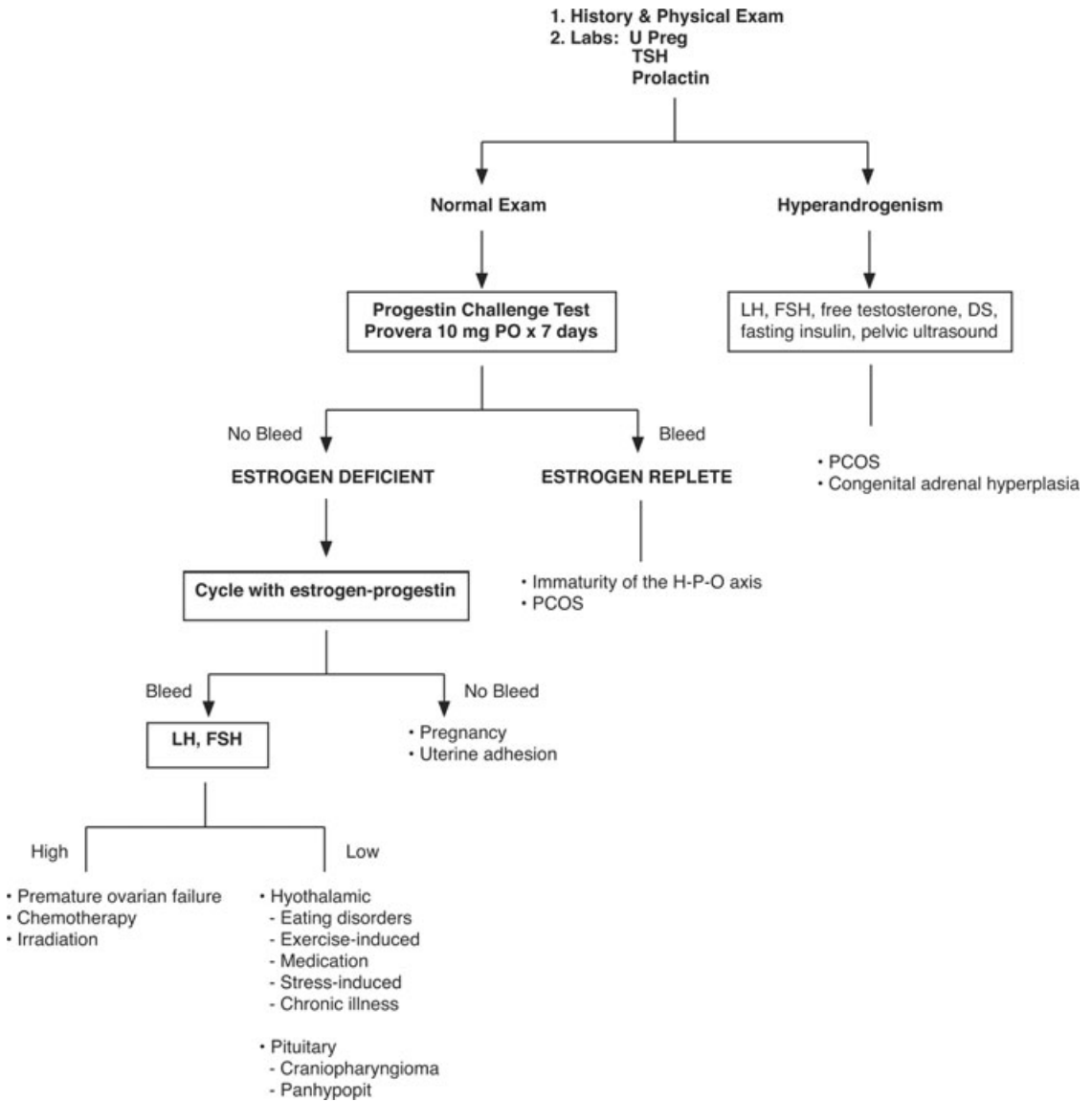
pregnancy, thyroid disease or hyperprolactinemia. If an elevated prolactin level is found and the patient is not on medication known to increase serum prolactin levels, an MRI should be obtained to rule out a prolactinoma. The history should elicit the age of the patient at thelarche, the age at which the patient's mother had menarche, whether or not the patient is sexually active, and any medications she is taking. Specific information should be obtained regarding weight loss, preoccupation with shape and weight, and the amount and intensity of exercise.

For the patient with primary amenorrhea, the physical examination should focus on pubertal development and possible genital outflow obstruction. The physician should remember that any cause of secondary amenorrhea can also cause primary amenorrhea. Any major discrepancy in Tanner staging of the breast and pubic hair development (e.g., Tanner V breast development in the absence of pubic hair or lack of breast development in the presence of adequate pubic hair) should arouse suspicion of a chromosomal abnormality such as androgen insensitivity (46,XY) or Turner syndrome (45,X). Visualization of the external genitalia should be performed to assess the patency of the

hymen. If indicated, a rectoabdominal examination can be performed to rule out any pelvic mass. A pelvic ultrasound will confirm the presence of a uterus and rule out any obstructive lesion.

For patients with secondary amenorrhea, the physical examination should focus on signs of hyperandrogenism and insulin resistance as well as evidence of weight loss. Height and weight should be accurately measured and BMI calculated. The presence of hyperandrogenism should focus the investigation in the direction of PCOS or congenital adrenal hyperplasia. Assessment of the pelvis may include a bimanual vaginal examination or a rectoabdominal examination. Whether or not the patient is estrogen-deficient or estrogen-sufficient can be determined by response to the progestin challenge test. After administration of a 7-day course of Provera, menstrual bleeding within a few days of discontinuing the medication indicates estrogen sufficiency. The most likely diagnosis is either immaturity of the HPO axis if the patient is within 2–3 years of menarche, or PCOS, especially if evidence of hyperandrogenism is present. If there is no response to the progestin challenge test, the patient is estrogen-deficient and the work-up should proceed as indicated in FIGURE 3.

1. History & Physical Exam
2. Labs: U Preg
             TSH
             Prolactin

Normal Exam   Hyperandrogenism

Progestin Challenge Test   LH, FSH, free testosterone, DS,
Provera 10 mg PO x 7 days   fasting insulin, pelvic ultrasound

No Bleed   Bleed

ESTROGEN DEFICIENT   **ESTROGEN REPLETE**   · PCOS
                                                       · Congenital adrenal hyperplasia

Cycle with estrogen-progestin   · Immaturity of the H-P-O axis
                                    · PCOS

Bleed   No Bleed

LH, FSH   · Pregnancy
                   · Uterine adhesion

High   Low

· Premature ovarian failure   · Hyothalamic
· Chemotherapy   - Eating disorders
· Irradiation   - Exercise-induced
                                - Medication
                                - Stress-induced
                                - Chronic illness

                              · Pituitary
                                - Craniopharyngioma
                                - Panhypopit

FIGURE 3. Management of secondary amenorrhea.

Some patients with premature ovarian insufficiency may respond to a progestin challenge test. In order to avoid missing this diagnosis, some experts recommend assessing the LH, FSH and estradiol levels even in the face of a positive response to the progestin challenge test.

Implications of Amenorrhea in Adolescents

In an adolescent who is estrogen-replete, prolonged amenorrhea secondary to anovualtion leads to hyper-plasia of the endometrium. Under the influence of unopposed estrogen stimulation, the endometrium becomes thick, outgrows its blood supply, and begins to slough off irregularly, leading to dysfunctional uterine bleeding (DUB). DUB is the most common cause of abnormal uterine bleeding in adolescents, accounting for 95% of cases presenting to a physician in the outpatient setting and approximately 80% of those hospitalized for this reason. The menstrual bleeding is characteristically painless. It may be mild and prolonged, but may also be excessive, leading to severe anemia and shock. DUB can be avoided by preventing the unopposed

estrogen effect on the endometrium. Administration of progesterone (Provera, 10 mg orally per day for 7 days) to an adolescent girl after 2–3 months of secondary amenorrhea will prevent DUB from occurring. Prolonged exposure to unopposed estrogen stimulation should be avoided because it predisposes to endometrial carcinoma later in life.

In the estrogen-deficient adolescent, the major medical morbidity is reduction in bone mineral density and increased risk of fracture. The adolescent years are critical for accretion of bone mass and any condition interfering with this process can result in failure to achieve peak bone mass, predisposing to a life-long increased risk of fractures. In anorexia nervosa, reduction in bone mass occurs after a relatively short duration of illness and can be severe.[74] More than 90% of adolescent and young adults with anorexia nervosa have reduction of bone mineral density at one or more sites.[74,75] Large population-based studies have shown that the long-term fracture risk in anorexia nervosa is increased two- to threefold compared with controls.[25,76] Treatment with estrogen replacement therapy does not reverse the bone loss.[77–80] Exercise-induced amenorrhea, the so-called "female athlete triad," is also associated with reduction in bone mass and increased fracture risk. With reduction of the intensity of training and improvement in nutritional intake, resumption of menses can occur and bone mineral density can improve. Prolonged administration of DMPA is associated with reduction in BMD which is reversible after discontinuing the medication.[81,82]

Directions for Future Research

Hypothalamic amenorrhea is the most common, but least well understood, cause of amenorrhea in adolescents. The discovery of leptin in 1994 has markedly improved our understanding of the pathophysiology of hypothalamic amenorrhea in adolescents, but much remains unknown. The promise of understanding the "hidden link" between nutritional status and reproductive function has not yet been entirely fulfilled. Future research should further explore the complex neuroendocrine regulation of hypothalamic function. What is the role of leptin in this process? Are the pathways for malnutrition, exercise-induced amenorrhea, and stress-induced amenorrhea similar or different? Many of the causative factors (e.g., low body weight, body composition, nutritional intake, exercise, and stress) are interrelated. What are the relative contributions of each? Future studies should include both biochemical and behavioral measures to better understand the mul-

tiple, overlapping processes that are occurring. Such components could include serum hormone markers, leptin values, exercise and nutrition variables, eating disorder behaviors, and measures of body composition and resting energy expenditure. Finally, the recent synthesis of recombinant leptin brings with it the hope of improved understanding of the pathophysiology of amenorrhea in adolescents. Recombinant leptin may have important therapeutic possibilities not only to treat amenorrhea, but also to improve bone mineral density in those adolescents who are estrogen-depleted.

Conflict of Interest

The authors declare no conflicts of interest.

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